

FOOD ADDITIVES: COMPETITIVE, REGULATORY, AND SAFETY PROBLEMS

HEARINGS
BEFORE THE
SELECT COMMITTEE ON SMALL BUSINESS
UNITED STATES SENATE
NINETY-FIFTH CONGRESS
FIRST SESSION
ON
FOOD ADDITIVES: COMPETITIVE, REGULATORY,
AND SAFETY PROBLEMS

PART 1

JANUARY 13 AND 14, 1977



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FOOD ADDITIVES: COMPETITIVE, REGULATORY, AND SAFETY PROBLEMS

THURSDAY, JANUARY 13, 1977

U.S. SENATE,
SELECT COMMITTEE ON SMALL BUSINESS,
Washington, D.C.

The committee met, pursuant to notice, at 9:30 a.m., in room 1818, Dirksen Senate Office Building, Hon. Gaylord Nelson, chairman, presiding.

Present: Senator Nelson.

Also present: Benjamin Gordon, staff economist; Karen Young, research assistant; and Judith Robinson, Senator Nelson's staff.

OPENING STATEMENT OF HON. GAYLORD NELSON, A U.S. SENATOR FROM THE STATE OF WISCONSIN, AND CHAIRMAN, SENATE SMALL BUSINESS COMMITTEE

Senator NELSON. The committee will come to order.

We are today opening 2 days of hearings to assess where we are with respect to the marketing, regulation, and safety of food additives.

This is the first time since 1972, when the Senate Select Committee on Nutrition and Human Needs conducted hearings, that the current regulatory and commercial status of food additives has been aired in public congressional hearings.

Concern about the safety of a number of additives led to the examination of the subject by this committee and the General Accounting Office.

Safety questions raised about certain additives include the potential to cause cancer, birth defects, genetic damage, and other harmful effects on human health.

The GAO, in three separate reports to us, revealed a large number of problems relating to: (1) Food additives testing—the state-of-the-art for screening and evaluation of safety; (2) the desirability for an independent testing system, free from economic bias on the part of product promoters; (3) the need for new statutory suspension authority for regulatory agencies to utilize when safety questions about additives—and other substances to which the public is widely exposed—arise; (4) the need to set some guidelines for reaching regulatory decisions based on benefits versus risks; and (5) the need to ascertain what research is being conducted into alternatives for additives that pose safety questions.

“No segment of the environment to which humans are exposed is as chemically complex as food, and, in large part, the chemical and

biological properties of food components and their relationships to human illness and disease are poorly understood," says the second National Institute of Environmental Health Sciences Task Force report on "Human Health and the Environment: Some Research Needs," which is about to be issued.

We have reached the point in the United States where more processed, convenience, snack, and franchised food products are purchased than fresh foods.

This means that consumers are consuming more chemicals that make it possible to process foods with a long shelf and shipping life.

Many of these substances are safe and economic to use as stabilizers, emulsifiers, antioxidants, flavoring and coloring agents, nonnutritive sweeteners, texturizers, and for other purposes.

However, by 1970, the use of food additives had more than doubled in two decades to more than 1 billion pounds a year, according to industry figures. The average American, the industry estimates, eats 5 pounds of additives a year. More kinds of food additives are approved for use in the United States than in any other nation.

The FDA regulates more than 2,100 additives for uses in food directly, and more than 10,000 additives in packaging and other indirect uses.

Unfortunately, many chemicals long used in the food supply are being shown to be harmful under new scientific scrutiny; many were never adequately pretested prior to approval and widespread use; and the number of new chemicals or new uses of them continue to proliferate without a real assessment of their nutritional value and sometimes with potential health hazards.

The questions must be asked:

Where are we now, with respect to the chemicalization of our food supply?

Can the food supply to the Nation and the world be expanded safely with or without the large numbers of chemicals that are now going into the food?

What synergistic effects are chemicals in the food supply having on human health when they are combined with the mass of other chemicals and substances in the environment, including drugs?

If we are going to continue the widespread use of these chemicals and, in fact, increase their use, there must be carefully developed scientific evidence showing that they are safe, necessary and free from long-term possibly irrevocable, effects on the human race. The technology now exists to do so.

Foods must be safe and wholesome before they are placed on the grocery shelf. Labeling and nutritional education cannot prevent cancer or birth defects if the food supply causes insidious hazards. Scientists are concerned that long-term, low-doses of chemicals may cause not only cancer, but birth defects or genetic damage.

Scientific knowledge now exists to screen these chemicals for such effects.

We are here today to begin building a record documenting the extensive use, marketing, regulation and safety of food additives.

These hearings may lead to legislation, designed to meet the needs of the times with respect to mass human exposure and the state-of-the-art for testing.

As information is generated in the hearings, the committee will consider whether to conduct further hearings, and will accept material for the hearing record that contributes new and relevant information.

Our first witness this morning is Mr. Gregory J. Ahart, Director of Human Resources Division of the General Accounting Office.

Mr. Ahart, if you would identify your associates, so that the reporter will have a correct record.

STATEMENT OF GREGORY J. AHART, DIRECTOR, HUMAN RESOURCES DIVISION, U.S. GENERAL ACCOUNTING OFFICE, ACCOMPANIED BY ALBERT B. JOJOKIAN, GAO; AND STUART FLEISHMAN, GAO

Mr. AHART. Thank you, Mr. Chairman.

On my immediate right is Mr. Albert B. Jojokian, and on his right is Mr. Stuart Fleishman, who works with Mr. Jojokian.

Mr. Chairman and members of the committee, we are pleased to appear here today to discuss our reports on the Food and Drug Administration's (FDA's) regulation of three color and food additives—Red No. 2, saccharin, and aspartame. In addition we have issued a report to the Congress on chemical carcinogens including food additives and we have recently initiated a broad scale review of FDA's regulation of food additives. We will discuss these also.

Our reviews concerning the three additives were directed primarily toward developing information on (1) The history of FDA's regulation of them, (2) the current status of testing the safety of the additives, and (3) whether the regulatory actions taken by FDA on the three additives complied with the Federal Food, Drug, and Cosmetic Act as amended (21 U.S.C. 301).

REGULATION OF RED NO. 2

Red No. 2 is the name given to a certified lot of the dye generically known as amaranth. The composition and purity of amaranth varies. FDA has established composition and purity specifications that amaranth must meet before it can qualify for use in food, drugs, and cosmetics. Only amaranth meeting such specifications is classified as Red No. 2.

Since July 12, 1960, the color additive amendments to the Food, Drug and Cosmetic Act have required FDA to establish regulations listing color additives that are safe for use in food, drugs, or cosmetics. Such regulations may list color additives for use generally in food, drugs, or cosmetics or may prescribe the conditions under which the color additives may be safely used.

The act provides that a color additive is deemed unsafe and should not be listed in a regulation permitting its use in food, drugs, or cosmetics if it is found by FDA to induce cancer in man or animal.

The Food, Drug and Cosmetic Act, as amended in 1960, placed all color additives commercially established at that time, including Red No. 2, on a provisional list to allow their use for a reasonable period until their safety could be reviewed and regulations for their use could be issued.

Senator NELSON. How many food additives were being used at that time?

Mr. AHART. Mr. Jojokian, do you know about how many there were?

Mr. JOJOKIAN. I do not know the exact figure, but I understand there are 1,300 additives, intentional additives, and I believe 600 unintentional additives.

Senator NELSON. One thousand three hundred intentional?

Mr. JOJOKIAN. That is an estimate.

Senator NELSON. Those were additives added directly to the food?

Mr. JOJOKIAN. Yes, that is correct.

Senator NELSON. When you say unintentional, you mean in the packaging, which may infiltrate the food?

Mr. JOJOKIAN. That is correct.

Senator NELSON. What are the true figures now?

Mr. JOJOKIAN. I believe there are about that many today.

Senator NELSON. But we have been adding additives every year.

Mr. AHART. I do not think we have the exact information as to how many there were then or how many there are now.

We would be glad to furnish that for the record if we could get hold of the data.

Senator NELSON. If the act required in 1960, that many of the additives being used at that time be placed on a "GRAS" or provisional list, how would the FDA put them on a list if they did not know what they were?

Mr. AHART. I assume that information is available, and we just do not have it here.

Senator NELSON. All right.

[The information follows:]

An FDA official told GAO that FDA did not have information on the total number of food additives that were in use in 1960. According to the October 12, 1960, Federal Register, 131 color additives were placed on the provisional list at that time.

Mr. AHART. The 1960 amendments provided that the provisional listing was to terminate no later than 2½ years from the effective date of enactment—July 12, 1960—or January 12, 1963.

Senator NELSON. For purpose of the appropriate place in the record, the staff advised me there are now about 2,100 approved direct additives, contrasted with the 1,300 that you just mentioned.

Mr. AHART. Thank you, Mr. Chairman. The amendments also provided, however, that FDA could postpone the termination date if such action was consistent with the objective of carrying to completion, in good faith, as soon as reasonably practicable, the scientific investigations necessary for making a determination as to the additives' safety.

We found that FDA had permitted the use of Red No. 2 in food, drugs, and cosmetics for 15 years without making a final determination of its safety. FDA postponed termination of the provisional listing for Red No. 2, 14 times on the basis of requests from manufacturers or industry associations to allow completion of scientific investigations concerning its safety.

Since 1970 several scientific studies involving animals, including some performed or sponsored by FDA, raised questions concerning the safety of Red No. 2 in food. In some of these studies Red No. 2 or

amaranth was shown in test animals to be toxic to reproductive systems or to be carcinogenic.

Mr. GORDON. You described the statute as allowing use for a "reasonable" period until safety could be reviewed; that a termination date of January 12, 1963 was given; that postponement of termination date was permitted if such action was consistent with the objective of carrying to completion in good faith, as soon as reasonably practicable, the scientific investigations necessary for making a determination as to the additives' safety.

Would you say that the 15 years during which this product was allowed to be on the market without proof of safety is a reasonable period?

Mr. AHART. In my judgment, Mr. Gordon, it is not.

It seems unnecessarily long to us. The statute itself provided the provisional use of color additives for a reasonable period of time with a limit of 2½ years unless FDA extended the time to allow completion of safety studies, and it seems to me the Congress did not have in mind stretching the period to anything as long as 15 years before you could reach a conclusion regarding safety.

Mr. GORDON. Could you see the public benefiting from this delay?

Mr. AHART. I assume there is some public benefit.

At least it is my understanding that Red No. 2 has some economic benefits over some alternatives that would be available.

In other words, it would be cheaper to the manufacturer, and the reduction, however large it is, if passed on to the consumer, there might be some public benefit in that regard.

Senator NELSON. When you are talking about economic benefit, that is not the function of the Food and Drug Administration.

The function of the FDA is to protect the public health.

Mr. AHART. That is correct.

Senator NELSON. And to make sure what is on the market is safe. Considerations of economic benefits certainly are not within the purview of the FDA, is that correct?

Mr. AHART. I do not think I indicated it was. I was asked to answer the general question whether there was any public benefit from the continued use of Red No. 2 during this long period of time.

Senator NELSON. I see.

Mr. AHART. Because of its concern about the safety of Red No. 2, FDA in July 1972 issued a proposal to limit human exposure to the color additive. However, at the time our report was issued on October 20, 1975, FDA had not made a final determination regarding its safety.

Because we believed that continued use of Red No. 2 before resolving its safety exposed the public to unnecessary risk, we recommended that the Secretary of the Department of Health, Education, and Welfare (HEW) direct the Commissioner of FDA to promptly establish the safety of Red No. 2 or prevent its use in food, drugs, and cosmetics.

Senator NELSON. What date was that?

Mr. AHART. That was in October 1975, Mr. Chairman. October 20, 1975.

On January 19, 1976, FDA announced a ban on the use of Red No. 2 in food, drugs, and cosmetics. FDA took the action because new evi-

dence showed that Red No. 2 caused a statistically significant increase in the number of malignant tumors in test animals and because of what it termed "the absence of other data to allow a definitive judgment of safety."

REGULATION OF SACCHARIN

In our report on saccharin we pointed out that saccharin was "generally recognized as safe" for use in food until about 1970 when studies raised questions about its potential to cause cancer in test animals.

Saccharin is an acid and pure saccharin generally is unsuitable for use in foods and beverages because it is only slightly soluble. It is most often combined with either sodium, calcium, or ammonium salts which neutralize the acid and produce a more readily soluble compound.

The Food, Drug and Cosmetic Act, as amended by the Food Additives Amendment of 1958 (21 U.S.C. 348), requires FDA to establish regulations prescribing the conditions under which a food additive may be safely used. The act defines "food additive" as any substance which becomes or may be expected to become a component of food, either directly or indirectly or which may otherwise affect the characteristics of the food. The proposed use of a food additive whose safety is not generally recognized by qualified scientists must be approved by FDA.

Food additives "generally recognized as safe" are referred to as GRAS substances. Such substances added to food are not considered food additives and are exempt from the requirement for FDA approval.

Senator NELSON. You say they are not considered food additives. They, in fact, are food additives, however.

Mr. AHART. The GRAS food additives are not considered food additives subject to FDA's approval.

Approval is not required.

Senator NELSON. But they are additives which were at the time generally accepted as safe, is that right?

Mr. AHART. That is correct, Mr. Chairman.

Senator NELSON. Were there about 600 of them at the time, or thereabouts, on the GRAS list?

Mr. FLEISHMAN. We do not know the exact figure. There is a very large number that the FDA is presently reviewing, something like 675.

Senator NELSON. In a review of the GRAS list, if they are found to be unsafe, they come under the same statutory provisions as new additives, do they not?

Mr. JOJOKIAN. If new evidence raises safety questions about a GRAS substance, FDA then may issue an interim regulation until those questions are resolved.

Senator NELSON. And if they are found to be unsafe, the same statutory provisions apply, as would apply to new additives?

Mr. JOJOKIAN. That would be correct. If they come under an interim regulation, then they are considered a food additive.

Senator NELSON. When did the FDA start its review of the GRAS list, what year was that?

Mr. JOJOKIAN. I believe 1970.

Senator NELSON. Are you familiar with how far along the FDA is in that review?

Mr. AHART. We have just started a survey, and plan to do a review on FDA's regulations of food additives in general.

I do not think we have the specific information that you are asking for.

We are not that far along in our review.

Senator NELSON. Go ahead.

Mr. AHART. The FDA's food additive regulations define GRAS substances as those which experts determine, based on scientific data or reasoned judgment founded in experience with common food use, pose "no significant risk of harm if used as intended."

Senator NELSON. Now, in fact, the placement of these additives on the GRAS list did not necessarily mean there had been any scientific studies made of the health hazard question?

Mr. AHART. That would be correct.

Senator NELSON. In other words, if they had been in the marketplace for a long time, and the literature did not disclose any health hazard, they were listed on the GRAS list, is that roughly correct?

Mr. AHART. That is correct.

Senator NELSON. So that these additives to the GRAS list were simply those that had been used for quite some time, and there appeared to be no known risk in the scientific community, is that correct?

Mr. AHART. That is correct.

Senator NELSON. But it was not based on individual studies of these items on the GRAS list, individual scientific studies of these items on the GRAS list, is that correct?

Mr. AHART. That would be correct for many of the substances, I am sure.

Senator NELSON. There may be some. The definition is generally recognized as safe?

Mr. AHART. Yes.

Senator NELSON. So a good many of them, the vast majority, were on the list, simply because they were generally recognized as safe, because there were no known health hazards about them that had been yet disclosed, is that correct?

Mr. AHART. That would be correct, yes.

If an important question of safety has been raised regarding a GRAS substance, it may be removed from GRAS status.

An interim food additive regulation may be issued to permit its use while the safety question is being resolved, provided there is reasonable certainty that the substance is not harmful and that no harm to the public health will result from its continued use.

On February 1, 1972, FDA removed saccharin and its various salt forms from the GRAS status and issued an interim food additive regulation limiting the use of saccharin in foods.

The interim regulation stated that preliminary results from studies on long-term feeding of saccharin to animals conducted by FDA and others indicated "possible adverse effects." According to the regulation, if the experimental findings indicate that continued use of saccharin poses a "significant risk" to the public health, action would be taken as warranted to minimize the risk. The regulation authorized saccharin's use as a sweetening agent only in special dietary food and for certain technological purposes such as reducing bulk and enhancing flavors in chewable vitamin tablets. This authority for saccharin's use was to expire June 30, 1973.

However, on May 25, 1973, FDA issued a Federal Register notice extending saccharin's interim regulation indefinitely. The Federal Register identified several completed or nearly completed long-term feeding studies made of three different animal species. These study results showed a statistically significant incidence of bladder tumors in the male offspring of test animals fed saccharin.

Senator NELSON. Why was that done?

Mr. AHART. As I indicate here, the Register pointed out there were studies being made on three different species of animals, and at the completion of those studies, they would make a determination of safety of saccharin.

I assume it was extended so those studies could be completed, so they would have better data upon which to base a judgment.

Senator NELSON. But during this interim period, there has been a question of safety?

Mr. AHART. The question of safety was raised in 1970.

The Federal Register indicated that these studies were referred to the National Academy of Sciences for review and that a final determination of saccharin's safety would be made after FDA received recommendations from the Academy.

Senator NELSON. When were the studies referred to the National Academy of Sciences?

Mr. AHART. Some time prior to May 25, 1973, Mr. Chairman.

I do not know if we have a specific date.

Senator NELSON. You state that the Federal Register identified several completed, or nearly completed long-term feeding studies made on three different animal species.

Now, that was in May of 1973, 3½ years ago. Are all of those studies now completed?

Mr. AHART. I think those studies are completed. There are other studies being conducted.

Senator NELSON. You mean studies that have been initiated since the long-term studies you referred to here?

Mr. AHART. Well, I am not sure just when they were initiated. I think some of them may have been initiated prior to that time, some of them initiated since.

I will refer to additional studies later in my statement, Mr. Chairman.

Senator NELSON. But you refer to the fact that there are several long-term studies nearly completed, as of May 25, 1973.

Mr. AHART. That is correct.

Senator NELSON. Are you saying that there are new studies that have been initiated since then?

Mr. AHART. There are additional studies that were not included in these referred to here, initiated since that time, yes.

Senator NELSON. And you say these studies have been referred to the National Academy of Sciences for review.

Do you have any information as to the status of the National Academy's review of the studies referred to?

Mr. AHART. Yes, it is the next part of my statement, Mr. Chairman. We will get to it.

In December 1974, the Academy submitted to FDA its report on the safety of saccharin which pointed out problems with the studies

and concluded that existing studies had "not established conclusively whether saccharin is or is not carcinogenic when administered orally to test animals." The Academy recommended that certain additional studies be made to resolve the question of whether saccharin is carcinogenic or otherwise unsafe in the human diet.

In hearings on FDA's fiscal year 1976 appropriations before a subcommittee of the House Committee on Appropriations, the Acting Director of FDA's Bureau of Foods stated that most tests recommended in the Academy's 1974 report were being made by the health protection branch of the Canadian Government. He estimated that the tests would be completed in 3 years and that in the meantime "saccharin will continue to be interim listed for use as a food additive until such time as conclusive evidence is obtained that saccharin is or is not carcinogenic."

Mr. GORDON. Mr. Ahart, you used the word conclusive.

Why does the evidence have to be conclusive?

I would suggest that, even if the evidence were not conclusive, but at the very least, highly suggestive, would not that be enough?

Mr. AHART. Well, I would think that certainly FDA can act on evidence that is less than conclusive. I think the issuance of an interim regulation on saccharin in 1972, and a more recent notice proposing further action concerning saccharin were based on less than conclusive evidence.

I think that you are right that FDA would be in position to act with less than conclusive evidence as to the lack of safety of—

Senator NELSON. What is the standard statutory requirement?

Mr. AHART. The statute requires it be proven safe, be shown to be safe, so I would think if there was a need for conclusive evidence, it would be on the fact it was safe, not on the fact it was unsafe.

The language used by the FDA as to conclusive evidence relates to whether it is or is not carcinogenic.

Mr. GORDON. Also, Mr. Ahart, in your report to Senator Nelson of August 16, 1976, you stated that studies have shown that saccharin rapidly crosses the placental barrier in pregnant animals. Wouldn't providence dictate, then, that the public should not be exposed to this substance, or, at the very least, it's use should be tightly controlled?

Mr. AHART. I would think, Mr. Gordon, if in fact FDA has found there is danger to the fetus if the mother has consumed saccharin, it should be labeled so that the mother can make a choice whether she would want to undertake that risk.

SAFETY FACTOR USED FOR SACCHARIN QUESTIONABLE

The level of saccharin allowed in food under FDA's interim food additive regulation is based on a safety factor of 30 to 1 rather than the conventional 100-to-1 safety factor. Use of a safety factor less than 100 to 1 for saccharin, which was removed as a GRAS substance because questions were raised about its potential to cause cancer, seems questionable.

In determining whether the proposed use of a food additive is safe, the Food, Drug and Cosmetic Act (21 U.S.C. 348(c) (5) (C)) requires FDA to consider safety factors generally recognized by qualified experts as appropriate for the use of animal experimentation data.

FDA's regulations provide that except where evidence is submitted which justifies use of a different safety factor, a food additive for use by man will not be granted a tolerance that will exceed one-one hundredth of the maximum amount demonstrated to be without harm to experimental animals.

We believe that while resolution of safety questions are pending, saccharin's authorized levels of use in food should be based on the conventional margin of safety provided by FDA's regulations.

IMPURITIES IN SACCHARIN SHOULD BE LIMITED TO LOWEST ACHIEVABLE LEVELS

We noted also that the levels of o-toluenesulfonamide (OTS), an impurity in saccharin with possible cancer-causing potential, was not being limited to the lowest level achievable under present manufacturing technology. FDA limits the level of OTS to 100 parts per million. We were told that this limit was established in 1974 because—substantial levels of the impurity were identified in saccharin samples used in two studies; the impurity has possible carcinogenic potential; and industry was capable of reducing its levels to 100 parts per million.

According to a 1974 National Academy of Sciences report to FDA, impurities in saccharin, especially OTS, may have been the possible cause of the bladder tumors observed in certain studies.

Technology advancements have since made it possible to reduce the levels of OTS in saccharin to less than 50 parts per million and as low as 1 to 3 parts per million. The scientific community questioned the prudence of allowing saccharin on the market with levels of impurities that exceeded levels which industry could reasonably achieve.

CONCLUSIONS AND RECOMMENDATIONS

We believe that allowing an interim food additive regulation to remain in effect for several years while safety questions concerning the additive are being resolved seems contrary to FDA's intent of permitting use of such additive for a limited period. Potential hazards from the use of saccharin could be further minimized by applying the conventional 100 to 1 safety factor and by reducing the levels of OTS in saccharin to the lowest level practically achievable under present manufacturing technology.

Because saccharin has been used under an interim food additive regulation for about the past 4 years and because safety questions about it are not expected to be resolved soon, we recommended that the Secretary of HEW direct the FDA Commissioner to promptly reassess: The justification for continued use of free saccharin and its three salt forms under the interim food additive regulation; and the need for issuing a permanent regulation or possibly discontinuing their use in food.

We also recommended that if continued use under the interim regulation is justified, consideration be given to the need for increasing the safety factor to the conventional level set forth in FDA's regulations and to reducing the permissible levels of OTS in saccharin to the lowest achievable levels.

On December 10, 1976, HEW advised us that the FDA Commissioner had reassessed the justification for the interim listing of sac-

charin for use as a food additive. He concluded that continuation of the interim listing was appropriate. On January 7, 1977, FDA published two notices concerning saccharin. One extended the interim regulation to permit saccharin's continued use until Canadian studies on its safety are completed and evaluated. The other notice proposed to amend the interim food additive regulation to establish a tolerance of 25 parts per million for toluenesulfonamide. FDA does not believe a change in the safety factor is necessary.

REGULATION OF ASPARTAME

Our third report concerned aspartame, an artificial sweetener that was developed by G. D. Searle & Co.

On February 9, 1973, Searle submitted to FDA a petition proposing the issuance of a food additive regulation to provide for the use of aspartame in foods. The petition included general information on the characteristics and specifications of aspartame, its proposed uses, and summaries of scientific animal and human studies regarding its safety.

After reviewing the petition, FDA considered certain aspects of the animal study data submitted in support of aspartame's safety to be incomplete and suggested to Searle that the petition be withdrawn unless the issues could be promptly resolved. Searle submitted additional support data and on July 26, 1974, FDA published a regulation approving the use of aspartame in certain foods.

OBJECTIONS FILED AGAINST ASPARTAME

The Food, Drug and Cosmetic Act provides that individuals adversely affected by a food additive regulation may object and request a formal public hearing. FDA received three statements of objection relating to the aspartame regulation. One statement raised objections to a labeling requirement for cold cereals containing aspartame but did not contain a request for a hearing. The other statements raised questions about the possibility of aspartame causing brain damage in infants and young children and requested a hearing to resolve those questions.

After reviewing the objections FDA considered the uses of aspartame authorized by the regulations safe but recognized there was a difference of opinion and agreed to convene a hearing to address the safety issues raised by the objectors.

Plans to convene a hearing were suspended, however, as subsequent testing data submitted by Searle indicated that diketopiperazine (DKP), a manufacturing byproduct in aspartame, could be carcinogenic. FDA did not take regulatory action to prevent the marketing of aspartame because Searle and General Foods Corp., a comarketer, voluntarily agreed to withhold it from the market until DKP's carcinogenic potential was resolved.

FDA QUESTIONS DATA SUBMITTED BY SEARLE

Besides aspartame, Searle also manufactures a number of drugs which FDA has approved for marketing. In July 1975, FDA raised questions about Searle's performance of animal experiments and its reporting of safety data to FDA concerning two drugs—flagyl, used

to treat infections and aldactone, an antihypertension drug. Because of the importance and sensitivity of these questions, the FDA Commissioner, on July 23, 1975, established a task force to: Review the practices followed by Searle in conducting animal experiments, analyzing the experiments' data, and submitting the data to FDA; determine if there is evidence that any practices of Searle in carrying out the above functions violated the Food, Drug and Cosmetic Act or any other laws of the United States; and recommend an appropriate course of action based on the investigation's findings.

FDA officials said that the investigation was directed primarily toward evaluating drug data submitted to FDA since 1968. They stated that the review of aspartame data was included as part of the investigation, however, because (1) of the additive's recent approval, (2) of its potential for wide use in foods, and (3) its inclusion would provide a broader product base to evaluate Searle's practices.

ASPARTAME REGULATION STAYED

Preliminary results of the task force investigation indicated possible discrepancies in the data and the research summaries submitted to FDA supporting aspartame's safety. On December 5, 1975, FDA stayed the regulation approving the use of aspartame.

In joint hearings held on January 20, 1976, before the Senate Subcommittees on Health and on Administrative Practice and Procedure, Committees on Labor and Public Welfare, and the Judiciary, the FDA Commissioner disclosed preliminary task force findings. He stated that 11 studies submitted supporting the food additive petition for aspartame had been reviewed and numerous problems had been noted. These problems included poor methods of distribution and identification of control and treated animals, poor records of weighings, poor animal husbandry practices, discrepancies between Searle's pathology sheets and pathology summaries submitted to FDA, and problems in the design of some of the studies. The Commissioner stated that a final decision on whether to revoke the regulation approving the use of aspartame would be made after the task force had officially completed its investigation and added that aspartame would not be permitted to be marketed until all questions about its safety had been aired and resolved.

An FDA Bureau of Foods official told us that as of January 1, 1977, no decision had been made on whether to revoke the regulation.

TESTING FOOD ADDITIVES FOR CARCINOGENICITY

In addition to these reports, we issued a report to the Congress on June 16, 1976, on Federal efforts to protect the public from cancer-causing chemicals.

In this report we discussed the need for a Federal policy concerning carcinogens. Federal agencies have problems accepting and applying the results of animal tests to people, because (1) the National Cancer Institute has only recently developed minimum testing guidelines for determining a chemical's carcinogenicity and other agencies have not officially adopted the guidelines as a basis for carcinogenicity testing, and (2) there are no scientific principles to help Federal agencies

apply animal test results to humans. As a result, some carcinogens are not regulated at all while others are regulated differently by the various regulatory agencies.

Senator NELSON. What known carcinogens are not regulated at all?

Mr. AHART. I think there are 36 known carcinogens that have been identified by the National Cancer Institute.

It is my understanding that 15 of those are subject to some kind of regulation by the Federal Government, but because of variances in the regulatory practices, and the reason why they are regulated, our study indicated that probably 32 of the 36 are subject to public exposure of some type, so out of the 36 known ones, 32 have some public exposure to them.

Senator NELSON. What is the number that is unregulated?

Mr. AHART. Fifteen of them are subject to some regulation, so that would leave 21 of them that are not subject to regulation.

Senator NELSON. Do you have a list of those carcinogens?

Mr. AHART. Yes, in an appendix to our June 16, 1976, report, there is a list of the 36 identified known carcinogenic substances.

Senator NELSON. And how many of them are food additives?

Mr. AHART. I would have to check the list. I do not know if I have the number. I would expect the known carcinogens, Mr. Chairman, are not approved for use as food additives.

Senator NELSON. Even indirectly?

Mr. AHART. Well, not even indirectly, if they are known carcinogens, the law would not allow them to be approved for use as a food additive, even as unintentional food additives.

Senator NELSON. And you have discussed in your report in what way there is exposure to human beings by these, as you state?

Mr. AHART. We do talk about a couple of examples in the report that we have studied in some detail to find out just what the effects were.

One of these is asbestos which is regulated by OSHA in the workplace.

It is also regulated in the air by EPA. It is not regulated, however, in water.

Another one is benzedrine, which is regulated by OSHA in the workplace. They prescribe it to be used only in a closed system so there would be no exposure to the workers.

EPA, however, does not regulate benzedrine.

Senator NELSON. All right.

Mr. AHART. All agencies responsible for protecting the public from carcinogens should, we believe, cooperate to develop a uniform policy for identifying and regulating carcinogenic chemicals and the products in which they appear. The policy should also deal with such issues as the conditions under which regulatory agencies will allow public exposure to carcinogens.

We pointed out in the report that under the Food, Drug and Cosmetic Act the safety of certain products and substances, including food additives, is to be assured before they are approved for commercial use. We found that in some cases, however, they did not receive the kind of long-term tests that experts agree are needed to detect cancer-causing potential.

According to officials in FDA's Division of Food and Color Additives, all intentional food additives must receive long-term tests to

detect carcinogenicity before FDA will approve them. Intentional additives are to, (1) improve nutritional value, (2) maintain freshness, (3) improve esthetic appeal, or (4) aid in processing.

Unintentional additives are used mainly in packaging foods and, according to the FDA officials, receive long-term testing only when the consumer would be exposed to more than 1 or 2 parts per million of the additive in the food unless FDA had valid reasons to suspect that the additive might be carcinogenic. FDA officials explained that the long-term tests were expensive, and because virtually none of the unintentional additives migrate from the packaging material to the food, the amount of the additive which may be ingested is virtually nil. FDA's principle in this regard is the higher the anticipated human exposure, the greater the amount of toxicological data required to assure human safety. According to an April 1970 report to the Surgeon General by the Ad Hoc Committee on the Evaluation of Low Levels on Environmental Chemical Carcinogens:

1. No level of exposure of a chemical carcinogen should be considered toxicologically insignificant for humans, and
2. No chemical substance should be assumed safe for human consumption without proper negative lifetime biological assays of adequate size.

HEW said that, although extending carcinogenicity testing to unintentional food additives that have only remote possibilities of risk might be reassuring, it did not foresee any benefit to the public great enough to justify the substantial costs of such a policy.

Senator NELSON. You state that no chemical substance should be assumed safe for human consumption without proper negative lifetime biological assays of adequate size.

Are you saying that is what the law requires, or is that an opinion?

Mr. AHART. This is the opinion of the committee which I have identified here as the Ad Hoc Committee on the Evaluation of Low Levels of Environmental Chemical Carcinogens in its report in April 1970 to the Surgeon General. This was their judgment.

Senator NELSON. Whose lifetime, that of a human being?

Mr. AHART. I assume they were talking about animal tests, lifetime studies in animals.

Senator NELSON. All right.

Mr. AHART. We do not agree that FDA can assure safety for unintentional additives when the additive migrates to the food and leaves a residue of less than 1 or 2 parts per million.

Based on the Ad Hoc Committee's criteria, we do not believe that FDA can assure that all food additives are safe unless the additives receive carcinogenicity testing.

Accordingly, we recommended that the Secretary, HEW, require FDA to have all approved and proposed food additives tested for carcinogenicity.

Senator NELSON. Is not that what they are required to do with all new additives proposed to be marketed now?

Mr. AHART. I think the law requires they reach a judgment that there is assurance that they are safe.

I do not think the law specifically requires they carry on long-term studies on animals in each case.

If they can assure themselves of safety, this would not be required; however, the Ad Hoc Committee's judgment was that you cannot assure safety unless you have carried out the long-term studies, so there is a judgment on merit.

Senator NELSON. We will ask the FDA about that, but are you saying that despite the law on carcinogenicity, that the FDA, when an application is made to market food additives, and proposed data show carefully controlled scientific studies that it is safe, that that data need not include careful studies as to whether or not it is a carcinogen?

Mr. AHART. Well, as I understand the FDA's present policy, if it is for direct additives, intentional additives, the long-term studies for carcinogen qualities would be required.

Senator NELSON. Required for all additives?

Mr. AHART. For intentional additives, Mr. Chairman.

Senator NELSON. So this sentence in which you say, "We recommend the Secretary of HEW require FDA to have all approved and proposed food additives tested for carcinogenicity," you are saying that is already done for direct additives?

Mr. AHART. It is done if it is an intentional additive; yes.

Senator NELSON. So this sentence, which implies to me it was not required for intentional additives, does not mean that at all. It means if it is an unintentional additive, which it might infiltrate the food supply, it is recommended that they be tested for purposes of determining if they are carcinogenic. Is that what you are saying?

Mr. AHART. Yes; our recommendation was directed to the unintentional additive.

Senator NELSON. All right.

Mr. AHART. Because our work to date on food additives has pointed out certain problems concerning the regulation of food and color additives, we have recently initiated a broad survey of FDA's programs to regulate these additives. During this survey we will attempt to determine whether current legislation and FDA regulatory practices adequately protect consumers with respect to substances which are added to food.

Mr. Chairman, that concludes my prepared statement. We will be pleased to answer any questions that you or other members of the committee may have.

Thank you, Mr. Chairman.

Senator NELSON. You have underway now, I understand from your testimony, a broad survey of food additives, including the GRAS list?

Mr. AHART. Yes; we will include the GRAS list in the study, Mr. Chairman.

Senator NELSON. And what are the parameters of that study?

Mr. AHART. Right now, we are in what we call a survey phase.

We are gathering information on the totality of the regulation of food additives.

We will be looking at the regulation of potential additives, of food additives; we will be looking at the use of interim regulations, and we will be looking at what FDA is doing in terms of the GRAS list to be sure it does not include anything which is harmful to the public. It is a fairly broad look at the total area of regulating food additives.

Senator NELSON. How many personnel of the GAO are working on this question?

Mr. JOJOKIAN. Six, Mr. Chairman.

Senator NELSON. Six?

Mr. JOJOKIAN. Yes.

Senator NELSON. And when was the study initiated?

Mr. JOJOKIAN. This month, the beginning of this month, Mr. Chairman.

Senator NELSON. Beginning of January of this year?

Mr. JOJOKIAN. Yes.

Senator NELSON. When do you anticipate the study will be concluded?

Mr. JOJOKIAN. We do not have a date for reporting on this, but we expect to complete our survey work within the next few months to determine whether we should do any further work in this area.

Mr. AHART. I think it will probably be more than a year, Mr. Chairman, before we will be issuing a report as a result of this work.

Senator NELSON. More than a year?

Mr. AHART. Probably more than a year.

Senator NELSON. Thank you very much, Mr. Ahart, for your very useful testimony.

We appreciate your taking the time to come.

Mr. AHART. Thank you, Mr. Chairman.

[The prepared statement of Mr. Ahart follows:]

UNITED STATES GENERAL ACCOUNTING OFFICE
WASHINGTON, D.C. 20548

FOR RELEASE ON DELIVERY
Expected at 9:30 a.m. EST
January 13, 1977

STATEMENT OF
GREGORY J. AHART, DIRECTOR, HUMAN RESOURCES DIVISION
BEFORE THE
SENATE SELECT COMMITTEE
ON SMALL BUSINESS
on
FOOD AND DRUG ADMINISTRATION'S
REGULATION OF FOOD ADDITIVES

Mr. Chairman and Members of the Committee, we are pleased to appear here today to discuss our reports on the Food and Drug Administration's (FDA's) regulation of three color and food additives--Red No. 2, saccharin and aspartame. In addition we have issued a report to the Congress on chemical carcinogens including food additives and we have recently initiated a broad scale review of FDA's regulation of food additives. We will discuss these also.

Our reviews concerning the three additives were directed primarily toward developing information on (1) the history of FDA's regulation of them, (2) the current status of testing the safety of the additives, and (3) whether the regulatory actions taken by FDA on the three additives complied with the Federal Food, Drug, and Cosmetic Act (FD&C Act), as amended (21 U.S.C. 301).

REGULATION OF RED NO. 2

Red No. 2 is the name given to a certified lot of the dye generically known as amaranth. The composition and purity of amaranth varies. FDA has established composition and purity specifications that amaranth must meet before it can qualify for use in food, drugs, and cosmetics. Only amaranth meeting such specifications is classified as Red No. 2.

Since July 12, 1960, the Color Additive Amendments to the FD&C Act have required FDA to establish regulations listing color additives that are safe for use in food, drugs, or cosmetics. Such regulations may list color additives for use generally in food, drugs, or cosmetics or may prescribe the conditions under which the color additives may be safely used.

The act provides that a color additive is deemed unsafe and should not be listed in a regulation permitting its use in food, drugs, or cosmetics if it is found by FDA to induce cancer in man or animal.

The FD&C Act, as amended in 1960, placed all color additives commercially established at that time, including Red No. 2, on a provisional list to allow their use for a reasonable period until their safety could be reviewed and regulations for their use could be issued. The 1960 amendments provided that the provisional listing was to terminate no later than 2-1/2 years from the effective date of enactment (July 12, 1960), or January 12, 1963. The amendments also provided, however, that FDA could postpone the termination date if such action was consistent with the objective of carrying to completion, in good faith, as soon as reasonably practicable, the scientific investigations necessary for making a determination as to the additives' safety.

We found that FDA had permitted the use of Red No. 2 in food, drugs, and cosmetics for 15 years without making a final determination of its safety. FDA postponed termination of the provisional listing for Red No. 2, 14 times on the basis of requests from manufacturers or industry associations to allow completion of scientific investigations concerning its safety.

Since 1970 several scientific studies involving animals, including some performed or sponsored by FDA, raised questions concerning the safety of Red No. 2 in food. In some of these studies Red No. 2 or amaranth was shown in test animals to be toxic to reproductive systems or to be carcinogenic.

Because of its concern about the safety of Red No. 2, FDA in July 1972 issued a proposal to limit human exposure to the color additive. However, at the time our report was issued on October 20, 1975, FDA had not made a final determination regarding its safety.

Because we believed that continued use of Red No. 2 before resolving its safety exposed the public to unnecessary risk, we recommended that the Secretary of the Department of Health, Education, and Welfare (HEW) direct the Commissioner of FDA to promptly establish the safety of Red No. 2 or prevent its use in food, drugs, and cosmetics.

On January 19, 1976. FDA announced a ban on the use of Red No. 2 in food, drugs, and cosmetics. FDA took the action because new evidence showed that Red No. 2 caused a statistically significant increase in the number of malignant tumors in test animals and because of what it termed "the absence of other data to allow a definitive judgment of safety."

REGULATION OF SACCHARIN

In our report on saccharin we pointed out that saccharin was "generally recognized as safe" for use in food until about 1970 when studies raised questions about its potential to cause cancer in test animals.

Saccharin is an acid and pure saccharin generally is unsuitable for use in foods and beverages because it is only slightly soluble. It is most often combined with either sodium, calcium, or ammonium salts which neutralize the acid and produce a more readily soluble compound.

The FD&C Act, as amended by the Food Additives Amendment of 1958 (21 U.S.C. 348), requires FDA to establish regulations prescribing the conditions under which a food additive may be safely used. The act defines "food additive" as any substance which becomes or may be expected to become a component of food, either directly or indirectly, or which may otherwise affect the characteristics of

the food. The proposed use of a food additive whose safety is not generally recognized by qualified scientists must be approved by FDA.

Food additives "generally recognized as safe" are referred to as GRAS substances. Such substances added to food are not considered food additives and are exempt from the requirement for FDA approval.

Saccharin's safety not established

FDA's food additive regulations (21 C.F.R. 121.1(k)) define GRAS substances as those which experts determine, based on scientific data or reasoned judgment founded in experience with common food use, pose "no significant risk of harm if used as intended." If an important question of safety has been raised regarding a GRAS substance, it may be removed from GRAS status. An interim food additive regulation may be issued to permit its use while the safety question is being resolved, provided there is reasonable certainty that the substance is not harmful and that no harm to the public health will result from its continued use.

On February 1, 1972, FDA removed saccharin and its various salt forms from the GRAS status and issued an interim food additive regulation limiting the use of saccharin in foods.

The interim regulation stated that preliminary results from studies on long-term feeding of saccharin to animals conducted by FDA and others indicated "possible adverse effects." According to the regulation, if the experimental findings indicate that continued use of saccharin poses a "significant risk" to the public health, action would be taken as warranted to minimize the risk. The regulation authorized saccharin's use as a sweetening agent only in special dietary food and for certain technological purposes such as reducing bulk and enhancing flavors in chewable vitamin tablets. This authority for saccharin's use was to expire June 30, 1973.

However, on May 25, 1973, FDA issued a Federal Register notice extending saccharin's interim regulation indefinitely. The Federal Register identified several completed or nearly completed long-term feeding studies made of three different animal species. These study results showed a statistically significant incidence of bladder tumors in the male offspring of test animals fed saccharin.

The Federal Register indicated that these studies were referred to the National Academy of Sciences for review and that a final determination of saccharin's safety would be made after FDA received recommendations from the Academy. In December 1974 the Academy submitted

to FDA its report on the safety of saccharin which pointed out problems with the studies and concluded that existing studies had "not established conclusively whether saccharin is or is not carcinogenic when administered orally to test animals." The Academy recommended that certain additional studies be made to resolve the question of whether saccharin is carcinogenic or otherwise unsafe in the human diet.

In hearings on FDA's fiscal year 1976 appropriations before a subcommittee of the House Committee on Appropriations, the Acting Director of FDA's Bureau of Foods stated that most tests recommended in the Academy's 1974 report were being made by the Health Protection Branch of the Canadian Government. He estimated that the tests would be completed in 3 years and that in the meantime "saccharin will continue to be interim listed for use as a food additive until such time as conclusive evidence is obtained that saccharin is or is not carcinogenic."

Safety factor used for
saccharin questionable

The level of saccharin allowed in food under FDA's interim food additive regulation is based on a safety factor of 30 to 1 rather than the conventional 100 to 1 safety factor. Use of a safety factor less than 100 to 1 for saccharin, which was removed as a GRAS substance

because questions were raised about its potential to cause cancer, seems questionable.

In determining whether the proposed use of a food additive is safe, the FD&C Act (21 U.S.C. 348(c)(5)(C)) requires FDA to consider safety factors generally recognized by qualified experts as appropriate for the use of animal experimentation data. FDA's regulations provide that except where evidence is submitted which justifies use of a different safety factor, a food additive for use by man will not be granted a tolerance that will exceed 1/100th of the maximum amount demonstrated to be without harm to experimental animals.

We believe that while resolution of safety questions are pending, saccharin's authorized levels of use in food should be based on the conventional margin of safety provided by FDA's regulations.

Impurities in saccharin
should be limited to
lowest achievable levels

We noted also that the levels of o-toluenesulfonamide (OTS), an impurity in saccharin with possible cancer-causing potential, was not being limited to the lowest level achievable under present manufacturing technology. FDA limits the level of OTS to 100 parts per million. We were told that this limit was established in 1974 because

- substantial levels of the impurity were identified in saccharin samples used in two studies,
- the impurity has possible carcinogenic potential, and
- industry was capable of reducing its levels to 100 parts per million.

According to a 1974 National Academy of Sciences report to FDA, impurities in saccharin, especially OTS, may have been the possible cause of the bladder tumors observed in certain studies.

Technology advancements have since made it possible to reduce the levels of OTS in saccharin to less than 50 parts per million and as low as 1 to 3 parts per million. The scientific community questioned the prudence of allowing saccharin on the market with levels of impurities that exceeded levels which industry could reasonably achieve.

Conclusions and recommendations

We believe that allowing an interim food additive regulation to remain in effect for several years while safety questions concerning the additive are being resolved seems contrary to FDA's intent of permitting use of such additive for a limited period. Potential hazards from the use of saccharin could be further minimized by applying the conventional 100 to 1 safety factor and by reducing

the levels of OTS in saccharin to the lowest level practically achievable under present manufacturing technology.

Because saccharin has been used under an interim food additive regulation for about the past 4 years and because safety questions about it are not expected to be resolved soon, we recommended that the Secretary of HEW direct the FDA Commissioner to promptly reassess

- the justification for continued use of free saccharin and its three salt forms under the interim food additive regulation and
- the need for issuing a permanent regulation or possibly discontinuing their use in food.

We also recommended that if continued use under the interim regulation is justified, consideration be given to the need for increasing the safety factor to the conventional level set forth in FDA's regulations and to reducing the permissible levels of OTS in saccharin to the lowest achievable levels.

On December 10, 1976, HEW advised us that the FDA Commissioner had reassessed the justification for the interim listing of saccharin for use as a food additive. He concluded that continuation of the interim listing was appropriate, and that no change in the safety factor was necessary. FDA is evaluating the feasibility of lowering the permissible levels of OTS in saccharin.

REGULATION OF ASPARTAME

Our third report concerned aspartame, an artificial sweetener that was developed by G. D. Searle and Company.

On February 9, 1973, Searle submitted to FDA a petition proposing the issuance of a food additive regulation to provide for the use of aspartame in foods. The petition included general information on the characteristics and specifications of aspartame, its proposed uses, and summaries of scientific animal and human studies regarding its safety.

After reviewing the petition, FDA considered certain aspects of the animal study data submitted in support of aspartame's safety to be incomplete and suggested to Searle that the petition be withdrawn unless the issues could be promptly resolved. Searle submitted additional support data and on July 26, 1974, FDA published a regulation approving the use of aspartame in certain foods.

Objections filed against aspartame

The FD&C Act provides that individuals adversely affected by a food additive regulation may object and request a formal public hearing. FDA received three statements of objection relating to the aspartame regulation. One statement raised objections to a labeling requirement for cold cereals containing aspartame but did not contain a request for a hearing. The other

statements raised questions about the possibility of aspartame causing brain damage in infants and young children and requested a hearing to resolve those questions.

After reviewing the objections FDA considered the uses of aspartame authorized by the regulations safe but recognized there was a difference of opinion and agreed to convene a hearing to address the safety issues raised by the objectors.

Plans to convene a hearing were suspended, however, as subsequent testing data submitted by Searle indicated that diketopiperazine (DKP), a manufacturing byproduct in aspartame, could be carcinogenic. FDA did not take regulatory action to prevent the marketing of aspartame because Searle and General Foods Corporation, a co-marketer, voluntarily agreed to withhold it from the market until DKP's carcinogenic potential was resolved.

FDA questions data
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Besides aspartame, Searle also manufactures a number of drugs which FDA has approved for marketing. In July 1975 FDA raised questions about Searle's performance of animal experiments and its reporting of safety data to FDA concerning two drugs--flagyl, used to treat infections and aldactone, an antihypertension drug. Because of the importance and sensitivity of these

questions, the FDA Commissioner, on July 23, 1975, established a task force to

- review the practices followed by Searle in conducting animal experiments, analyzing the experiments' data, and submitting the data to FDA;
- determine if there is evidence that any practices of Searle in carrying out the above functions violated the FD&C Act or any other laws of the United States; and
- recommend an appropriate course of action based on the investigation's findings.

FDA officials said that the investigation was directed primarily toward evaluating drug data submitted to FDA since 1968. They stated that the review of aspartame data was included as part of the investigation, however, because (1) of the additive's recent approval, (2) of its potential for wide use in foods, and (3) its inclusion would provide a broader product base to evaluate Searle's practices.

Aspartame regulation stayed

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An FDA Bureau of Foods official told us that as of January 1, 1977, no decision had been made on whether to revoke the regulation.

TESTING FOOD ADDITIVES
FOR CARCINOGENICITY

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In this report we discussed the need for a Federal policy concerning carcinogens. Federal agencies have problems accepting and applying the results of animal tests to people because (1) the National Cancer Institute has only recently developed minimum testing guidelines for determining a chemical's carcinogenicity and other agencies have not officially adopted the guidelines as a basis for carcinogenicity testing and (2) there are no scientific principles to help Federal agencies apply animal test results to humans. As a result, some carcinogens are not regulated at all while others are regulated differently by the various regulatory agencies. All agencies responsible for protecting the public from carcinogens should, we believe, cooperate to develop a uniform policy for identifying and regulating carcinogenic chemicals and the products in which they appear. The policy should also deal with such issues as the conditions under which regulatory agencies will allow public exposure to carcinogens.

We pointed out in the report that under the FD&C Act the safety of certain products and substances, including food additives, is to be assured before they are approved for commercial use. We found that in some cases, however, they did not receive the kind of long-term tests that experts agree are needed to detect cancer-causing potential.

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According to an April 1970 report to the Surgeon General by the Ad Hoc Committee on the Evaluation of Low Levels of Environmental Chemical Carcinogens

--no level of exposure of a chemical carcinogen should be considered toxicologically insignificant for humans and

--no chemical substance should be assumed safe for human consumption without proper negative lifetime biological assays of adequate size.

HEW said that, although extending carcinogenicity testing to unintentional food additives that have only remote possibilities of risk might be reassuring, it did not foresee any benefit to the public great enough to justify the substantial costs of such a policy.

We do not agree that FDA can assure safety for unintentional additives when the additive migrates to the food and leaves a residue of less than 1 or 2 parts per million. Based on the Ad Hoc Committee's criteria, we do not believe that FDA can assure that all food additives are safe unless the additives receive carcinogenicity testing.

Accordingly, we recommended that the Secretary, HEW, require FDA to have all approved and proposed food additives tested for carcinogenicity.

CURRENT GAO WORK

Because our work to date on food additives has pointed out certain problems concerning the regulation of food and color additives, we have recently initiated a broad survey of FDA's programs to regulate these additives. During this survey we will attempt to determine whether current legislation and FDA regulatory practices adequately protect consumers with respect to substances which are added to food.

Mr. Chairman, that concludes my prepared statement. We will be pleased to answer any questions that you or other members of the Committee may have.

Senator NELSON. Our next witness is Mr. Sherwin Gardner, Acting Commissioner for the Food and Drug Administration.

Mr. Gardner, could you please identify for the reporter your associates.

STATEMENT OF SHERWIN GARDNER, ACTING COMMISSIONER, FOOD AND DRUG ADMINISTRATION, PUBLIC HEALTH SERVICE, U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE; ACCOMPANIED BY DR. HOWARD R. ROBERTS, ACTING DIRECTOR, BUREAU OF FOODS, FDA; RICHARD A. MERRILL, CHIEF COUNSEL, FDA; MR. RONK, BUREAU OF FOODS, FDA; AND DR. BLUMEN-THAL, DIRECTOR, DIVISION OF TOXICOLOGY, FDA

Mr. GARDNER. Yes, Mr. Chairman.

On my right is Dr. Howard R. Roberts, Acting Director, Bureau of Foods, and on my left is Mr. Richard A. Merrill, Chief Counsel, Food and Drug Division.

In addition, Senator, there are several members of the Agency who are in the audience, in the event that questions are raised which they might be able to answer. We will identify them when they are called upon.

Senator NELSON. All right.

Let's see, you have a statement, plus an appendix.

Your statement is 35 pages, and then you have an appendix.

Can you summarize your statement?

Mr. GARDNER. I did not intend to go through the appendix, but I do think that most of the statement is something that needs to be read into the record. So with your permission, we will do that, and if I find a place where we can summarize, I will try to do that.

Senator NELSON. All right.

Your statement and the appendix will be printed in full in the record. Where you can summarize, and economize on the time, we would appreciate it, and if you wish to comment on any of the testimony of the GAO, we would appreciate having your comments in the record.

Mr. GARDNER. Thank you, Mr. Chairman.

I appreciate this opportunity to appear before your committee to discuss the current legal authority of the Food and Drug Administration (FDA) to regulate food and color additives and the activities of the Agency in this important and highly publicized area.

Your letter of invitation and our discussions with the committee staff indicate a broad interest in the regulation of food additives. Therefore, my statement is directed to a discussion of the Agency's authority and program activities for food and color additives. I am also attaching to my statement individual appendices which discuss the specific subjects you inquired about in your letter of invitation. On the assumption that these specific subjects are likely to consume more of the discussion following my prepared statement, I believe it appropriate to focus my prepared remarks on the general legal, administrative and scientific context in which debate about the safety of particular compounds takes place.

It is apparent, from a review of the history of the various amendments to the Federal Food, Drug, and Cosmetic Act—from the Food Additives Amendment of 1958 to the Medical Device Amendments of 1976, that Congress has consistently expected that the Agency would make its decisions based on the best science available at the time. Dramatic changes in science and technology have occurred, however, since 1958. Each of the various amendments makes clear that the burden of establishing the safety and performance of the regulated products is on the manufacturer. FDA has the responsibility to make scientific judgments to approve or disapprove the use of these products.

One of the most difficult tasks facing FDA today is to evaluate the safety of individual food substances on an *ad hoc* basis, and, at the same time, to develop a mechanism for conducting safety reviews for all substances in an orderly manner to assure that safety decisions are valid in terms of current scientific standards.

Senator NELSON. What do you mean, one of your most difficult tasks is to evaluate the safety of individual food substances on an *ad hoc* basis?

Mr. GARDNER. Senator, what happens usually is that someone will raise a question about a substance.

Senator NELSON. Now, so that I have it clear in my mind, are you talking now about a new substance that is proposed to market, as a direct food additive, for example?

Mr. GARDNER. It could be a substance that is already marketed on the basis of an existing approval.

Senator NELSON. Leaving aside the GRAS list for a moment, under the current law, if a manufacturer proposes to market a food additive, what is the standard for proof of safety that is required by the Food and Drug Administration?

Mr. GARDNER. For marketing a new additive?

Senator NELSON. A new additive at this time.

Mr. GARDNER. Well, that would depend to some extent on the scientific judgments about the substance, and what sorts of tests are needed to prove its safety.

Senator NELSON. All right.

I did not have a chance to review the statute, it has been 4 years since the last hearings that I chaired before the Nutrition Committee, so I cannot recall.

Is the standard roughly the same as the standard to market a prescription drug, that is to say that there have to be an adequately controlled scientific study or studies to prove its safety, and in the case of drugs, efficacy.

Mr. MERRILL. Let me respond to that, Mr. Chairman.

The statute requires the Agency to make a determination that a substance is reasonably certain to be safe for its intended use.

The tests that the Agency is permitted to require are not nearly so specifically defined in the food additive amendments, as in the drug provisions. The Agency has a good deal more freedom to demand new tests as new scientific information suggests. We are not tied to a phrase like adequate and well-controlled studies.

Senator NELSON. What is the statutory language? What is the operative language? I have it here.

Mr. MERRILL. Is your concern with the language that describes the kinds of tests, rather than the standard of judgment that the Agency is to apply?

Senator NELSON. Yes; any evaluator of a study will have to exercise judgment as to whether or not he is satisfied that the study is based on sound protocol, convincing, and whatever else it asserts.

What I am trying to find out is, what is the standard used by the Food and Drug Administration for food additives' safety?

I see the statute here, but your being a lawyer, I know that 10 lawyers will interpret it 10 ways.

Mr. MERRILL. There are two parts to the question, I think, Senator.

One is, what do we require manufacturers to submit to us. The statutory language that deals with that appears in section 409(b) of the statute, which lists a number of items of information that a manufacturer is required to supply, such as, the name and information concerning the additive, including its chemical identity, its composition, statements of the condition of proposed use of the additive, all relevant data bearing on physical and other technical effects, a description of practical methods of determining the quantity of additive in or on food, if that is important, and finally, full reports of investigations made with respect to safety for use of such additive, including full information as to the methods and controls used in conducting the investigation.

Senator NELSON. I would ask that that section 409(b) be printed in the record.

Mr. MERRILL. We should also include at that point, if I may interject, the regulations that the Agency has issued, which describe and somewhat amplify the requirements of food additives.

Senator NELSON. How long are the regulations? You mean the regulations adopted by the FDA, to implement the provisions of the statute?

Mr. MERRILL. That is correct.

Senator NELSON. How long are those regulations? I do not want to put something here that would be too long. I will put it in the appendix if it is too long.

Mr. MERRILL. The citation would be sufficient. I could not tell you the precise length.

Senator NELSON. If you can give us the citation, that may be sufficient.

Mr. MERRILL. I will supply that for the record, if that is satisfactory. [The statute and citation follows:]

TITLE 21 - CODE OF FEDERAL REGULATIONS

Section	121.51
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FOOD ADDITIVES

Unsafe Food Additives

Sec. 409 [348]. (a) A food additive shall, with respect to any particular use or intended use of such additives, be deemed to be unsafe for the purposes of the application of clause (2) (C) of section 402(a), unless—

(1) it and its use or intended use conform to the terms of an exemption which is in effect pursuant to subsection (i) of this section; or

(2) there is in effect, and it and its use or intended use are in conformity with, a regulation issued under this section prescribing the conditions under which such additive may be safely used.

While such a regulation relating to a food additive is in effect, a food shall not, by reason of bearing or containing such an additive in accordance with the regulation, be considered adulterated within the meaning of clause (1) of section 402(a).

Petition To Establish Safety

(b) (1) Any person may, with respect to any intended use of a food additive, file with the Secretary a petition proposing the issuance of a regulation prescribing the conditions under which such additive may be safely used.

(2) Such petition shall, in addition to any explanatory or supporting data, contain—

(A) the name and all pertinent information concerning such food additive, including, where available, its chemical identity and composition;

(B) a statement of the conditions of the proposed use of such additive, including all directions, recommendations, and suggestions proposed for the use of such additive, and including specimens of its proposed labeling;

(C) all relevant data bearing on the physical or other technical effect such additive is intended to produce, and the quantity of such additive required to produce such effect;

(D) a description of practicable methods for determining the quantity of such additive in or on food, and any substance formed in or on food, because of its use; and

(E) full reports of investigations made with respect to the safety for use of such additive, including full information as to the methods and controls used in conducting such investigations.

(3) Upon request of the Secretary, the petitioner shall furnish (or, if the petitioner is not the manufacturer of such additive, the petitioner shall have the manufacturer of such additive furnish, without disclosure to the petitioner), a full description of the methods used in, and the facilities and controls used for, the production of such additive.

(4) Upon request of the Secretary, the petitioner shall furnish samples of the food additive involved, or articles used as components thereof, and of the food in or on which the additive is proposed to be used.

(5) Notice of the regulation proposed by the petitioner shall be published in general terms by the Secretary within thirty days after filing.

Senator NELSON. Now, did I understand you to say the FDA has more leeway respecting food additives than it does respecting prescription drugs, or over-the-counter drugs?

Mr. MERRILL. I did say that. I think the point is that the statute does not contain a description of the kind of studies.

Senator NELSON. All right.

What you are really saying is that the minimum standard respecting prescription and over-the-counter drugs is more specific in its statutory requirements than it is respecting food additives?

Mr. MERRILL. It is for effectiveness studies, Mr. Chairman. It is not for safety.

The provisions in the Food and Drug Act, with respect to the safety of drugs use the language in all tests reasonably appropriate, which is not very definitive.

Congress in 1972 introduced the adequate and well-controlled studies requirement for proof of effectiveness, which is of course a good deal more focused and explicit.

Senator NELSON. Does the language of the statute respecting additives give the FDA authority to require any tests that it decides may be appropriate?

Mr. MERRILL. Yes, indeed.

Senator NELSON. You may require very elaborately controlled scientific studies if, in the judgment of the FDA, the proposed chemical additive may indicate the need for them?

Mr. MERRILL. Not only can we do, but the statute also permits us, as a new phenomenon of concern to scientists, to require tests to determine whether a chemical can be responsible for that hazard as well; I think the statute in that sense is very flexible.

Senator NELSON. Is whatever you are trying to establish subject to challenge?

Mr. MERRILL. Yes.

Senator NELSON. I suppose it is, if it were totally unreasonable?

Mr. MERRILL. Sure, I think a manufacturer who was confronted with the requirement for 73-year study of some kind in animals might well want to take us to court and challenge the reasonableness of that requirement.

Senator NELSON. So, back to your sentence on page 2, saying, "One of the most difficult tasks is to evaluate safety of food additives, of food substances on an ad hoc basis." What does that mean in that context?

Mr. GARDNER. What I was trying to get at is that questions have been raised about currently approved and marketed substances, or questions raised about substances newly submitted to the Agency. We have broader implications for all classes of similar substances, and that is the difficulty which the Agency has to contend with. New questions arise because scientific methods and standards have changed over time; new discoveries are made about substances, and that requires us in the middle of the stream, so to speak, to go back and look anew at other substances about which the same source of questions could and should be raised.

It is difficult in that sense, and it precludes us from making an orderly and systematic approach to safety evaluations.

Senator NELSON. All right.

Mr. GARDNER. The statute does not explicitly require the Agency to review periodically its safety decisions about products it regulates. Indeed, it would be much simpler if FDA could view its initial product decisions as final. However, it is not possible to rely indefinitely on the scientific judgments of a particular time, even if they are based on the best available data. Continual review of scientific regulatory decisions is necessary because, over time, several factors come into play that can make the data available at the time of the initial decision obsolete. Some of these factors are:

New information on risk associated with certain substances; new patterns of use of products; product defect information; changes in scientific knowledge and standards; and technological advancement in manufacturing and measuring techniques affecting residues in foods.

Senator NELSON. Is the staff correct that there are about 2,100 direct food additives now?

The testimony of the GAO was that in 1960 there were about 1,300.

Mr. Gordon says that there are now about 2,100. Is that an accurate figure?

Mr. GARDNER. Dr. Roberts said yes, that is the correct figure.

Senator NELSON. I am advised it is in the FDA testimony some place.

Now, is there a listing, do you have a listing of all of these additives by class, and what their purpose, function, role is as a direct food additive?

For example, how many are emulsifiers?

Dr. ROBERTS. We could supply this, Senator Nelson, for the record. Breaking these down, for example, there are on the order of 400 regulated direct food additives.

There are about 1,650 flavors and spices, and we could give you those categories, as well as specific details.

Senator NELSON. Are the flavors and spices considered not to be health hazards in any way? You said controlled food additives.

Dr. ROBERTS. If they are permitted, they are not considered unsafe, that is correct.

Senator NELSON. Are not some of them part of the GRAS list?

Dr. ROBERTS. Yes, sir. There are both GRAS and regulated flavors.

Mr. MERRILL. Perhaps I can interject.

We use that phraseology frequently—

Senator NELSON. What phraseology?

Mr. MERRILL. We use the phraseology "regulated food additives," and it bothers me, too.

What we mean is that it is an ingredient added to food for which there is a food additive regulation on the books.

Senator NELSON. They are all subject to regulation?

Mr. MERRILL. They are all subject to regulation of a variety of kinds, as the testimony points out.

Senator NELSON. But beginning with the GRAS list, none of them were, is that correct?

Mr. MERRILL. Prior to 1958, they were regulated—if we put aside pesticides for a moment—they were regulated in the sense that FDA, if it found an ingredient about which there were safety concerns, to indicate enforcement action against foods that contained it, and additives that were subject to after-the-fact regulation.

Senator NELSON. I would like to have the record show what these additives are, and what their function is.

This is the kind of question I am raising. If an additive, let us say a color, as a consequence of long-term use and careful scientific studies, appears to be perfectly safe, and there is no known hazard, is there any sound reason for permitting in the marketplace another one that does the same thing, unless there are scientific studies that show it is even safer, or unless there is some substantial economic benefit?

Mr. GARDNER. I think you are asking questions which bear on relative safety and relative benefit for the use of different additives, and that is not a matter in which we are authorized to make judgments.

Senator NELSON. I know that.

We have regulations to deal with that question as to prescription drugs.

I am asking you what would be your view about the value of some legislation in this area respecting adequacy?

Mr. GARDNER. I would not look forward to that role for the Food and Drug Administration, having to make judgments about relative benefits of different kinds of additives.

I think that the marketplace which is now the way that kind of question is decided, is probably the most appropriate one.

Senator NELSON. The marketplace cannot make any determination about safety. That is the responsibility of the FDA.

Mr. GARDNER. That is right, and the judgments that are made by the FDA with respect to safety are whether or not the substance is reasonably safe for use in foods.

That is a role we have now, and I am not suggesting that that change.

Senator NELSON. I know you are not suggesting a change, and I know you said you would not like the role, but would it be in the public interest? —

That is the other question.

Mr. GARDNER. I do not think that that would be a very effective use of public resources.

Senator NELSON. We have had lots of testimony over the years with respect to a similar problem in principle: That is, that there are vast numbers of prescription drugs put in the marketplace, which are no more effective, and on which we do not have the safety experience, and some of which are less effective and less safe than ones already in use. Why would it not make sense to ask, when you have an additive that is going to perform the same function, that is no more effective, or less effective, and on which the safety question is not as clear, to ask, what is the purpose of allowing it to be marketed?

The marketplace cannot determine that. It is a safety and efficacy question. We have marketed drugs for years and years and years, that were ineffective. As a matter of fact, for hundreds of years, many drugs put into the marketplace, which physicians swore by, were found later to be ineffective or unsafe. Even in the past few years over 6,000 drugs were taken off the market because there was no evidence of their efficacy.

So the marketplace cannot determine that.

Mr. GARDNER. Well, I do not think you can really separate out the question of relative safety and relative benefit, because when you start making judgments about which sweetener or which color is safer, then you cannot escape looking at the way in which those products are used,

or in which they might be used. That gets to usage and economic benefits, and then we are back into the benefit business, which I think is a quagmire for the Government to get involved in.

Senator NELSON. Well, yes, it is, but the problem is there. You have hundreds and hundreds of compounds and chemicals and non-prescription drugs and prescription drugs in the market. In fact, I think you have by name a hundred-thousand non-prescription drugs in the marketplace; 20,000 prescription drug products but only 700 different compounds; food additives are up to 2,100. There is no way in the world to make studies about the long-term safety of all of them, and there is no way that we will make studies finding out how they interact with each other, the food additives that are chemicals, with drugs being taken by the same individual, and so forth. All we are doing is compounding the problem, without any social benefit that I can see.

I am wondering if we should start addressing ourselves to that: Why add a chemical that may very well be carcinogenic, if we do not know if it is more effective; or one that does nothing, or perhaps is not cheaper than one that has been on the marketplace for 25 years, and for which we have pretty good studies that indicate that there is no safety question there?

Mr. GARDNER. Well, I would agree with you, Senator, that that is a good question, and one that the Congress should consider anew. I say anew, because apparently there is an implied suggestion that Congress considered that when it enacted the food additive legislation that is now on the books.

Senator NELSON. Well, it is a question that ought to be addressed by the scientific community.

After all, the legislation, if there is legislation in this field, ought to be based upon good scientific advice.

It is there, in the FDA and the NIH (National Institutes of Health), the National Academy of Sciences, and among the thousands of biomedical scientists all over the country. It seems to me that is where the guidance, technical and scientific advice, should be coming from, not from legislators who have neither the resources, nor are they scientifically qualified to make independent judgments on these questions.

Mr. GARDNER. I think it would be appropriate and proper for the Congress to get advice from scientists, but ultimately that turns out to be a public policy decision, which I do not think is solely in the province of scientific decision.

Senator NELSON. No, I do not think it is either. But in order to make a public policy decision, you need to have the information.

Well, we will go on. I should not take anymore time with it. You were at the top of page 3.

Mr. GARDNER. I was about to say that the periodic review of original scientific decisions is essential and an implied requirement of law if we are to carry out properly our regulatory responsibilities.

THE REVIEW PROCESS

In FDA, product reviews are conducted in two ways: By classes of products, and by specific product evaluation.

The product class review approach is effective and efficient. It permits a contemporary comprehensive review of similar or related sub-

stances and it assures a uniform and consistent application of policy and scientific standards. Specific product evaluations usually occur on an ad hoc basis in response to applications from manufacturers for approval of products prior to marketing or in response to new scientific findings.

There are six major product class reviews now in progress in the Agency: The drug efficacy study for prescription products; the OTC drug review; the safety and efficacy study of biological products; the review of low level antibiotics used in animal feeds; and the GRAS review of food substances; and the cyclic review of food additives.

I would like to submit for the record a brief description of each of these class review projects.

Specific product evaluations have a varied character, ranging from the addition and/or deletion of an indication or warning statement on a drug label to the removal of a previously approved product from the market. Included in this latter category, for example, are actions related to diethylstilbestrol, cyclamates, Food, Drug and Cosmetic Red No. 2 and many others.

Based on our experience with product class review programs, it will not surprise us to find that some food additives now considered to be safe by qualified scientists will have questions raised about their safety during the course of one of our food additive review programs. Such questions are inherent in the nature of scientific inquiry; in science there are no closed subjects.

When a question of safety occurs about a compound that FDA previously has approved, the Agency must review the question in a reasoned and scientific manner. This approach is rooted in commonsense, because it is not difficult to raise questions about the safety of a food substance. If unevaluated questions produced an immediate and uncritical response, our food supply would be in constant chaos, with products continually being banned and then later returned to the grocer's shelves. This kind of institutionalized "wolf-crying" would destroy the credibility of any agency, as well as undermine our food supply.

Of at least equal importance, an objective, orderly review is necessary to provide the legal and scientific basis for any regulatory action that may need to be taken. FDA's regulatory actions will not be accepted by the scientific community, the courts, and the public unless they are supported by sound scientific evidence. Moreover, Congress has prescribed specific standards and procedures to be applied in making major regulatory decisions.

In short, with the exception of situations involving the sudden appearance of contaminated lots of food or other specific, unequivocal threats, there is a need for orderly review of new information about familiar products. Action without scientific basis, even in response to widely publicized but ungrounded allegations, would ultimately mean less, not more, protection for the American consumer.

FDA'S FOOD SAFETY ACTIVITIES

To put the safety evaluation of food substances into perspective, I think it would be useful to describe briefly for the committee FDA's food safety program.

Nearly all of FDA's food budget is directed to problems concerned with food safety. Out of a total of approximately \$77 million for fiscal year 1977, approximately \$72 million is devoted to food safety. Program activities include, among others, surveillance for: (1) Microbiological contamination and toxins, (2) heavy metals, (3) industrial chemicals, (4) mycotoxins and natural poisons, and (5) manufacturing and storage conditions. Our program entails a variety of approaches to various problems geared to the likelihood and degree of hazard from a given problem and its prevalence. For example, in fiscal year 1976 we made nearly 19,000 establishment inspections of domestic firms and over 61,000 examinations of import shipments. In addition, during this same period, we analyzed nearly 37,000 domestic and imported food samples.

I am providing for the record a description of the major projects by which we manage our food safety activities. This will illustrate further the broad range of our current food safety activities, of which the GRAS review project and the cyclic review project of food and color additives are two important parts.

Before describing these projects in detail, I believe it will be useful first to outline the statutory requirements that govern the regulation of food additives.

REGULATORY CONTROLS FOR FOOD ADDITIVES

Substances added to human food are regulated under several not entirely congruent provisions of the Federal Food, Drug, and Cosmetic Act. While the public perceives "food additives" as embracing all artificial chemicals added to food, the legal definition of the term excludes many chemical substances that are regulated under other provisions of the law and includes many natural ingredients, such as spices and many added vitamins.

The statutory standards for the evaluation of substances added to food, and the procedures for applying them, are a product of a series of amendments made by Congress to the basic 1938 act between 1954 and 1968. The result is a complex regulatory framework in which the consequences of scientific findings about a substance and the procedures for terminating use of a substance that FDA has found to be unsafe often depend on the legal category into which it falls.

The act broadly defines a food additive to include " * * any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food." This expansive threshold definition includes both natural and artificial substances. Consumption of artificial food additives is substantially exceeded by consumption of natural food additives.

In addition to ingredients added directly to food, the definition of "food additive" also includes those substances that may reasonably be expected to migrate from food packaging and other food contact surfaces to food. As analytical methods have become capable of measuring increasingly tiny amounts of a compound, we have discovered that more and more substances can "reasonably be expected" to migrate to food in small quantities, and, thus, this category of food additives has been growing rapidly.

Accordingly, under modern methods of processing and packaging food, the vast majority of the components, intended and expected, of the Nation's food supply are potentially classifiable as food additives and therefore are subject to premarket testing and approval. However, in addition to the broad inclusive language just described, the statutory definition of food additive includes a number of important exceptions for certain substances.

These exceptions are of two basic kinds. One kind includes substances added to food that are subjected to premarket approval requirements under other provisions of the act, such as color additives, pesticide residues, and animal drugs. The other kind includes two important categories of added food ingredients for which Congress has prescribed somewhat different controls.

The most important of these latter two exceptions, which embraces the larger number of substances, is the category of so-called GRAS substances. Any substance that is "generally recognized as safe among experts qualified by scientific training and experience to evaluate its safety" is not classified as a food additive and is therefore excluded from the requirements of section 409 of the act for premarket testing and approval. A substance may be classified by experts as GRAS on either of two bases:

1. For a substance used in food before January 1, 1958, by either scientific procedure or experience based on common use in food.
2. For any substance, by scientific procedures.

FDA has adopted regulations that particularize these two criteria for GRAS classification. We have defined "experience based on common use in food" to require "a substantial history of consumption of a substance by a significant number of consumers in the United States." We have defined "scientific procedures" even more narrowly, and require the same quality and quantity of scientific evidence for GRAS status as for approval of a regulated food additive. The application of these definitions to substances being evaluated in our GRAS review project will help insure that all GRAS ingredients in food are generally safe for consumption.

Senator NELSON. Let me ask a question here.

As to the problem raised respecting some challenge to an additive that is in the marketplace, and the problem that would arise if, in response, you removed it from the marketplace, and so forth, I can agree that that creates a very serious problem, and it is easy to challenge the safety of any product.

However, is it not quite another question when a manufacturer proposes to introduced a new additive? There is no disruption of the marketplace, if the FDA requires very careful scientific studies on a new use for a substance, including carcinogenicity, or all other aspects of the health hazards. That does not disrupt the marketplace. You can do something about slowing up the proliferation of chemicals used by careful scientific requirements, can you not, and should you not?

Mr. GARDNER. Yes, we are making sure that appropriate scientific tests are required to assure safety.

I would not want the record to show we did that as a means of deliberately slowing introduction of new additives.

Senator NELSON. Well, that aside, you raised the question of the problem of somebody's challenging the safety of a product, and your

responding by taking it out of the marketplace, and disrupting the market. That would affect the credibility of the FDA.

I am saying that you do not have that problem, when somebody comes along, and says, "I have a new additive here," but that additive does nothing that others already do not do.

You know it does not. They know it does not. The other additives have had long experience in testing, and you know of no safety hazard.

It is my conclusion that no social purpose is accomplished at all by adding a new substance to the food supply, if it is not any cheaper economically, it does not do the job better in coloring, in emulsifying, but is just a new chemical, that may or may not be a hazard.

Under the circumstance, is not there some obligation, regardless of the relative efficacy question, simply to say, all right, as to safety alone, we are going to require very careful and extensive tests?

Mr. GARDNER. We would require very extensive and careful testing, but the standards required for that product for demonstration of safety would not be different from the standards we would require for similar products.

Senator NELSON. Well, counsel simply said you have lots of flexibility in that area, and it would seem to me your obligation is quite different for a product that has been in the marketplace, is being widely used, and for which you may very well say, "we will require some studies before we remove it from the marketplace."

It is quite a different matter before letting it in the marketplace, to require very extensive and careful studies, is it not?

Mr. MERRILL. I think it is, and I think the record bears out we do demand exactly that of the new products coming on. The standards for approval, as science has progressed, have become more rigorous, more demanding.

I think Mr. Gardner's point is that we try to apply the best science available which we would apply across the board.

It is important that the record show that we often know a good deal more about the safety of relatively more compounds, simply because science has provided us a good deal more information, we have done a good deal more testing, less experience in use perhaps, but a good deal more testing, precisely because the scientific criteria have improved so much since 1960.

Senator NELSON. I was not raising that question. I was raising the question about a new product that you did not know more about.

For example, what is your policy respecting testing on a new product, as to whether it is a carcinogenic, or mutagenic?

Mr. GARDNER. That would depend upon the product.

Senator NELSON. It would depend upon what?

Mr. GARDNER. On the particular substance.

We need to get scientific judgment about the character of that substance, and from that judgment, determine whether mutagenic or carcinogenic tests would be required.

Senator NELSON. Are you saying now that for any new product, you consult the scientific community to determine what kind of tests ought to be made as to that particular chemical?

Mr. GARDNER. I think I can say yes, but I would like Mr. Ronk, who is in the Bureau of Foods, and responsible for the food additive activity, to elaborate on that answer.

Mr. RONK. We would require generally based on anticipated exposure that that material would have. If it would be a direct food additive, where we anticipated the food exposure would be more than—

Senator NELSON. Let me clarify that more precisely. If it is a direct food additive to be broadly consumed, as contrasted with an indirect additive, is it automatic that you require mutagenic and teratogenic testing?

Mr. GARDNER. It would be. We would automatically require the teratogenic plus other tests.

Senator NELSON. And mutagenic?

Mr. GARDNER. We would ask for the mutagenic information, but we would not require the same thing.

Senator NELSON. So that is now the practice of the FDA as to any new food additive that is to be widely consumed?

Mr. GARDNER. That is right.

Senator NELSON. And when did the FDA start that policy?

Mr. GARDNER. Well, I have been in my job since 1972. As I view our records, that policy started around the time of the Pfizer Committee in 1969, which recommended that we look at the physiology and the teratogenic kinds of tests.

Senator NELSON. I take it the teratogenic test is relatively simple to conduct. Am I wrong on that?

Mr. GARDNER. I do not believe any test is; I would not classify it as simple to conduct.

Senator NELSON. I did not say that. I said relatively.

Mr. GARDNER. Relative in terms of the long-term teratogenic study, yes. So if we are going to say the ultimate in difficulty would be the long-term study which takes 2½ and 3 years to perform, yes.

Senator NELSON. I am going by memory, and I may be wrong, but about 1969, we took testimony on testing for teratogenicity. I do not know what the language was, but I believe the witness said it was practical and feasible to make such tests, is that correct?

Mr. GARDNER. Yes; if you used standard protocol.

Senator NELSON. Go ahead.

Mr. GORDON. May I ask a question at this point?

What is the cost of the GRAS review to date?

Mr. GARDNER. I believe that figure is \$18 million, and it is alluded to somewhere in our testimony.

The second major exception to the food additive definition covers substances approved for use in food prior to 1958 by either FDA or the Department of Agriculture. This category of substances—which are described as “prior sanctioned”—are, in a sense, “grandfathered” on the basis of prior approvals by FDA or USDA. We endeavor to apply essentially the same scientific standards in evaluating prior sanctioned substances that we apply to food additives and GRAS substances and, if new evidence warrants, will rely on the adulteration provisions of the act to limit their use or to remove them from the food supply.

Nonetheless, the different legal status established by Congress for prior sanctioned substances requires a different, potentially more cumbersome regulatory approach.

Also excepted from the food additive definition are three categories of products that are subject to premarket testing and approval under other provisions of the act or other laws.

Pesticide chemicals on raw agricultural products are excepted from the definition because their use is regulated under the pesticide residue provisions of the act and under the Federal Insecticide, Fungicide, and Rodenticide Act. The Environmental Protection Agency (EPA) sets standards and tolerances for pesticide use; FDA enforces the tolerances for pesticides set by EPA.

Color additives are excepted from the food additive definition because they are specifically regulated—and subject to premarket testing—under the Color Additive Amendments of 1960. In enacting those amendments, Congress provided that the Agency could provisionally list colors in use in 1960 while scientific investigations were undertaken to enable decisions to be made about their safety. As the committee knows, the Agency has for several years provisionally listed certain colors while studies were being undertaken and additional data gathered to enable the Agency to evaluate fully the safety of the colors. Recently, the Agency published a final, definite schedule for resolving the status of the remaining colors on the provisional list.

The final category of products excepted from the food additive definition because they are subject to premarket testing and approval under other sections of the act are new animal drugs. Before 1968, new animal drugs were regulated under sections 505 and 409 of the act. Congress enacted the animal drug amendments in 1968 which unified the regulation of new animal drugs under a single scheme.

Mr. Chairman, it is important that this committee and the public appreciate that the procedures for terminating the approval of any of these products—that is, for removing them from the food supply—differ, just as the procedures for approving the use of added food ingredients vary. Thus, for example, the use of a provisionally listed color can be terminated rapidly—we need only issue a notice in the Federal Register—while the use of a permanently listed color additive can be terminated only after a lengthy administrative process that may include a formal evidentiary hearing. The approval of a food additive cannot be terminated as rapidly as a provisionally listed color additive, but the procedure is not as cumbersome as that required to remove a permanently listed color additive from the marketplace. Finally, the procedures for terminating the approval of a pesticide or new animal drug each differ. In short, Congress has provided some half dozen different procedures for terminating the approval of the types of products which are frequently all lumped by the public under the heading of “food additives”.

Senator NELSON. Let me ask you a question there. In order to remove a drug, or suspend an additive, you would have to make a finding of an imminent hazard? What is the finding you have to make?

Mr. MERRILL. I am sorry, but the explanation will take a few moments.

The imminent hazard standard applies only to new drugs and new animal drugs, and permits us to withdraw approval without or before holding the hearing. But to do so we must persuade the Secretary that he should find that a product constitutes an imminent hazard.

Senator NELSON. This does not apply to food additives?

Mr. MERRILL. It does not apply to food additives or to color additives.

Senator NELSON. What does apply to food additives?

Mr. MERRILL. With respect to provisionally listed color additives—I will talk about these colors first because that process we found is the speediest—the Agency may withdraw the certificates and terminate the provisional listing essentially overnight on the basis of a discovery of a hazard.

Senator NELSON. What do you have to find?

Mr. MERRILL. I can give you the precise—

Senator NELSON. Paraphrase it.

Mr. MERRILL. Essentially if the public health so requires. It is a fairly soft standard.

Senator NELSON. Are you saying that if the FDA concludes or suspects a health hazard, you have the authority to suspend it overnight?

Mr. MERRILL. If it is a provisionally listed color.

Senator NELSON. How does something become provisionally listed?

Mr. MERRILL. If it were on the provisional list at the time—

Senator NELSON. You are not talking about the GRAS list?

Mr. MERRILL. No; only provisionally listed color additives. That is why it is complicated. The law does not treat similar things alike.

Provisionally listed color additives can effectively be removed overnight, if there is any suggestion of real concern.

If it is a permanently listed color additive, the process is the most cumbersome that the law currently provides. The filing of objections to an action by an agency to terminate the listing of the color additive immediately stays that order, and authorizes the petitioner an administrative hearing before the order can become effective.

Senator NELSON. What factors would cause the FDA to provisionally list an additive?

Mr. MERRILL. The color additives that are provisionally listed are there because they were in commercial use in 1960.

Senator NELSON. Could you now put one that is on the market, and about which you have reservations, on the provisional list? You can or cannot do that?

Mr. MERRILL. I don't think we have ever done that, Mr. Chairman.

Mr. Pape confirms my impression that the provisional list was a transitional provision, designed for things in use to enable a full safety assessment to be made.

It is a mechanism that will expire with the completion of the studies on the compounds now on the provisional list.

Senator NELSON. So, under the provisions of the current law, if the FDA, based on some evidence called to their attention, concludes that there is a health hazard respecting a now marketed color additive, of the same dimension and judgment as one that is on the provisional list, it still cannot remove or suspend the use of that additive in the same way it can if the additive was on the provisional list?

Mr. MERRILL. That is correct, and let me amplify that. If we were dealing only with color additives, which are not on the permanent list, the procedure would be nightmarish in my personal judgment. If a color is provisionally listed, it is very prompt. If it were a human drug, about which we have safety concerns, unless they arise to the level of an imminent hazard, we cannot take the kind of action against that product that we can against a provisionally listed color additive.

Senator NELSON. Well, should not the law have some carefully drawn provisions which permit the FDA the authority, when evidence by some reasonable standard indicates a health problem, to suspend the drug, or the additive?

Mr. MERRILL. I think the answer to that is very clearly yes. We say in the testimony, and in recommendations for legislation, that the whole area ought to be addressed and a standard summary removal authority established.

Senator NELSON. Have you looked at the legislation we introduced on that precise point?

Mr. MERRILL. I have indeed.

Senator NELSON. Is it drafted in a way that would meet the problem in your judgment, or should it be amended?

Mr. MERRILL. I would prefer to comment by letter for the record on that. My general impression is the bill does address the problem and would be satisfactory.

My concern would be, does it cut across all of the pertinent provisions of the statute.

Senator NELSON. We would like the benefit of your judgment on that legislation that we introduced several years ago, because we intend to introduce it again.

Go ahead.

Mr. GARDNER. Along those lines, it is also important to note that it is not only the procedures that apply to these products that are different; Congress has also in some instances provided different substantive standards for evaluating their safety. Most notable is the Delaney clause, which prohibits the use of any food additive—direct or indirect—or any color additive shown to cause cancer in man or animal. A modified version of the Delaney clause also applies to new animal drugs intended to be used in food producing animals. On the other hand, the Delaney clause does not apply to prior sanctioned substances or pesticide residues.

FOOD ADDITIVE REGULATION : SCOPE AND PRESENT DAY TRENDS

The number of food substances in all of the foregoing categories that fall within our regulatory jurisdiction is very large. There are over 400 nonflavor GRAS substances; approximately 1,650 flavors and spices, some of which are GRAS and some regulated additives; about 400 regulated direct food additives and on the order of 10,000 GRAS and regulated indirect additives. Additionally, there are some 65 regulated and 52 "provisionally listed" color additives (including drug and cosmetic colors). Pesticides, as we have noted, are regulated by the EPA and animal drugs are covered by a separate agency program.

Senator NELSON. For purposes of keeping the record clear, when you say regulated and unregulated, you are using that term in the context of the testimony of counsel, Mr. Merrill, is that correct?

Mr. GARDNER. Yes.

A consequence of the dramatic improvement in the tools for safety evaluation has been the relatively more stringent requirements now imposed on petitioners seeking approval of food additives than were considered necessary in the early 1960's. There has been a significant reduction in the addition of new additives to the food supply. For

example, an average of 44 direct food additives were approved annually in the period 1959 through 1963. In the 1972-76 period, approximately ten food additives per year were approved. A table showing the number of food additive applications received and approved from 1958 to the present is attached as appendix J.

STATUTORY CRITERIA FOR APPROVAL

As I noted earlier, a primary purpose of the Food Additives Amendment of 1958 was to place the responsibility for demonstrating the safety of food additives on the producers and users of food additives, rather than the Government. Before enactment of this amendment, pretesting and approval of food substances was not required. Substances with unknown or uncertain toxicity could be added to foods. The Government had the burden of proving in court that an added substance was poisonous or deleterious before regulatory action could be taken to remove the substance from food use.

Any food additive must now undergo strict testing to establish the safety of the intended use. Information must be presented to FDA in the form of a petition and includes the identity of the new additive, its chemical composition, its manufacturing methods, and the analytical methods to be used to detect and measure its presence in the food supply at the levels of expected use. Data must be provided to demonstrate that the proposed analytical method is sufficiently reliable and capable of determining compliance with the regulations.

Data also must be provided to demonstrate that the additive will accomplish the intended physical or technical effect in the food, and that the level sought for approval is no higher than that reasonably necessary to accomplish this effect. This is the so-called functionality requirement.

Finally, data must be provided to establish that the additive is safe for its intended use. This requires scientific evidence ordinarily derived from animal feeding studies using the proposed additive at various levels in the diets of two or more species of animals.

In reaching a decision on the safety of any substance to be added directly to food for man, a number of factors are considered:

1. Available toxicology data.
2. Estimated average daily intake of the substance by those whose diet normally includes the foods containing such substances.
3. Estimated average daily intake of the population consuming the maximum amounts.
4. Adequacy of the safety factor employed.
5. Relationship to other substances of closely similar chemical structure whose metabolism and physiological or pharmacological activity is known.

For a new proposed indirect additive—one which migrates from the package to a food—the safety decision is based on a combination of migration studies in food simulating solvents, in addition to toxicological and other safety data, and exposure information. In cases where there is virtually no migration of components to the food simulating solvent, short-term toxicity tests generally suffice. Where a compound possesses high biological activity and there is more migration, lifetime feeding studies may be required.

The criteria for establishing the safety of color additives are generally the same as those for food additives. In short, the safety of the color must be established by appropriate studies in test animals and in nonanimal laboratory testing—for example, testing required to establish the chemical identity of the color—before it will be listed for use by FDA.

It is important to note that the criteria for evaluating an application for approval of a food or color additive, do not include the “benefits” from the use of the additive. The term “functionality,” which as I indicated previously is one of the requirements for approval of a food additive, requires FDA to determine whether the additive proposed for use performs its intended function. Thus, FDA must be satisfied that an emulsifier emulsifies and that a stabilizer stabilizes. But the Agency is not authorized to determine whether society needs another emulsifier or stabilizer. FDA does not, and I doubt whether the Agency should or even could, as a routine matter, make the kind of value judgments that would be necessary if only “beneficial” food additives could be approved. That is a judgment that already has been made by the Congress in a collective way when it enacted the Food Additives Amendment.

GRAS REVIEW

Another key issue which concerned Congress and led to passage of the 1958 amendment was the need to establish priorities in testing substances. Congress clearly was concerned that testing of new unknown substances be given priority over testing of ingredients that had been in use for years. While it was important to test the older ingredients as well, these could be reviewed more gradually as resources become available. The GRAS review project was an inevitable outcome of the amendment which gave formal recognition to the concept of generally recognized as safe.

One of FDA's first actions after passage of the 1958 amendment was initially to determine which of the many food ingredients then in use were GRAS and which were food additives and thus subject to the pre-market testing requirements of the new law. To make these determinations, during the 1958–62 period, the Agency surveyed a number of experts and published advisory lists of GRAS substances, codified in 21 CFR 121.101. The composite list included substances which were sanctioned for food use by the FDA or USDA prior to 1958, and those substances that had a history of use in food prior to 1958 without any known or expected health hazard. It is clear, however, that this list was developed without a thorough scientific review, as we know it, of most of these ingredients.

Very few substances were added to the published FDA GRAS list between 1962 and 1970. The Agency did, however, issue individual advisory opinion letters during this period, with the effect that at least 100 additional unpublished substances were considered to be GRAS for specific food uses.

In his Consumer Message of October 30, 1969, President Nixon directed the Secretary of Health, Education, and Welfare to initiate a full review of all GRAS ingredients. To implement this mandate, FDA developed an approach that has followed four sequential phases:

1. *The collection phase.*—An industrial user survey to establish consumer exposure to each substance and a literature search of 50 years of scientific literature to collect all of the safety information relative to each substance.

2. *The collation phase.*—The organization of the literature and consumption data into a “scientific literature review.”

3. *The evaluation phase.*—The development of a tentative evaluation of the “scientific literature review” to determine whether expert food safety scientists agree that the substance is generally recognized as safe for its intended use or whether some limitation is required in the interest of safety, the release of the evaluation for review and comment at a public hearing, and the development of a final evaluation based on the tentative findings and the public comments.

4. *The implementation phase.*—The issuance of proposals and final regulations necessary to implement the evaluations.

The FDA contracted with the Food Protection Committee of the National Academy of Sciences (NAS) to survey the food industry and determine the aggregate national production of all GRAS ingredients, the amount of each ingredient used in any particular food, and the anticipated consumption of these ingredients by U.S. consumers. This survey was completed for all published and unpublished GRAS ingredients, and for FDA regulated flavor ingredients in 1972. The NAS also has continued to update and refine consumption information on GRAS ingredients on an as needed basis.

The search of the world scientific literature, from 1920 to the present time, and the summary of available safety data for each GRAS ingredient or group of ingredients were accomplished with contract support. Announcement of these contracts, and solicitation of unpublished safety data, have also been published in the Federal Register. To date, 118 scientific literature reviews, covering 356 GRAS substances, have been prepared for the published nonflavor GRAS list plus a few chemically related unpublished nonflavor GRAS ingredients; 28 literature reviews covering the remaining 83 unpublished nonflavor GRAS ingredients are now in preparation.

We also have had scientific literature reviews prepared for approximately 700 of the existing 1,650 natural flavors and species and synthetically produced flavor ingredients, because our original intent was to evaluate all flavor ingredients used in food, rather than to narrowly evaluate only the 226 predominately natural spices and essential flavoring oils on the GRAS list. Our approach to this safety evaluation of flavors has also been to examine synthetically produced components of flavor formulations first, and then to evaluate natural spices, and their essential oils.

An auxiliary source of information in the GRAS review has come from laboratory studies on the potential mutagenesis and teratogenesis of GRAS substances. The testing has been done predominantly under contract. The compounds selected for testing were also selected on a priority basis in accordance with their suspected effects and to provide as much information as possible for substances which cannot be tested with limited resources. To date, 81 representative nonflavor ingredients have been tested for teratogenic effects in at least two species of animals; 52 ingredients have been tested for mutagenic effects in

3 test systems and by the end of this fiscal year 235 nonflavor substances will have been tested for mutagenesis potential.

The evaluation phase of our GRAS contract program has been accomplished through a contract with the Federation of American Societies for Experimental Biology (FASEB). The federation has established a select committee of food scientists to review the scientific literature, consumption information, and test data for each ingredient; and to issue advisory opinions to the FDA on the safety of the ingredients. The results of the committee's work for any substance under review can lead to any number of the following five recommendations:

1. There are sufficient data to assure continued safe use of the ingredient under present or expected levels of use. This provides a basis to affirm the GRAS status of the ingredient when used in accordance with good manufacturing practices.

2. There are sufficient data to assure continued safe use of the ingredient only at present levels of use. This provides a basis for affirming the substance as GRAS but with limitations on its use corresponding to present use levels.

3. Questions about the safety of the ingredient have arisen in the review which should be resolved. In such a case we would promulgate a regulation, requiring that studies be undertaken for the ingredient if its use in food is to continue.

4. The data indicate such substantial questions about the safety of the ingredient for use in food that prohibition of its use is appropriate.

5. There are insufficient data to establish conditions of safe use for an ingredient. This is a basis for either prohibition of use for the ingredient or a regulation requiring additional testing as a condition for continued approval.

We have committed ourselves to an open process that permits public participation in the GRAS review. It is our view that all data and information leading to the development of each federation recommendation and Agency decision should be made public. We have, therefore, announced in the Federal Register the public availability of all consumption, testing, and literature review material as it is completed on each group of ingredients. We have also provided that the federation's tentative evaluations for each ingredient are announced in the Federal Register for comment and argument before the federation prepares a final recommendation for the Agency.

Our estimate is that we have reached the halfway point in the GRAS review of nonflavor ingredients. We have completed all data and information retrieval phases of the project and advisory opinions have been provided by FASEB for about 200 of the total 439 substances. Our remaining task is to implement these advisory opinions by regulations.

Thus far, the total cost of the GRAS review program has been approximately \$18 million. During this fiscal year, we received specific funds for the reevaluation of currently used food additives as part of a budget supplement to increase our efforts in the monitoring of the quality of bioresearch data submitted to the Federal Government. The funds for food additives will be used, in part, to hire additional scientific personnel to review FASEB opinions and for personnel to prepare implementing regulations and the associated Federal Register documents.

ADDITIVE REVIEW

Soon after the GRAS review started, we began to develop a plan for the reevaluation of the safety of ingredients that have been subject to food additive regulation by FDA since 1958. By September of 1972 the basic concepts for a cyclic review project for all ingredients added to foods had been developed to take account of changes in scientific standards. As mentioned earlier, the safety decisions for compounds approved more than 10 years ago were based on less stringent toxicological standards than would apply to similar petitions submitted today.

FDA has already conducted reevaluations of certain food additives. Approved food additive petitions may be subjected to reevaluation for a number of reasons, for example:

Questions on safety concerning an approved petition may arise as the result of review of a new petition for a similar substance or literature reviews by our scientists.

Review of a new petition for an additional use of a compound may lead to reevaluation of the original petition, especially where submission of the two petitions is separated by a number of years.

In these and other ways, reevaluation of food additives by contemporary safety standards has already begun. However, such reevaluations are not comprehensive or systematic. They depend almost entirely on "chance" factors and as such do not provide either the Agency or the public with a reliable basis for assuming the safety of food additives under current knowledge.

The primary goal of the additive review is to develop a current toxicological profile for each food and color additive, which is integrated with fairly precise estimates of the amount of each of these additives a consumer is exposed to in his daily diet. This safety profile of toxicity and exposure information will be updated periodically, thus providing practical assurance that permitted food additives are not responsible for the induction of acute or chronic disease effects.

We will, as a part of the periodic evaluation, ask for additional safety information to be developed so that as new lines of investigation are explored, the limited risks that these uses present will be further reduced. This information will give us a clearer picture than we have now about the potential of each food ingredient to produce cancer, adverse effects on reproduction, effects on the fetus, or heritable genetic damage, or other types of chronic disease. The second major thrust is a program to insure the integrity and quality of the biological information submitted to the Agency in support of regulated food and color additives.

ELEMENTS OF THE ADDITIVE REVIEW PROJECT

The project consists of four elements:

1. Review of substances added directly to foods. This element includes the reevaluation of the safety of all flavors, colors, and regulated direct food additives. These total some 2,100 compounds.

2. Review of substances which are added indirectly, that is, migrate, to food. This element will embrace the reevaluation of the safety of the regulated, GRAS, and prior sanctioned packaging materials. There are about 10,000 indirect additives.

3. Completion of the GRAS review. Included will be the existing nonflavor items included on GRAS lists, as well as the prior sanctioned items. Also included is the ongoing program of evaluation of GRAS affirmation petitions.

4. The Bio-Research Monitoring Program. This project is part of the Agency's comprehensive program to assure the integrity of the data submitted to the Government in support of safety decisions. This program is being implemented through the publication of Good Laboratory Practice Regulations and monitoring of the approximately 130 nonclinical laboratories through an inspection program.

It is not practical in this statement to outline in great detail all the elements of this many-faceted program. I will, however, state our major objectives, and outline the more important procedural steps which place the program in a time perspective.

The objectives for each of the first three action elements for classes of additives are similar. They are:

1. Determine how much of each additive the consumer is ingesting.
2. Assemble the toxicological and other safety data available for each additive to evaluate whether (or not) the data meet today's criteria.
3. Develop acceptable protocols for the variety of toxicological and other safety tests we use to decide the safety of additives.
4. Develop a set of evaluation criteria to describe as precisely as we can the minimum amount of safety information that we will accept to allow the use of an ingredient in food regardless of how low the exposure may be.

The fourth element, bioresearch monitoring, is different in character from the evaluation per se of food additives. It does, however, have an important influence on our ability to make sound judgments about the safety of additives. For some time, FDA has been concerned about the absence of industrywide standards for the conduct of non-clinical laboratory studies. Intensive investigations of certain laboratories have revealed significant quality control problems in some of them. FDA's reliance on the basic accuracy and integrity of the data submitted as a result of studies performed in these laboratories is essential to the review and approval of food and color additives. The submission of faulty, erroneous, or distorted data increases the potential for reaching invalid judgments about the safety of these additives.

Mr. Chairman, when this program is fully operational, it will assure that studies in support of petitions—for food additives, new drugs, and so forth—submitted to the Federal Government, are conducted in an appropriate scientific manner, and that the data from such studies are accurate, complete and reliable. Other approaches, such as the Government's being responsible for the conduct of safety studies—commonly known as “third party testing”—have been proposed to address these concerns. However, we believe that our bioresearch monitoring is, to date, the most effective and efficient approach to solving the problem. We believe that the cost of “third party testing” would not result in any substantial benefits. It would merely add another Federal superstructure for designating laboratories, collecting fees and hearing disputes. Because FDA is responsible in either case for the final evaluation of the data and making the decision on whether

the product is approvable, we believe our current program is more appropriate.

To implement the bioresearch monitoring program, the Agency has published proposed Good Laboratory Practice for Nonclinical Laboratory Studies regulations. We have initiated a pilot inspection program designed to determine how widespread are the problems uncovered in the initial investigations. We will develop baseline data to measure conformance of the laboratories to the proposed regulations and to determine what improvements are needed. The pilot effort will also enable us to obtain the necessary knowledge to improve the quality of the proposed regulations and the methods of inspection. It will be a learning process for both the Agency and the industry.

We believe there are about 130 nonclinical laboratories doing toxicity testing on food and color additives. When the bioresearch monitoring program becomes fully operational, we will be routinely inspecting each laboratory at least once every 2 years. Where problems are uncovered or suspected, we will, of course, make more frequent inspections. Our goal is to improve the performance of nonclinical laboratories, where necessary, and to maintain compliance at a high level.

Returning to the overall food additive review project, there are three major near-term tasks:

1. An industry additive use survey for all flavors, colors, and direct regulated food additives. This survey will provide the information needed to calculate the exposure pattern of any additives in a particular food. A prototype of this survey was conducted in 1970 for those food ingredients which were considered GRAS. This more comprehensive survey that we are discussing this morning, a survey of all direct addition food additives and color additives, is expected to begin in April.

2. We are currently establishing internal working groups to develop the standards by which we will evaluate the safety of any ingredient added to food. These standards will include definitions of test protocols to be followed when evaluating the safety of food and color additives.

3. In March a team of Agency scientists will begin development of a priority list for the approximately 2,100 direct food, color and flavor additives. The reevaluation process will begin with collection of available information for each individual ingredient. This information will be evaluated using the safety standards described above. Concurrently, a review of the scientific literature of colors, flavors and direct food additives will be made by the Bureau of Foods. This review will help to establish a toxicology profile for each ingredient, and thereby help establish a priority order for evaluation of the approximately 2,100 ingredients.

Completion of these activities should enable us within 18 months to make preliminary judgments regarding these 2,100 substances and will lead to the establishment of a priority list.

We recently received, on contract, a set of criteria for evaluating the safety of flavors which we will soon publish for public comment. These criteria were developed by the Federation of American Societies for Experimental Biology's "Special Committee on Flavor Evaluation

Criteria"—SCOFEC. Included in these recommendations are the use of short-term *in vitro* screening test.

Although the status of screening tests is being debated, we will define an appropriate role for the Ames type tests to screen compounds to predict their potential carcinogenicity.

Before any indepth evaluation takes place, we will classify the substances under review into three categories:

1. Those ingredients which cannot be adequately searched in the literature because they have problems in nomenclature.

Action—FDA will require industry to furnish information.

2. Those ingredients for which no toxicological or other biological safety information can be discovered.

Action—FDA will request data through Federal Register announcement. If information is not available, we will require industry to carry out studies to allow for continued use.

3. Those ingredients, which have an adequate preliminary safety profile.

Action—FDA will do an "indepth" review of the information and reach a current safety conclusion, recognizing that further scientific advances or reports of experiences may subsequently require change.

Ingredients for which there is an adequate preliminary safety profile will be designated for immediate evaluation.

CONCLUSION

Mr. Chairman, the process of evaluation, approval, and reevaluation of food additives that I have described is one of the most important responsibilities of the FDA. It is also the most visible activity of the Agency, which confirms the importance of the hearings you are conducting this week.

Our two major ongoing projects for reviewing the safety of food ingredients, like other programs in our Bureau of Foods, are designed to permit FDA to provide continued assurance that consumers are not exposed to unsafe foods. These projects are a reflection of the fact that the scientific standards for evaluating the safety of chemicals have changed dramatically in the nearly two decades since the passage of the Food Additives Amendment in 1958.

We have always sought to apply the best contemporary standards for assessing the safety of substances added to food, and in the process we have often found ourselves applying new rules to old ingredients. The distinctive feature of the GRAS review and the additive review is that they represent an effort to apply new standards, not simply as questions are raised about familiar compounds, but to all ingredients on a rational, systematic basis. This is a costly, exacting, but indispensable process.

The application of contemporary standards to products approved 15 years ago will cause us to revise some old decisions, and undoubtedly produce some public anxiety. I believe it is important, Mr. Chairman, for Congress and the public to come to understand that new questions always can—and indeed should—be raised about ingredients previously assumed to be safe. Continuing reevaluation, in the light of new evidence and new standards, must become a routine part of FDA's

work, and an accepted characteristic of the regulatory process, rather than a cause for accusation and alarm. The time for alarm would come if the safety of approved products were never questioned. That would signify that we were not doing our job and that scientists had ceased to be inquisitive.

In short, the raising of questions about the safety of previously approved products is a sign of a system that is working, not a signal that it has failed.

Thank you, Mr. Chairman. My colleagues and I will be happy to answer any questions you and other members of the committee may have.

Senator NELSON. We thank you very much for your statement.

[The prepared statement and supplemental information submitted by Mr. Gardner follows:]

STATEMENT

BY

SHERWIN GARDNER

ACTING COMMISSIONER

FOOD AND DRUG ADMINISTRATION

PUBLIC HEALTH SERVICE

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

BEFORE THE

SELECT COMMITTEE ON SMALL BUSINESS

UNITED STATES SENATE

JANUARY 13, 1977

Mr. Chairman:

I appreciate this opportunity to appear before your Committee to discuss the current legal authority of the Food and Drug Administration (FDA) to regulate food and color additives and the activities of the Agency in this important and highly publicized area.

Your letter of invitation and our discussions with the Committee staff indicate a broad interest in the regulation of food additives. Therefore, my statement is directed to a discussion of the Agency's authority and program activities for food and color additives. I am also attaching to my statement individual appendices which discuss the specific subjects you inquired about in your letter of invitation. On the assumption that these specific subjects are likely to consume more of the discussion following my prepared statement, I believe it appropriate to focus my prepared remarks on the general legal, administrative and scientific context in which debate about the safety of particular compounds takes place.

It is apparent, from a review of the history of the various amendments to the Federal Food, Drug, and Cosmetic Act--from the Food Additives Amendment of 1958 to the Medical Device Amendments of 1976, that Congress has consistently expected that the Agency would make its decisions based on the best science available at the time. Dramatic changes in science and technology have occurred, however, since 1958. Each of the various amendments makes clear that the burden of establishing the safety and performance of the regulated products is on the manufacturer. FDA has the responsibility to make scientific judgments to approve or disapprove the use of these products.

One of the most difficult tasks facing FDA today is to evaluate the safety of individual food substances on an ad hoc basis, and, at the same time, to develop a mechanism for conducting safety reviews for all substances in an orderly manner to assure that safety decisions are valid in terms of current scientific standards.

The statute does not explicitly require the Agency to review periodically its safety decisions about products it regulates. Indeed, it would be much simpler if FDA could view its initial product decisions as final. However, it is not possible to rely indefinitely on the scientific judgments of a particular time, even if they are based on the best available data. Continual review of scientific regulatory decisions is necessary because, over time, several factors come into play that can make the data available at the time of the initial decision obsolete. Some of these factors are:

- new information on risk associated with certain substances;
- new patterns of use of products;
- product defect information;
- changes in scientific knowledge and standards; and
- technological advancement in manufacturing and measuring techniques affecting residues in foods.

Thus, periodic review of original scientific decisions is essential and an implied requirement of law if we are to carry out properly our regulatory responsibilities.

THE REVIEW PROCESS

In FDA, product reviews are conducted in two ways:

- by classes of products, and
- by specific product evaluation.

The product class review approach is effective and efficient. It permits a contemporary comprehensive review of similar or related substances and it assures a uniform and consistent application of policy and scientific standards. Specific product evaluations usually occur on an ad hoc basis in response to applications from manufacturers for approval of products prior to marketing or in response to new scientific findings.

There are six major product class reviews now in progress in the Agency:

- the drug efficacy study for prescription products;
- the OTC drug review;
- the safety and efficacy study of biological products;
- the review of low level antibiotics used in animal feeds;
- and
- the GRAS review of food substances;
- the cyclic review of food additives.

I would like to submit for the record a brief description of each of these class review projects.

Specific product evaluations have a varied character, ranging from the addition and/or deletion of an indication or warning statement on a drug label to the removal of a previously approved product from the market. Included in this latter category, for example, are actions related to diethylstilbestrol, cyclamates, FD&C Red No. 2 and many others.

Based on our experience with product class review programs, it will not surprise us to find that some food additives now considered to be safe by qualified scientists will have questions raised about their safety during the course of one of our food additive review programs. Such questions are inherent in the nature of scientific inquiry; in science there are no closed subjects.

When a question of safety occurs about a compound that FDA previously has approved, the Agency must review the question in a reasoned and scientific manner. This approach is rooted in common sense, because it is not difficult to raise questions about the safety of a food substance. If unevaluated questions produced an immediate and uncritical response, our food supply would be in constant chaos, with products continually being banned and then later returned to the grocer's shelves. This kind of institutionalized "wolf-crying" would destroy the credibility of any agency, as well as undermine our food supply.

Of at least equal importance, an objective, orderly review is necessary to provide the legal and scientific basis for any regulatory action that may need to be taken. FDA's regulatory actions will not be accepted by the scientific community, the courts, and the public unless they are supported by sound scientific evidence. Moreover, Congress has prescribed specific standards and procedures to be applied in making major regulatory decisions.

In short, with the exception of situations involving the sudden appearance of contaminated lots of food or other specific, unequivocal threats, there is a need for orderly review of new information about familiar products. Action without scientific basis, even in response to widely publicized but ungrounded allegations, would ultimately mean less, not more, protection for the American consumer.

FDA's FOOD SAFETY ACTIVITIES

To put the safety evaluation of food substances into perspective, I think it would be useful to describe briefly for the Committee FDA's food safety program.

Nearly all of FDA's food budget is directed to problems concerned with food safety. Out of a total of approximately \$77 million for Fiscal Year 1977, approximately \$72 million is devoted to food safety. Program activities include, among others, surveillance for:

(1) microbiological contamination and toxins, (2) heavy metals, (3) industrial chemicals, (4) mycotoxins and natural poisons, and (5) manufacturing and storage conditions. Our program entails a variety of approaches to various problems geared to the likelihood and degree of hazard from a given problem and its prevalence. For example, in fiscal year 1976 we made nearly 19,000 establishment inspections of domestic firms and over 61,000 examinations of import shipments. In addition, during this same period, we analyzed nearly 37,000 domestic and imported food samples.

I am providing for the record a description of the major projects by which we manage our food safety activities. This will illustrate further the broad range of our current food safety activities, of which the GRAS review project and the cyclic review project of food and color additives are two important parts.

Before describing these projects in detail, I believe it will be useful first to outline the statutory requirements that govern the regulation of food additives.

Regulatory Controls for Food Additives

Substances added to human food are regulated under several not entirely congruent provisions of the Federal Food, Drug, and Cosmetic Act. While the public perceives "food additives" as embracing all artificial chemicals added to food, the legal definition of the term excludes many chemical substances that are regulated under other provisions of the law and includes many natural ingredients, such as spices and many added vitamins.

The statutory standards for the evaluation of substances added to food, and the procedures for applying them, are a product of a series of amendments made by Congress to the basic 1938 Act between 1954 and 1968. The result is a complex regulatory framework in which the consequences of scientific findings about a substance and the procedures for terminating use of a substance that FDA has found to be unsafe often depend on the legal category into which it falls.

The Act broadly defines a food additive to include ". . . any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food." This expansive threshold definition includes both natural and artificial substances. Consumption of artificial food additives is substantially exceeded by consumption of natural food additives.

In addition to ingredients added directly to food, the definition of "food additive" also includes those substances that may reasonably be expected to migrate from food packaging and other food contact surfaces to food. As analytical methods have become capable of measuring increasingly tiny amounts of a compound, we have discovered that more and more substances can "reasonably be expected" to migrate to food in small quantities, and, thus, this category of food additives has been growing rapidly.

Accordingly, under modern methods of processing and packaging food, the vast majority of the components, intended and expected, of the Nation's food supply are potentially classifiable as food additives and therefore are subject to premarket testing and approval.* However, in addition to the broad inclusive language just described, the statutory definition of food additive includes a number of important exceptions for certain substances.

These exceptions are of two basic kinds. One kind includes substances added to food that are subject to premarket approval requirements under other provisions of the Act, such as color additives, pesticide residues, and animal drugs. The other kind includes two important categories of added food ingredients for which Congress has prescribed somewhat different controls.

The most important of these latter two exceptions, which embraces the larger number of substances, is the category of so-called GRAS substances.

Any substance that is "generally recognized as safe among experts qualified by scientific training and experience to evaluate its safety" is not classified as a food additive and is therefore excluded from the requirements of section 409 of the Act for premarket testing and approval. A substance may be classified by experts as GRAS on either of two bases:

1. For a substance used in food before January 1, 1958, by either scientific procedure or experience based on common use in food; or
2. For any substance, by scientific procedures.

FDA has adopted regulations that particularize these two criteria for GRAS classification. We have defined "experience based on common use in food" to require "a substantial history of consumption of a substance by a significant number of consumers in the United States." We have defined "scientific procedures" even more narrowly, and require the same quality and quantity of scientific evidence for GRAS status as for approval of a regulated food additive. The application of these definitions to substances being evaluated in our GRAS review project will help ensure that all GRAS ingredients in food are generally safe for consumption.

The second major exception to the food additive definition covers substances approved for use in food prior to 1958 by either FDA or the Department of Agriculture. This category of substances -- which are described as "prior sanctioned" -- are, in a sense, "grandfathered" on the basis of prior approvals by FDA or USDA. We endeavor to apply essentially the same scientific standards in evaluating prior sanctioned substances that we apply to food additives and GRAS substances and, if new evidence warrants, will rely on the adulteration provisions of the Act to limit their use or to remove them from the food supply. Nonetheless, the different legal status established by Congress for prior sanctioned substances requires a different, potentially more cumbersome regulatory approach.

Also excepted from the food additive definition are three categories of products that are subject to premarket testing and approval under other provisions of the Act or other laws.

Pesticide chemicals on raw agricultural products are excepted from the definition because their use is regulated under the pesticide residue provisions of the Act and under the Federal Insecticide, Fungicide, and Rodenticide Act. The Environmental Protection Agency (EPA) sets standards and tolerances for pesticide use; FDA enforces the tolerances for pesticides set by EPA.

Color additives are excepted from the food additive definition because they are specifically regulated -- and subject to premarket testing -- under the Color Additive Amendments of 1960. In enacting those Amendments, Congress provided that the Agency could provisionally list colors in use in 1960 while scientific investigations were undertaken to enable decisions to be made about their safety. As the Committee knows, the Agency has for several years provisionally listed certain colors while studies were being undertaken and additional data gathered to enable the Agency to evaluate fully the safety of the colors. Recently, the Agency published a final, definite schedule for resolving the status of the remaining colors on the provisional list.

The final category of products excepted from the food additive definition because they are subject to premarket testing and approval under other sections of the Act are new animal drugs. Before 1968, new animal drugs were regulated under sections 505 and 409 of the Act. Congress enacted the Animal Drug Amendments in 1968 which unified the regulation of new animal drugs under a single scheme.

Mr. Chairman, it is important that this Committee and the public appreciate that the procedures for terminating the approval of any of these products -- that is, for removing them from the food supply -- differ, just as the procedures for approving the use of added food ingredients vary. Thus, for example, the use of a provisionally listed color can be terminated rapidly -- we need only issue a notice in the Federal Register -- while the use of a permanently listed color additive can be terminated only after a lengthy administrative process that may include a formal evidentiary hearing. The approval of a food additive cannot be terminated as rapidly as a provisionally listed color additive, but the procedure is not as cumbersome as that required to remove a permanently listed color additive from the marketplace. Finally, the procedures for terminating the approval of a pesticide or new animal drug each differ. In short, Congress has provided some half dozen different procedures for terminating the approval of the types of products which are frequently all lumped by the public under the heading of "food additives".

It is also important to note that it is not only the procedures that apply to these products that are different; Congress has also in some instances provided different substantive standards for evaluating their safety. Most notable is the Delaney Clause, which prohibits the use of any food additive -- direct or indirect -- or any color additive shown to cause cancer in man or animal. A modified version of the Delaney Clause also applies to new animal drugs intended to be used in food producing animals. On the other hand, the Delaney Clause does not apply to prior sanctioned substances or pesticide residues.

Food Additive Regulation: Scope and Present Day Trends

The number of food substances in all of the foregoing categories that fall within our regulatory jurisdiction is very large. There are over 400 nonflavor GRAS substances; approximately 1,650 flavors and spices, some of which are GRAS and some regulated additives; about 400 regulated direct food additives and on the order of 10,000 GRAS and regulated indirect additives. Additionally, there are some 65 regulated and 52 "provisionally listed" color additives (including drug and cosmetic colors). Pesticides, as we have noted, are regulated by the EPA and animal drugs are covered by a separate Agency program.

A consequence of the dramatic improvement in the tools for safety evaluation has been the relatively more stringent requirements now imposed on petitioners seeking approval of food additives than were considered necessary in the early 1960's. There has been a significant reduction in the addition of new additives to the food supply. For example, an average of 44 direct food additives were approved annually in the period 1959 through 1963. In the 1972-1976 period, approximately ten food additives per year were approved. A table showing the number of food additive applications received and approved from 1958 to the present is attached as Appendix J.

Statutory Criteria for Approval

As I noted earlier, a primary purpose of the Food Additives Amendment of 1958 was to place the responsibility for demonstrating the safety of food additives on the producers and users of food additives, rather than the Government. Before enactment of this Amendment, pretesting and approval of food substances was not required. Substances with unknown or uncertain toxicity could be added to foods. The Government had the burden of proving in court that an added substance was poisonous or deleterious before regulatory action could be taken to remove the substance from food use.

Any food additive must now undergo strict testing to establish the safety of the intended use. Information must be presented to FDA in the form of a petition and includes the identity of the new additive, its chemical composition, its manufacturing methods, and the analytical methods to be used to detect and measure its presence in the food supply at the levels of expected use. Data must be provided to demonstrate that the proposed analytical method is sufficiently reliable and capable of determining compliance with the regulations.

Data also must be provided to demonstrate that the additive will accomplish the intended physical or technical effect in the food, and that the level sought for approval is no higher than that reasonably necessary to accomplish this effect. This is the so-called "functionality" requirement.

Finally, data must be provided to establish that the additive is safe for its intended use. This requires scientific evidence ordinarily derived from animal feeding studies using the proposed additive at various levels in the diets of two or more species of animals.

In reaching a decision on the safety of any substance to be added directly to food for man, a number of factors are considered:

1. Available toxicology data.
2. Estimated average daily intake of the substance by those whose diet normally includes the foods containing such substances.
3. Estimated average daily intake of the population consuming the maximum amounts.
4. Adequacy of the safety factor employed.
5. Relationship to other substances of closely similar chemical structure whose metabolism and physiological or pharmacological activity is known.

For a new proposed indirect additive -- one which migrates from the package to a food -- the safety decision is based on a combination of migration studies in food simulating solvents, in addition to toxicological and other safety data, and exposure information. In cases where there is virtually no migration of components to the food simulating solvent, short-term toxicity tests generally suffice. Where a compound possesses high biological activity and there is more migration, lifetime feeding studies may be required.

The criteria for establishing the safety of color additives are generally the same as those for food additives. In short, the safety of the color must be established by appropriate studies in test animals and in nonanimal laboratory testing (e.g., testing required to establish the chemical identity of the color) before it will be listed for use by FDA.

It is important to note that the criteria for evaluating an application for approval of a food or color additive, do not include the "benefits" from the use of the additive. The term functionality, which as I indicated previously is one of the requirements for approval of a food additive, requires FDA to determine whether the additive proposed for use performs its intended function. Thus, FDA must be satisfied that an emulsifier emulsifies and that a stabilizer stabilizes. But the Agency is not authorized to determine whether society needs another emulsifier or stabilizer. FDA does not, and I doubt whether the Agency should or even could, as a routine matter, make the kind of value judgments that would be necessary if only "beneficial" food additives could be approved. That is a judgment that already has been made by the Congress in a collective way when it enacted the Food Additives Amendment.

GRAS Review

Another key issue which concerned Congress and led to passage of the 1958 Amendment was the need to establish priorities in testing substances. Congress clearly was concerned that testing of new unknown substances be given priority over testing of ingredients that had been in use for

years. While it was important to test the older ingredients as well, these could be reviewed more gradually as resources became available. The GRAS review project was an inevitable outcome of the Amendment which gave formal recognition to the concept of generally recognized as safe.

One of FDA's first actions after passage of the 1958 Amendment was initially to determine which of the many food ingredients then in use were GRAS and which were food additives and thus subject to the premarket testing requirements of the new law. To make these determinations, during the 1958-1962 period, the Agency surveyed a number of experts and published advisory lists of GRAS substances, codified in 21 CFR 121.101. The composite list included substances which were sanctioned for food use by the FDA or USDA prior to 1958, and those substances that had a history of use in food prior to 1958 without any known or expected health hazard. It is clear, however, that this list was developed without a thorough scientific review, as we know it, of most of these ingredients.

Very few substances were added to the published FDA GRAS list between 1962 and 1970. The Agency did, however, issue individual advisory opinion letters during this period, with the effect that at least 100 additional unpublished substances were considered to be GRAS for specific food uses.

In his Consumer Message of October 30, 1969, President Nixon directed the Secretary of Health, Education, and Welfare to initiate a full

review of all GRAS ingredients. To implement this mandate, FDA developed an approach that has followed four sequential phases:

1. The Collection Phase - An industrial user survey to establish consumer exposure to each substance and a literature search of 50 years of scientific literature to collect all of the safety information relative to each substance.
2. The Collation Phase - The organization of the literature and consumption data into a "scientific literature review."
3. The Evaluation Phase - The development of a tentative evaluation of the "scientific literature review" to determine whether expert food safety scientists agree that the substance is generally recognized as safe for its intended use or whether some limitation is required in the interest of safety, the release of the evaluation for review and comment at a public hearing, and the development of a final evaluation based on the tentative findings and the public comments.
4. The Implementation Phase - The issuance of proposals and final regulations necessary to implement the evaluations.

The FDA contracted with the Food Protection Committee of the National Academy of Sciences (NAS) to survey the food industry and determine the aggregate national production of all GRAS ingredients, the amount of each such ingredient used in any particular food, and the anticipated consumption

of these ingredients by United States consumers. This survey was completed for all published and unpublished GRAS ingredients, and for FDA regulated flavor ingredients in 1972. The NAS also has continued to update and refine consumption information on GRAS ingredients on an as needed basis.

The search of the world scientific literature, from 1920 to the present time, and the summary of available safety data for each GRAS ingredient or group of ingredients were accomplished with contract support. Announcement of these contracts, and solicitation of unpublished safety data, have also been published in the Federal Register. To date, 118 scientific literature reviews, covering 356 GRAS substances, have been prepared for the published nonflavor GRAS list plus a few chemically related unpublished nonflavor GRAS ingredients. Twenty-eight literature reviews covering the remaining 83 unpublished nonflavor GRAS ingredients are now in preparation.

We also have had scientific literature reviews prepared for approximately 700 of the existing 1,650 natural flavors and spices and synthetically produced flavor ingredients, because our original intent was to evaluate all flavor ingredients used in food, rather than to narrowly evaluate only the 226 predominately natural spices and essential flavoring oils on the GRAS list. Our approach to this safety evaluation of flavors has also been to examine synthetically produced components of flavor formulations first, and then to evaluate natural spices and their essential oils.

An auxiliary source of information in the GRAS review has come from laboratory studies on the potential mutagenesis and teratogenesis of GRAS substances. The testing has been done predominantly under contract. The compounds selected for testing were also selected on a priority basis in accordance with their suspected effects and to provide as much information as possible for substances which cannot be tested with limited resources. To date, 81 representative nonflavor ingredients have been tested for teratogenic effects in at least two species of animals; 52 ingredients have been tested for mutagenic effects in three test systems and by the end of this fiscal year 235 nonflavor substances will have been tested for mutagenesis potential.

The evaluation phase of our GRAS contract program has been accomplished through a contract with the Federation of American Societies for Experimental Biology (FASEB). The Federation has established a select committee of food scientists to review the scientific literature, consumption information, and test data for each ingredient; and to issue advisory opinions to the FDA on the safety of the ingredients. The results of the committee's work for any substance under review can lead to any number of the following five recommendations:

- There are sufficient data to assure continued safe use of the ingredient under present or expected levels of use. This provides a basis to affirm the GRAS status of the ingredient when used in accordance with good manufacturing practices;

- There are sufficient data to assure continued safe use of the ingredient only at present levels of use. This provides a basis for affirming the substance as GRAS but with limitations on its use corresponding to present use levels;
- Questions about the safety of the ingredient have arisen in the review which should be resolved. In such a case we would promulgate a regulation, requiring that studies be undertaken for the ingredient if its use in food is to continue;
- The data indicate such substantial questions about the safety of the ingredient for use in food that prohibition of its use is appropriate;
- There are insufficient data to establish conditions of safe use for an ingredient. This is a basis for either prohibition of use for the ingredient or a regulation requiring additional testing as a condition for continued approval.

We have committed ourselves to an open process that permits public participation in the GRAS review. It is our view that all data and information leading to the development of each Federation recommendation and Agency decision should be made public. We have, therefore, announced in the Federal Register the public availability of all consumption, testing, and literature review material as it is completed on each group of

ingredients. We have also provided that the Federation's tentative evaluations for each ingredient are announced in the Federal Register for comment and argument before the Federation prepares a final recommendation for the Agency.

Our estimate is that we have reached the half-way point in the GRAS review of nonflavor ingredients. We have completed all data and information retrieval phases of the project and advisory opinions have been provided by FASEB for about 200 of the total 439 substances. Our remaining task is to implement these advisory opinions by regulations.

Thus far, the total cost of the GRAS review program has been approximately \$18 million. During this fiscal year, we received specific funds for the reevaluation of currently used food additives as part of a budget supplement to increase our efforts in the monitoring of the quality of bio-research data submitted to the Federal Government. The funds for food additives will be used, in part, to hire additional scientific personnel to review FASEB opinions and for personnel to prepare implementing regulations and the associated Federal Register documents.

ADDITIVE REVIEW

Soon after the GRAS review started, we began to develop a plan for the reevaluation of the safety of ingredients that have been subject to food additive regulation by FDA since 1958. By September of 1972 the basic concepts for a cyclic review project for all

ingredients added to foods had been developed to take account of changes in scientific standards. As mentioned earlier, the safety decisions for compounds approved more than ten years ago were based on less stringent toxicological standards than would apply to similar petitions submitted today.

FDA has already conducted reevaluations of certain food additives. Approved food additive petitions may be subjected to reevaluation for a number of reasons, for example:

- Questions of safety concerning an approved petition may arise as the result of review of a new petition for a similar substance or literature reviews by our scientists.
- Review of a new petition for an additional use of a compound may lead to reevaluation of the original petition, especially where submission of the two petitions is separated by a number of years.

In these and other ways, reevaluation of food additives by contemporary safety standards has already begun. However, such reevaluations are not comprehensive or systematic. They depend almost entirely on "chance" factors and as such do not provide either the Agency or the public with a reliable basis for assuming the safety of food additives under current knowledge.

The primary goal of the additive review is to develop a current toxicological profile for each food and color additive, which is integrated with fairly precise estimates of the amount of each of these additives a consumer is exposed to in his daily diet. This safety profile of toxicity and exposure information will be updated periodically, thus providing practical assurance that permitted food additives are not responsible for the induction of acute or chronic disease effects.

We will, as a part of the periodic evaluation, ask for additional safety information to be developed so that as new lines of investigation are explored, the limited risks that these uses present will be further reduced. This information will give us a clearer picture than we have now about the potential of each food ingredient to produce cancer, adverse effects on reproduction, effects on the fetus, or heritable genetic damage, or other types of chronic disease. The second major thrust is a program to insure the integrity and quality of the biological information submitted to the Agency in support of regulated food and color additives.

Elements of the Additive Review Project

The project consists of four elements:

1. Review of substances added directly to foods. This element includes the reevaluation of the safety of all flavors, colors, and regulated direct food additives. These total some 2,100 compounds.

2. Review of substances which are added indirectly, i.e., migrate, to food. This element will embrace the reevaluation of the safety of the regulated, GRAS, and prior sanctioned packaging materials. There are about 10,000 indirect additives.
3. Completion of the GRAS review. Included will be the existing nonflavor items included on GRAS lists, as well as the prior sanctioned items. Also included is the ongoing program of evaluation of GRAS affirmation petitions.
4. The Bio-Research Monitoring Program. This project is part of the Agency's comprehensive program to assure the integrity of the data submitted to the Government in support of safety decisions. This program is being implemented through the publication of Good Laboratory Practice Regulations and monitoring of the approximately 130 nonclinical laboratories through an inspection program.

It is not practical in this statement to outline in great detail all the elements of this many faceted program. I will, however, state our major objectives, and outline the more important procedural steps which place the program in a time perspective.

The objectives for each of the first three action elements for classes of additives are similar. They are:

- determine how much of each additive the consumer is ingesting;
- assemble the toxicological and other safety data available for each additive to evaluate whether (or not) the data meet today's criteria;
- develop acceptable protocols for the variety of toxicological and other safety tests we use to decide the safety of additives; and
- develop a set of evaluation criteria to describe as precisely as we can the minimum amount of safety information that we will accept to allow the use of an ingredient in food regardless of how low the exposure may be.

The fourth element, bio-research monitoring, is different in character from the evaluation per se of food additives. It does, however, have an important influence on our ability to make sound judgments about the safety of additives. For some time, FDA has been concerned about the absence of industry-wide standards for the conduct of nonclinical laboratory studies. Intensive investigations of certain laboratories have revealed significant quality control problems in some of them. FDA's reliance on the basic accuracy and integrity of the data submitted as a result of studies performed in these laboratories is essential to the review and approval of food and color additives. The submission of faulty, erroneous, or distorted data increases the potential for reaching invalid judgments about the safety of these additives.

Mr. Chairman, when this program is fully operational, it will assure that studies in support of petitions (for food additives, new drugs, etc.) submitted to the Federal Government, are conducted in an appropriate scientific manner, and that the data from such studies are accurate, complete and reliable. Other approaches, such as the Government's being responsible for the conduct of safety studies (commonly known as "third party testing") have been proposed to address these concerns. However, we believe that our bio-research monitoring is, to date, the most effective and efficient approach to solving the problem. We believe that the cost of "third party testing" would not result in any substantial benefits. It would merely add another Federal superstructure for designating laboratories, collecting fees and hearing disputes. Because FDA is responsible in either case for the final evaluation of the data and making the decision on whether the product is approvable, we believe our current program is more appropriate.

To implement the bio-research monitoring program, the Agency has published proposed Good Laboratory Practice for Nonclinical Laboratory Studies regulations. We have initiated a pilot inspection program designed to determine how widespread are the problems uncovered in the initial investigations. We will develop baseline data to measure conformance of the laboratories to the proposed regulations and to determine what improvements are needed. The pilot effort will also

enable us to obtain the necessary knowledge to improve the quality of the proposed regulations and the methods of inspection. It will be a learning process for both the Agency and the industry.

We believe there are about 130 nonclinical laboratories doing toxicity testing on food and color additives. When the bio-research monitoring program becomes fully operational, we will be routinely inspecting each laboratory at least once every two years. Where problems are uncovered or suspected, we will, of course, make more frequent inspections. Our goal is to improve the performance of nonclinical laboratories, where necessary, and to maintain compliance at a high level.

Returning to the overall food additive review project, there are three major near-term tasks:

1. An industry additive use survey for all flavors, colors, and direct regulated food additives. This survey will provide the information needed to calculate the exposure pattern of any additives in a particular food. A prototype of this survey was conducted in 1970 for those food ingredients which were considered GRAS. This more comprehensive survey that we are discussing this morning, a survey of all direct addition food additives and color additives, is expected to begin in April.

2. We are currently establishing internal working groups to develop the standards by which we will evaluate the safety of any ingredient added to food. These standards will include definitions of test protocols to be followed when evaluating the safety of food and color additives.
3. In March a team of Agency scientists will begin development of a priority list for the approximately 2,100 direct food, color and flavor additives. The reevaluation process will begin with collection of available information for each individual ingredient. This information will be evaluated using the safety standards described above. Concurrently, a review of the scientific literature of colors, flavors and direct food additives will be made by the Bureau of Foods. This review will help to establish a toxicology profile for each ingredient, and thereby help establish a priority order for evaluation of the approximately 2,100 ingredients.

Completion of these activities should enable us within 18 months to make preliminary judgments regarding these 2,100 substances and will lead to the establishment of a priority list.

We recently received, on contract, a set of criteria for evaluating the safety of flavors which we will soon publish for public comment. These criteria were developed by the Federation of American Societies for Experimental Biology's "Special Committee on Flavor Evaluation Criteria" (SCOFEC). Included in these recommendations are the use of short-term in vitro screening test.

Although the status of screening tests is being debated, we will define an appropriate role for the Ames-type tests to screen compounds to predict their potential carcinogenicity.

Before any in-depth evaluation takes place, we will classify the substances under review into three categories:

Category 1 - Those ingredients which cannot be adequately searched in the literature because they have problems in nomenclature.

Action--FDA will require industry to furnish information.

Category 2 - Those ingredients which no toxicological or other biological safety information can be discovered.

Action--FDA will request data through Federal Register announcement. If information is not available, we will require industry to carry out studies to allow for continued use.

Category 3 - Those ingredients, which have an adequate preliminary safety profile.

Action--FDA will do an "in depth" review of the information and reach a current safety conclusion, recognizing that further scientific advances or reports of experiences may subsequently require change.

Ingredients for which there is an adequate preliminary safety profile will be designated for immediate evaluation.

Conclusion

Mr. Chairman, the process of evaluation, approval, and reevaluation of food additives that I have described is one of the most important responsibilities of the FDA. It is also the most visible activity of the Agency, which confirms the importance of the hearings you are conducting this week.

Our two major on-going projects for reviewing the safety of food ingredients, like other programs in our Bureau of Foods, are designed to permit FDA to provide continued assurance that consumers are not exposed to unsafe foods. These projects are a reflection of the fact that the scientific standards for evaluating the safety of chemicals have changed dramatically in the nearly two decades since the passage of the Food Additives Amendment in 1958.

We have always sought to apply the best contemporary standards for assessing the safety of substances added to food, and in the process we have often found ourselves applying new rules to old ingredients. The distinctive feature of the GRAS review and the additive review is that they represent an effort to apply new standards, not simply as questions are raised about familiar compounds, but to all ingredients on a rationale, systematic basis. This is a costly, exacting, but indispensable process.

The application of contemporary standards to products approved 15 years ago will cause us to revise some old decisions, and undoubtedly produce some public anxiety. I believe it is important, Mr. Chairman, for

Congress and the public to come to understand that new questions always can -- and indeed should -- be raised about ingredients previously assumed to be safe. Continuing reevaluation, in the light of new evidence and new standards, must become a routine part of FDA's work, and an accepted characteristic of the regulatory process, rather than a cause for accusation and alarm. The time for alarm would come if the safety of approved products were never questioned. That would signify that we were not doing our job and that scientists had ceased to be inquisitive.

In short, the raising of questions about the safety of previously approved products is a sign of a system that is working, not a signal that it has failed.

Thank you, Mr. Chairman. My colleagues and I will be happy to answer any questions you and other members of the committee may have.

APPENDIX A
PROVISIONAL COLOR LISTING

The Color Additive Amendments of 1960 to the Federal Food, Drug, and Cosmetic Act require that there be a separate regulation prescribing safe conditions of use for every color additive that is used in food, drugs, and cosmetics. Such regulations may be issued in response to a petition from an interested person or on the initiative of the Commissioner. Each regulation must be supported by data sufficient to establish its safety after considering, among other relevant factors: (1) probable consumption of the color; (2) cumulative effects of the color, if any; (3) safety factors; and (4) the availability of any needed practicable analytical methods.

The Color Additive Amendments also provided for the provisional listing of those color additives that were certified or in use as color additives prior to the effective date of the Amendments (July 12, 1960). Provisional listing of those colors already in use was intended to provide time for the orderly safety testing of color additives under the new law.

The provisional list was intended to expire initially two and one-half years after July 12, 1960. The Amendments, however, provided for the extension of the closing date for the provisional list to permit the completion of scientific investigations necessary to make final determinations on the listing of the colors.

The closing date for the provisional list of color additives has been extended from time to time since passage of the Amendments. It was extended from September 30, 1976 to December 31, 1976, on September 23, 1976.

Most recently on January 7, 1977, the closing date was extended to January 31, 1977.

On November 14, 1975, the Commissioner of FDA published a notice in the Federal Register of his intention to extend the closing date for use of provisionally listed colors to September 30, 1976 and to make final determination for many of the color additives by that date. The step was taken in an effort to resolve finally the status of the provisionally listed colors.

On September 23, 1976, FDA issued a notice of proposed rulemaking to extend the closing date for certain of the provisionally listed colors for specified time periods, and to terminate the provisional listing for certain uses of other colors including Red No. 4 and Carbon Black. (See Appendix C for status of Red No. 4.) The closing dates of the extensions were determined on a color-by-color basis and vary depending upon the type of data considered necessary to complete the data packages to support issuance of listing regulations for color additives within the parameters prescribed by the Amendments. Although none of the extensive available data for the color additives casts any doubts on their safety, these additional data must be submitted before a final conclusion can be made on their safety to the extent required by the Amendments.

With regard to the color additives that will remain on the provisional list, FDA proposed to require those who have petitioned for approval of these colors to:

- Agree in writing within 30 days of the effective date of the regulation to undertake the necessary tests.
- File research progress reports with FDA, and submit the final results of all tests within specific time periods.
- Notify FDA immediately of any findings that indicate a potential for any color additive to cause adverse effects.

Failure to abide by these requirements would result in the immediate removal of the color additive from the provisional list.

The comments to the proposal of September 23, 1976 are being considered and final regulations will be published in the Federal Register later this month.

As data become available that permit conclusions as to the safety of the colors within the parameters prescribed by the Amendments, listing regulations will be issued. Submission of the various types of data identified in the proposal of September 23, 1976 will complete the data-packages necessary for the individual colors and permit such conclusions.

If any scientific data become available that cast doubts upon the safety of a provisionally listed color additive, such provisional listing will be terminated immediately.

It is anticipated that no color will be provisionally listed after December 31, 1980. Continuation of the provisional list beyond that

date will not be considered unless there exist some unforeseeable and unavoidable occurrences or situations that make submission of the required data extremely impracticable if not virtually impossible.

The FDA will include color additives in the planned cyclic review of additives to assure continued confidence in their safety.

APPENDIX B
FD&C RED NO. 2

On February 12, 1976, the Food and Drug Administration (FDA) terminated provisional listing for FD&C Red No. 2 and, therefore, approval for the use of this color additive in foods, drugs, and cosmetics.

Red No. 2 has a long history of use in this country being among the first substances approved for use in food under the Food and Drugs Act of 1906. All of the available chemistry and safety data on the color had been reviewed before the issuance of Food Inspection Decision No. 76 on July 13, 1907. With passage of the Federal Food, Drug, and Cosmetic Act of 1938, the color was considered to be harmless for use in food, drugs, and cosmetics and listed among the color additives that would be batch certified by the Food and Drug Administration (FDA).

In the 1950's the safety of color additives in general was questioned following the occurrence of adverse effects in children who had consumed candy and popcorn containing excessive levels of certain color additives, although not Red No. 2. The various certified color additives, including Red No. 2, were subjected to extensive testing by the FDA, including chronic animal feeding studies. Red No. 2 was considered to be harmless, on the basis of the results of such studies, while several of the certified colors were found to produce adverse effects and were, therefore, delisted.

In 1960 under the Color Additive Amendments, all color additives then in use, including Red No. 2, were placed on provisional listing pending the completion and evaluation of scientific studies sufficient to demonstrate safety under the intended conditions of use. In the case of Red No. 2, these studies involved investigation of the potential dermal effects of the color additive when used in drugs or cosmetics. An evaluation of the data by FDA's Bureau of Foods on March 5, 1969, concluded that the color additive was safe for use in food, drugs and cosmetics. Permanent listing of the color additive at that time was precluded, and its provisional listing was extended as it had been over previous years.

In early 1971, reports were received indicating that FD&C Red No. 2 might produce adverse effects. These were reports of studies done with a color described only as amaranth by Russian investigators during the late 1960's and 1970. One such report indicated that the amaranth tested had produced carcinogenic (cancer causing) effects. This report has generally been discounted because of a lack of knowledge concerning the identity of the amaranth and the testing protocol used, along with the reported distribution of tumors in the test animals and the unusual absence of tumors in the control animals. Other Russian reports indicated amaranth had effects upon rat reproduction, but they were also questionable in terms of their

relevance to Red No. 2. The Russian studies did serve to emphasize a need for data concerning assessment of the potential for effects of certified synthetic organic color additives on the reproductive system. The Russian reports also raised public concern about the safety of Red No. 2.

In September 1971, FDA required industrial sponsors to provide data from reproduction studies for all provisionally listed colors subject to ingestion. In addition, because of the immediate question concerning safety of Red No. 2 and because of a request of the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives, FDA initiated a long-term chronic animal feeding study with rats that had been exposed to Red No. 2 from the time of conception (in utero). Industry also included Red No. 2 into their program of evaluating reproductive physiology of certified color additives.

The question concerning reproductive effects has been the subject of several studies to assess the potential for teratology, and other adverse effects on the reproductive system. Furthermore, the data from these studies have been evaluated at various times by three advisory committees in addition to FDA scientists. The general conclusion has been that Red No. 2 has had no adverse effects upon the reproductive system of test animals.

The chronic study that was initiated by FDA utilized rats from one of the early reproduction studies. Several problems occurred

with this study, however, that raised questions concerning its validity and usefulness. During the course of the study, there was a mixup among some of the animals at the various dosage levels. After a review of the matter, it was concluded that the study, if completed, could possibly give a determination of whether the color was carcinogenic and the study was permitted to go to completion.

The FDA study data concerning the chronic effects of Red No. 2 along with the other available data were presented to our Toxicology Advisory Committee for consideration of its potential carcinogenicity. At the committee's request, each of the available tissue slides of the test animals were reexamined and the incidence of malignant tumors statistically analyzed by a working group comprised of members of the committee, NCI and FDA. This group concluded that the recent FDA study could not be used to demonstrate safety and, in fact, raised questions concerning the safety of the color. The Toxicology Advisory Committee, during a meeting on March 8-9, 1976, affirmed the findings of the working group that the FDA study could not be used to demonstrate the safety of Red No. 2. The problems in the study led the majority of committee members to conclude that carcinogenicity had not been demonstrated either.

As noted above, FD&C Red No. 2 was provisionally listed following passage of the Color Additive Amendments. A color additive may be provisionally listed only as long as studies are being conducted

or evaluated to demonstrate its safety, or there are no indications of adverse effects upon the public health. Both of these factors were considered carefully before action was taken to terminate the provisional listing of FD&C Red No. 2. The questions of safety that were not answered by the FDA study, particularly the potential for carcinogenesis, can only be answered by completion of an adequate long-term animal feeding study with rats. We were unaware of any such studies currently underway or even planned. In view of the requirements for provisional listing, we could see no alternative but to remove the color additive from provisional listing and to deny the petition to list the color "permanently." A recall of products on the market was not considered necessary because of the lack of definitive evidence showing an adverse effect. FDA's action in denying the petition to list the color "permanently" is currently the subject of a formal evidentiary hearing provided for under section 706 of the Act.

APPENDIX C

FD&C RED NO. 4

On September 3, 1976, the Food and Drug Administration (FDA) terminated the provisional approval for Red No. 4. Red No. 4 may no longer be used to color any food or ingested drug. This color had been in use in food since 1929.

FDA decision on Red No. 4 was part of a more comprehensive Agency review of some 84 colors which were provisionally listed (as opposed to permanently listed) at that time. The origin of the provisional list dates back to 1960 action by Congress (see Appendix A).

A study completed by Hazleton Laboratories, Inc. in 1968, using beagles as the test animal, raised the question of safety about Red No. 4 for a second time. (Safety questions first were raised by results of an FDA study in 1964.) Evaluation of the Hazleton study indicated that lesions were present in the bladders of some of the test animals. Another effect observed in the study was atrophy of a portion of the adrenal glands. There is no dispute as to the occurrence of bladder lesions; there is a dispute as to the cause of the bladder lesions.

It was for this reason that we requested petitioners, as early as November 1969, that a third study be conducted to resolve the issue conclusively. However, these studies were never performed by industry.

The dispute over the bladder lesions has diverted attention from another effect which was observed in both studies--that is, atrophy of a portion of the adrenal glands. This is not a trivial effect; it has an important bearing on the safety of Red No. 4. There is no dispute as to the finding of adrenal atrophy. There is, however, some question as to whether the effect occurred at the lowest of the administered doses. In any event, the safety data now available to FDA do not establish an unequivocal no-effect level for the use of Red No. 4. To summarize briefly, Red No. 4 is a color for which:

--Safety questions have been raised by studies showing toxic effects attributed to ingestion of the color; the data available are not sufficient to enable the Commissioner to establish confidently a safe level of use.

--Additional studies are needed to resolve the questions, but these have not yet been undertaken and will require two to three years for completion and evaluation.

--Uncertainties about the safety of this color and the need for additional studies have been known for at least two years, but the petitioners have not taken action to initiate those studies.

Given these facts, we concluded that it would not be appropriate to continue the provisional listing status of Red No. 4 for use in foods or ingested drugs. We did, however, approve the permanent listing of Red No. 4 for use in externally applied drugs and cosmetics effective October 27, 1976.

We should emphasize, however, that all colors will continue to be the subject of attention and additional scientific evaluation as we update our knowledge about their safety. Indeed, the FDA has undertaken a massive review of the safety of all food and color additives to take account of newly acquired scientific knowledge and changes in safety standards that have occurred since these additives have been approved.

APPENDIX D

FD&C RED NO. 40

The color additive FD&C Red No. 40 is listed "permanently" for use in food, drugs, and cosmetics under sections 8.244, 8.4104, and 8.7201 of the color additive regulations, having been approved for use in regulations published in the Federal Register of April 10, 1971, and August 6, 1974.

After it was listed in 1971, FD&C Red No. 40 rapidly became a widely used color additive. In fiscal year 1972, for example, the first full year of its listing, FDA certified 892,282 pounds of the color. The amount of FD&C Red No. 40 certified remained reasonably steady until fiscal year 1976, when its use increased substantially; in that year, FDA certified 1,500,760 pounds of FD&C Red No. 40. This increase is no doubt attributable to the termination of the provisional listing of FD&C Red No. 2 published in the Federal Register of February 10, 1976. When its provisional listing was terminated, FD&C Red No. 2 was the second most widely used color additive. Before this termination, FD&C Red No. 40 was the fourth most widely used color additive. Today it ranks second in usage after FD&C Yellow No. 5.

In late 1974 and early 1975, the Allied Chemical Corporation, the patent holder and petitioner for use of FD&C Red No. 40, began two chronic feeding studies, one in the rat and one in

the mouse, on FD&C Red No. 40. Both studies are being conducted by Hazleton Laboratories, Inc., Falls Church, Virginia. Allied undertook the studies because neither the British nor Canadian governments would approve use of the color in their countries unless additional chronic studies were conducted. Moreover, FDA had advised Allied that it preferred new chronic studies rather than additional metabolism or reproduction studies if Allied were going to supplement the data already available on FD&C Red No. 40.

On February 25, 1976, representatives of Allied, Hazleton, and CFR Services, Inc. (a consultant to Allied) met with representatives of the Bureau of Foods of FDA to discuss the results of the studies to that point. Allied reported that the results of the study in the rat were unremarkable; the results of that study have remained unremarkable to this day. However, Allied did report the occurrence of lymphomas in some mice. The lymphomas were appearing earlier than expected, based on historical experience with the strain of mouse used, and they appeared to be dose related. It was suggested to Allied by FDA that an interim sacrifice of some animals in the mouse study might aid in determining whether there were any undetected lymphomas that could confirm or negate the statistical significance of the lymphomas already identified. At approximately the 52-week point in the study, animals were sacrificed in each group, reducing the original 50 animals per group to 30 animals per group. No lymphomas were detected in any of the sacrificed animals.

Allied began, at FDA's suggestion, a second chronic feeding study in the mouse to determine whether the early onset of lymphomas . . . apparently detected in the first mouse study would be duplicated. This second lifetime feeding study, which began in midsummer of this year, is being conducted using larger numbers of animals per group to provide more definitive information on this phenomena. The Food and Drug Administration has received three interim reports on this study. Although the results thus far are too preliminary for conclusions to be drawn, they do suggest a possible duplication of the early onset of lymphomas noted in the first study.

After it was advised of the preliminary results of the first mouse study, FDA insisted that monthly progress reports on the study be submitted to it. Thus, FDA has been closely monitoring the study since April 1976.

Although the studies are incomplete, the Acting Commissioner of Food and Drugs has ordered a review of the results of the chronic studies being conducted on FD&C Red No. 40 by a group of experts from FDA, NCTR, and NCI to aid FDA in determining whether regulatory action on FD&C Red No. 40 is appropriate, and to help develop a complete scientific and legal basis on which to base any necessary action. Consideration of the results of these studies by the working group will ensure that any decisions subsequently reached

by FDA regarding the safety and use of FD&C Red No. 40 are legally sound and scientifically supportable. The working group, is chaired by Dr. Albert C. Kolbye, Jr., Associate Director for Science in the Bureau of Foods, FDA.

It is not possible to establish a firm date on which the working group will file a final report with the Acting Commissioner and the Director of the Bureau of Foods, because the completion dates for the two mouse studies cannot now be precisely ascertained. The working group will report to the Acting Commissioner and the Director of the Bureau of Foods promptly, following completion of its review of the results currently available. A report is expected by the end of this month.

The working group held its first meeting on December 16 and 17, 1976. Although the working group is an internal Government body, and not an advisory committee within the meaning of the Federal Advisory Committee Act (5 U.S.C. Appendix I), the Acting Commissioner concluded that it would be beneficial to provide for public input into the working group's review. Accordingly, the morning session on December 16, 1976 was open to the public for the presentation of data and views.

Any further FDA decisions on Red No. 40 will be guided by the evaluations of the interagency work group and by the recommendations of the Bureau of Foods.

APPENDIX E

FD&C YELLOW NO. 5

FD&C Yellow No. 5 has been permitted for use in food in this country since 1916. It was among those colors listed for use in food, drugs, and cosmetics with passage of the Federal Food, Drug, and Cosmetic Act in 1938. It was among the provisionally listed color additives following passage of the Color Additive Amendments of 1960.

A color additive petition proposing the permanent listing of FD&C Yellow No. 5 for use in food, drugs, and cosmetics was submitted to FDA on February 25, 1965. The data in this petition were considered adequate to support listing of the color for certain uses and on July 7, 1969 a regulation was issued listing the color for use in food and ingested drugs. The data included the results of chronic toxicity tests conducted by the FDA with the color in mice, rats, and dogs. The color was not listed for use in externally applied drugs and in cosmetics because of questions concerning the adequacy of the data submitted to support these listings.

The provisional listing of FD&C Yellow No. 5 for use in externally applied drugs and in cosmetics has been extended until January 31, 1977. The proposal of September 23, 1976, described in Appendix A, would extend the closing date for provisional listing of FD&C Yellow No. 5 to December 31, 1980 pending submission of the results of new chronic feeding studies.

There have been a number of published reports of allergic-type reactions in certain susceptible individuals that establish a relation between their occurrence and the ingestion of FD&C Yellow No. 5. Following evaluation of these reports, FDA now is considering what would be the most appropriate action to assume consumer protection. We anticipate that we will be publishing a Federal Register notice on this very shortly.

APPENDIX F

SACCHARIN

In the June 29, 1971 Federal Register, FDA published a proposed regulation restricting the uses of saccharin and establishing limitations on daily intake. This action was based on a report by the National Academy of Sciences/National Research Council (NAS/NRC) which concluded that a daily intake of 1 gram per day for an adult weighing about 155 pounds would not constitute an appreciable hazard.

On February 1, 1972, FDA published in the Federal Register, a final order approving saccharin for limited use in foods. In that order FDA stated this action was only an interim measure pending the completion of studies underway at that time. The FDA also stated that if the results of those tests indicate that continued use of saccharin involved a significant risk to the public health, further action would be taken to minimize such risk.

In June 1972, FDA asked NAS/NRC to review the results of all experiments, either completed or ongoing, on the possible carcinogenicity of saccharin. Because no specific date could be established for the completion of the ongoing chronic feeding tests, FDA extended the effective date of the interim food additive regulation beyond June 30, 1973, until such time as the final report was received from NAS/NRC.

The NAS/NRC report was received in December 1974 and while FDA was reviewing it, several studies of saccharin were undertaken by the Canadian government. FDA was advised by Canadian officials that their final report is expected to be completed in early 1978.

In August of 1976, the GAO released a report on saccharin prepared at the request of Senator Nelson. The GAO recommended that FDA promptly reassess the justification for the continued use of saccharin under the interim regulation and the need for issuing a permanent regulation or possibly discontinuing the use of saccharin in food. GAO also urged that consideration be given to reducing the tolerance for OTS (ortho-toluenesulfonamide), an impurity in saccharin, if FDA determined that use of saccharin should continue under the interim regulation.

In the Commissioner's view, allowing continued limited use of saccharin under the interim regulation is appropriate because such use would not significantly increase the risk to the public health. Therefore, on January 7, 1977, FDA published in the Federal Register an order extending the effective date of the interim food additive regulation for saccharin until ongoing studies are completed and evaluated.

Concurrently, FDA published a proposal to amend the interim regulation for saccharin by establishing a tolerance for OTS, a major impurity in commercial saccharin. The tolerance proposed, 25 ppm, is the lowest level achievable under current manufacturing practices.

APPENDIX G

ASPARTAME AUTHENTICATION REVIEW

BACKGROUND

In response to a petition from G. D. Searle Co., FDA published a notice in the Federal Register of July 26, 1974 which concluded that the data in the Searle aspartame petition and other relevant material justified amending the food additive regulations to provide for the safe use of aspartame. Some 150 studies in all had been conducted and were submitted in support of the petition.

Subsequent to publication of the final regulation, the FDA received three objections and two requests for a hearing. The requests for a hearing were based on grounds questioning the calculations for children of both aspartame and glutamate and the correctness of assuming that the two compounds will not act synergistically to produce adverse neurological results, and on evidence on aspartame-induced brain damage in mice. The objectors later raised the issue of the finding of uterine polyps in rats in a long-term feeding study of diketopiperazine (a breakdown derivative of aspartame). A Public Board of Inquiry was to be scheduled to evaluate the issues raised by the objectors.

On April 29, 1975, Searle informed the FDA that they had arranged for an independent panel of experts to review the uterine tissues and that until the FDA had had an opportunity to evaluate the report of the panel, Searle would withhold products containing aspartame from the market. In addition, all of their customers who had purchased marketable quantities of aspartame had agreed to delay market plans.

In July 1975, the Commissioner established the Searle Investigation Task Force as a result of "recent investigations by the Agency (which) have raised questions about Searle Laboratories' conduct of animal experiments and the reporting of data to the FDA." In addition to the investigations with regard to two drug products, the Task Force investigated certain aspartame studies.

By notice in the Federal Register of December 5, 1975, the Commissioner stayed (effective immediately), the aspartame regulation because the "preliminary results of an audit of the records of certain animal studies conducted by or for the petitioner, including studies on aspartame, indicate the need for a comprehensive review of certain of the research data held by or for the petition." The Public Board of Inquiry was postponed by this same Federal Register notice until questions raised by the preliminary results of the audit were resolved.

The final report of the Searle Investigation Task Force concluded that "serious deficiencies" in Searle's operations and practices undermined "the basis for reliance on Searle's integrity in conducting high-quality animal research to accurately determine or characterize the toxic potential of its products." The investigation of two studies done by Searle's contractor, Hazleton Laboratories, demonstrated some of the same problems found at Searle.

THE NATURE OF AND FUNDING FOR THE AUTHENTICATION REVIEW

On July 23, 1976, the Commissioner conveyed to Searle his decision that certain aspartame studies must be authenticated utilizing a "review mechanism which, operating independently of but funded by Searle or other private sources" would promptly undertake the authentication. This decision was reached after considerable discussion within the Agency and careful review of several alternatives. One of the major issues was the propriety of using public funds for the purpose of authenticating data supporting the food additive petition. Careful estimates then, and detailed discussions since, led to the conclusion that the authentication review could be undertaken by the Agency itself only at substantial dollar costs and drains on limited manpower resources and that this should not be done to clear a particular product on behalf of a specific private commercial organization.

Based on the Agency's experience in using third-party organizations in advisory roles, (for instance, the use of the Federation of American Societies for Experimental Biology (FASEB) for the GRAS review,) the Agency felt that an objective, competent, third-party could be found that would possess the scientific capabilities to perform the authentication review. We also believed that a mechanism with such a third-party could be established so that FDA's continuing law enforcement responsibilities could be fully discharged.

The purpose of the authentication effort would simply be to determine whether the data from certain "control" studies submitted by Searle to the FDA in support of the food additive petition for aspartame are accurate, complete and reliable. As the Commissioner's letter to Searle pointed out, the authenticators would not "be invited or authorized to formulate judgments with respect to the question of whether aspartame is safe under the prescribed conditions of use." Instead, the FDA would discharge its responsibilities to undertake the evaluations necessary to the ultimate determination regarding the safety of aspartame and would indeed make that determination. Those scientific matters raised by the objectors would be submitted for resolution to the Board of Inquiry if the results of the authentication review confirmed the need for such proceedings.

The Commissioner's letter to Searle also amplified that the mechanism for any authentication review of the aspartame data must be a matter of public record because the ultimate conclusion with regard to the additive's safety would be sharply scrutinized and must, therefore, "be the product of sound, open, and thus credible procedures."

SELECTION OF AUTHENTICATORS

Searle initially proposed that individual experts would be convened in various fields to undertake the authentication effort, but the Agency believed such an approach to be unsatisfactory for several reasons. Instead, the FDA preferred that an objective, scientifically competent organization be found which was free of any possible

conflict of interest regarding Searle and that such an organization assume the responsibility for the authentication effort. Searle was told that it was inappropriate for them to make any contacts with organizations or individuals regarding the possibility of those organizations or individuals playing a part in the authentication effort, and further that FDA assumed the responsibility for reaching agreement with an organization that would ultimately do the authentication review. The assumption of that responsibility included the drafting of a contract acceptable to the FDA and the authenticating organization which was then presented to Searle for its review and approval.

The Universities Associated for Research and Education in Pathology (UAREP), an affiliated group within FASEB, was the group selected to authenticate the aspartame tests. After assuring that UAREP met the criteria with regard to scientific competence and that no conflict of interest existed in connection with Searle, FDA representatives discussed the possible contract with members of the UAREP Board of Trustees. That discussion focused on the fact that the purpose of the contract is to determine whether the data from certain studies submitted by Searle to the FDA in support of the food additive petition for aspartame are accurate, complete and reliable, and that UAREP was not being invited to make recommendations with respect to whether aspartame has been shown to be safe for human consumption under any conditions of use. The evaluations of the various studies and ultimate determination regarding the safety of

aspartame is clearly the FDA's responsibility, which the Agency will assume subsequent to receipt of UAREP's final written report.

Based on this and other understandings, the UAREP Board voted to undertake the authentication review and representatives of the FDA and UAREP have worked to produce a draft contract. The draft contract, approved by both the FDA and UAREP, has been forwarded to Searle for its review and approval.

APPENDIX H
HYPERKINESIS

Behavioral disorders related to the hyperkinetic syndrome are found in children of all socioeconomic groups and in most countries throughout the world. A conservative estimate would be that moderate and severe disorders are found in about 3 out of 100 elementary school children. More males than females appear to be affected. The major symptoms of the disorder are an increase of purposeless physical activity and a significantly impaired span of focused attention. The inability to control physical motion may generate other behavioral consequences. It has been suggested that there are several etiological subgroups within the syndrome.

In 1975, Dr. Ben F. Feingold stated in his book, Why Your Child is Hyperactive, that artificial colors and flavoring agents produce hyperactive behavioral symptoms in genetically predisposed children. In addition, he alleges total withdrawal of the artificial substances via the Feingold Kaiser-Permenente (K-P) diet can be of therapeutic value in the treatment of between 25 and 50 percent of the children with hyperkinesis. It should be noted that these reports are anecdotal.

The possible relationship between food additives and the hyperkinetic syndrome in children is an important health issue that is currently being studied by various agencies within the Department of Health, Education, and Welfare and by outside groups.

One recent study by an outside group was a study of the Feingold hypothesis by the Food Research Institute of the University of Wisconsin. This was a carefully designed study which represented the first systematic attempt to test the hypothesis in well-controlled settings. The preliminary findings of the researchers show no significant overall effect from the Feingold diet either as measured by classroom behavior or by parents.

The Interagency Collaborative Group on Hyperkinesis (ICGH), composed of scientists from FDA, NIH, NIMH, NIE, was established in the summer of 1975 to assess all the available data on the possible association between hyperkinesis and diet and to make recommendations for any additional research that appeared to be indicated. FDA scientists provided the leadership in organizing the ICGH and in the preparation of the First Report of the Preliminary Findings and Recommendation. This report was accepted by Dr. Theodore Cooper, Assistant Secretary for Health, in February 1976.

On February 23, 1976, the members of the ICGH prepared and approved three specific research proposals to carry out the recommendation of the ICGH report. These proposals are:

1. A Dietary Challenge Study of Artificial Food Colors and Flavors in Children (1-5 years old) with Behavioral Disturbances (to be carried out at the Kaiser-Permenente Center in California).

2. A ~~Multiple~~ Challenge Study of Artificial Food Colors and Flavors in School-Age Hyperkinetic Children (to be carried out at the University of Wisconsin).
3. Support to Obtain Data, Results and Interpretation of a Study of Food Additives and Hyperactivity in Children (the observational phase of this study was completed at the University of Wisconsin in December 1975).

The Bureau of Foods has provided \$37,506 for the funding of item 3 and the National Institutes of Health have provided \$106,800 for the funding of a challenge study in children ages 1-5 years (item 1).

APPENDIX I

STATE OF THE ART OF MASS SCREENING FOR MUTAGENICITY

INTRODUCTION

Mutagenicity unlike other fields of toxicology employs a myriad of indicator systems e.g., bacteria, plants, animal cells, insects, whole animals, each having specific indexes, parameters and philosophies regarding what demonstrates a significant or nonsignificant response. This poses problems for many investigators who wish to perform mutagenicity studies, and who must select among the various methods. Selecting the proper test method is especially difficult for those investigators possessing limited experience in the field. This situation is made worse by the fact that research in this area is continuing at a rapid pace and the test methods employed today may not be applicable tomorrow.

It should be recognized from the outset, however, that for a definitive evaluation of safety in regard to mutagenicity, animal testing is needed.

TYPES OF TEST SYSTEMS1. Bacterial

The strains of Salmonella typhimurium developed by Dr. Bruce Ames are among the most useful bacterial systems employed for the evaluation of mutagenicity substances. In addition to the basic strains detecting certain types of mutagenic events, other strains have been developed which can detect other mutagenic events. These fabricated strains

allow a greater penetration of substances into the organism and augment the expression of the mutagenic event.

Eschericia coli has also been employed for assessing the mutagenicity of chemicals. The various strains however have not undergone the extensive development of Salmonella, and have therefore been used in a limited capacity in the United States. It appears, however, that this strain of bacteria is equal to Salmonella in detecting chemical mutagens.

The work of Ames has contributed significantly to knowledge of the mutation process. Nevertheless, his efforts to develop strains having an exceedingly high susceptibility to mutagenesis has created a concern among some scientists that it will give positive results for many chemicals that are not truly hazardous to humans. This situation should be kept in mind when one employs these strains, for the possibility of

discovering so called "false positives" may be high. On the other hand, some known carcinogens have not been found to be mutagenic in these test systems. This problem, of false positives and false negatives, is of concern to FDA and other Government agencies. It needs to be resolved before the role of these tests in the regulatory process can be defined.

In comparing Salmonella and E. coli, one concludes that there is no need to use both in screening for mutagens. Either bacterial species may be utilized with artificial metabolic activation systems. Other bacterial systems can certainly be employed but due to inadequate information, it is not known to what degree of confidence negative results can be assessed.

2. Yeast

The most commonly used yeast strains are Saccharomyces cerevisiae and Schizosaccharomyces pombe. These organisms have the facility to detect not only gene mutations, but also chromosomal mutations. Yeast systems, however, appear to be less sensitive than bacteria in terms of detecting mutagenically active substances. This insensitivity in detection seems to stem from the relative impermeability of the cell membrane. Additionally, the activity of potent mutagens such as dimethylnitrosamine (DMN) is much less in yeast as compared to bacteria. Yeast strains are, however, more useful than Salmonella or Escherichia in analyzing the genetic bases of the mutational events detected.

The test systems discussed thus far are relatively easy to perform and can yield information in regards to the types of mutagenic lesions produced. One cannot, however, on the basis of significant mutagenicity in these tests declare a substance dangerous to man. They should be viewed as systems which demonstrate the potential of a substance to be mutagenic in man. They provide an information base, but cannot by themselves form the basis for decisions regarding mutagenicity to humans.

3. Mammalian Cells

Two major cell lines have been used for the assessment of gene mutations. They are the Chinese hamster ovary and mouse lymphoma.

Of the major procedures utilizing mammalian cells, the mouse lymphoma, which measure gene mutations, has been employed most extensively and has a larger data base with respect to the number of compounds that have been investigated. The technical procedure is somewhat involved and is not easily performed by a novice.

The Chinese hamster ovary cells can also assess gene mutations. Biochemical markers provide the means of identifying the mutant cells.

Since the ultimate objective in mutagenicity is to obtain data that is relevant to man, human diploid fibroblasts come closer to that objective than any other in vitro system which assesses gene mutations. It is a tedious and highly intricate procedure but is mentioned here due to its potential for providing very valuable information.

4. Drosophila Melanogaster

Drosophila melanogaster has been used quite extensively for assessing the mutagenicity of various chemicals. Both major classes of genetic damage can be assessed, i.e., gene as well as chromosomal mutations. Among the various procedures which can be performed in Drosophila are dominant lethality, chromosome loss and X-linked recessive lethality. The X-linked recessive lethal test is the most sensitive of the procedures in terms of the detection of chemical mutagens. Additionally, Drosophila appears to be able to activate metabolically chemicals in a manner similar to mammals. Therefore direct as well as indirect mutagens can be investigated.

TEST SYSTEMS DETECTING CHROMOSOMAL MUTATIONS

Chromosomal mutations are responsible for the greater portion of human diseases that are attributable to mutagenesis. Down's syndrome, and Klinefelters syndrome are well known diseases of chromosomal origin. Systems measuring this type of aberration are numerous and for the most part easy to perform.

Various types of mammalian cells in culture have been employed to investigate chemicals for cytogenetic abnormalities. They are too numerous to list individually. For example human lymphocytes obtained from volunteers can be utilized in two fashions: (1) after exposure of humans to a substance, these cells may be cultured, induced to divide and examined for chromosomal abnormalities, or (2) withdrawn from "normal" volunteers induced to grow, and subsequently exposed to chemical mutagens and observed for chromosomal abnormalities.

There has been some discussion among scientists in the field of mutagenicity in reference to the heritability of chromosomal breaks and gaps, and consequently the relevance of such effects to true genetic damage. Those who advocate use of in vitro cytogenetics do so on the basis that in instances where exchanges or translocations are not observed in combination with breaks and gaps, it is usually due to inadequate techniques or insufficient exposure time. There

is no doubt, however, that the technique is useful as a screen for potential mutagens, but cannot be thought of as a system which yields definitive information regarding heritability of the observed aberrations.

One area that has not been investigated is the relationship between gene and chromosomal mutations in the same cell line. The mouse lymphoma and Chinese hamster cells lend themselves very well to this possibility. Information of this type could establish that the quantitative and qualitative relationship of these effects are, and lead to a better understanding of these processes.

APPENDIX J

NUMBER OF FOOD ADDITIVE APPLICATIONS RECEIVED AND APPROVED
FROM 1958 TO PRESENT

<u>YEAR</u>	<u>RECEIVED</u>		<u>APPROVED</u>	
	Direct	Indirect	Direct	Indirect*
1958-1967	1441	1296	303	
1968	38	85	7	
1969	22	88	5	
1970	39	99	5	
1971	30	80	6	
1972	25	81	2	
1973	23	68	23	
1974	20	56	8	
1975(Sept)	14	14	11	

*No discreet number can be described for approved indirect additives because of overlapping listings.

Senator NELSON. We will have some questions that I will not ask at this time, which we would like to have for the record, which we will submit in writing, and I assume you will respond to.

Mr. GARDNER. We certainly will.

Senator NELSON. Just briefly, with respect to the Food and Drug Administration Task Force report on the Searle Co., when was that report finished?

Mr. MERRILL. I think the report was submitted to the Commissioner. I can supply the precise date for the record. It was in the spring of 1976, April 1.

Senator NELSON. Some time in the spring of 1976, the task force completed its report, or delivered its report to the Commissioner of the FDA. Is that correct?

Mr. MERRILL. That is correct.

Senator NELSON. And has the Commissioner taken any action, or recommended any legal action, respecting that task force report?

Mr. MERRILL. Well, a number of things have grown out of that.

There are matters of public record. One is that the drug Aldactone, on which some of the studies were conducted, had been labeled to include some additional warnings.

We do not have anyone here from the Bureau of Drugs who could describe the precise hazard we were concerned with, but that is one significant action.

We have made a recommendation with respect to other actions to the U.S. attorney, but I do not feel at liberty to describe its contents here.

Senator NELSON. You mentioned there is a public record, and you have made a recommendation. Have you made a recommendation for a reference of this question to a grand jury?

Mr. MERRILL. We have made a recommendation to the U.S. attorney in the northern district of Chicago that he consider that.

Senator NELSON. That he consider what?

Mr. MERRILL. A grand jury investigation.

Senator NELSON. When was that recommendation made?

Mr. MERRILL. I can supply that for the record.

Senator NELSON. Was it recently?

Mr. MERRILL. It was recently.

Senator NELSON. This year?

Mr. MERRILL. I cannot tell you precisely. It was January 10, 1977.

Senator NELSON. But in the past 2 or 3 weeks?

Mr. MERRILL. In the past month.

Senator NELSON. In the past 30 days?

Mr. MERRILL. Yes.

Senator NELSON. All right.

The Yellow Dye No. 5, you commented on that briefly in your statement.

Now, that is, as I understand it, the most widely used of all certified color additives, even more than Red Dye No. 2, is that correct?

Mr. MERRILL. Yes.

Mr. GARDNER. That is correct.

Senator NELSON. And my staff advises me that possibly up to 40 percent of all food that is colored contains Yellow No. 5; is that approximately correct?

Dr. ROBERTS. I have no basis for commenting on that.

Senator NELSON. The staff advises me that information was secured from the FDA.

Mr. GARDNER. We can go back and confirm this.

Senator NELSON. I just want the record to be correct, reasonably correct.

Mr. GARDNER. To the extent we have information, we will confirm it.

[Subsequent information was received and follows:]

Results of a limited survey conducted in 1973, on various food products, indicated that 153 out of 339 products tested contained Yellow No. 5. This is equivalent to approximately 45 percent.

Senator NELSON. The staff advises me the information furnished was possibly up to 40 percent of all products containing color additives contain Yellow No. 5, and that 1½ million pounds of it are in use today, and that studies by the FDA itself show it to cause an allergic reaction in some people similar to an allergic reaction to aspirin, which we would understand to mean that about 2 percent of the total population in the United States, or 400,000, are subject to such reaction.

My question is, what specifically is the Food and Drug Administration doing to inform the public, or those who might be allergic, about Yellow No. 5? What precisely is the Food and Drug Administration doing?

Mr. GARDNER. First, just to correct the record, the studies which demonstrate that effect are not the Agency studies. I believe those are in the scientific literature and being conducted by other people.

Senator NELSON. Well, the FDA collected the scientific studies?

Mr. GARDNER. That is correct, and with respect to the more important part of that question, the recommendation is being prepared, which I have yet to see, for proposing a labeling requirement, but I cannot comment on it because I have not seen the recommendation and obviously cannot answer or act on it.

Senator NELSON. Does the literature indicate serious allergic reactions in any of the population, or what is the nature of the allergic reactions?

Mr. GARDNER. Perhaps Dr. Blumenthal who is Director of our Division of Toxicology would like to respond.

Senator NELSON. Dr. Blumenthal?

Dr. BLUMENTHAL. As I recall, this will be subject to confirmation, but it is my recollection that the major reaction is urticaria itching, something like hives.

Senator NELSON. I was wondering about the seriousness. As you know much better than we do, people may have a very severe skin breakout that is serious, or mild. Are these people seriously allergic?

Dr. BLUMENTHAL. I would have to reserve my answer to some degree, because I have not reviewed this literature for some years. The literature relating to the reaction of people to Yellow No. 5 is really not extensive. Considering the number of people who are allergic to aspirin, one would expect that the medical literature would be full of people showing the combined response, but in fact, there is a great paucity of this kind of report, and most of the reports are perhaps 5, 6, 7 years old.

Now, my recollection is that urticaria is the major allergy's response—it may be major—but I would like to go back and check that specifically.

Senator NELSON. As to the literature, your reference is to the fact there is not much in literature on it, as compared with aspirin. Would I be correct to suggest that the reason may well be that a doctor more routinely asks about aspirin, and does not ask routinely about Yellow Dye No. 5, and, furthermore, if he did, the patient would not know whether he or she had consumed that?

Dr. BLUMENTHAL. What you say is obviously something that has a great deal of truth in it, although those individuals who have an allergic response, may go to an allergist. Here, we are talking about physicians who have been aware of these reports as long as we have.

Senator NELSON. I understand, but how many people get to an allergist.

Dr. BLUMENTHAL. I agree.

Senator NELSON. All right.

Thank you very much for your testimony, Mr. Gardner.

We will submit some additional questions. Thank you very much. We will have to move along.

Mr. GARDNER. Thank you.

[Subsequent information was received and follows:]

March 1, 1977

Sherwin Gardner, Acting Commissioner
Food and Drug Administration
Department of Health, Education & Welfare
Rockville, Maryland

Dear Commissioner:

The Senate Select Committee on Small Business appreciates the testimony supplied by the Food and Drug Administration for the hearings January 13, 1977 on food additives marketing, regulation and safety.

As we stated at the hearings, we are requesting additional information for the record. Since we would like to close the record in approximately one month, we would appreciate receiving the following information as soon as possible.

1. As requested at the hearings (see page 50 of the transcript), we would like to have a breakdown of how all additives are used, by category as to "regulated", "generally recognized as safe," "prior sanction," "provisionally listed color additives," and any other category, i.e. the number of additives used as preservatives, colors, flavors, etc. within each category.
2. During the hearings (see page 31 of transcript), we requested information as to whether any of the estimated 36 known carcinogens identified by the National Cancer Institute (as discussed in the GAO Report, "Federal Efforts to Protect the Public from Cancer-Causing Chemicals Are Not Very Effective") are food additives, either direct or indirect.

Subsequently, it has come to our attention that of some 200 chemicals backlogged in the NCI's Carcinogen Bioassay Program, 126 are regulated by the FDA as food additives, both direct and indirect (Food Chemical News, January 31, 1977, page 51).^{1/}

We would appreciate any information you may have that expands on the Food Chemical News list of such additives, and a response to the hearing question regarding the known carcinogens.

¹ See article, "FDA Regulates Half of Backlogged Chemicals in NCI Testing Program," Food Chemical News, January 31, 1977, page 491.

Sherwin Gardner, Acting Commissioner
 Food and Drug Administration
 March 2, 1977
 Page 2

3. Subsequent to the hearings, the FDA has proposed regulations regarding FD&C food color Yellow No. 5 (see page 136 of transcript). We would appreciate receiving:
 a) the FDA's public announcement of proposed action;
 b) the proposed regulations; c) any other material relevant to the specific issue.

4. With respect to saccharin, the FDA has proposed one action recommended by the GAO: reducing the amount of impurity allowed in saccharin to the lowest that is technically feasible. However, another recommendation of the GAO has not been acted upon, relating to the safety factor.

A. Why does the FDA propose continuing for saccharin a safety factor of 30-to-one instead of 100-to-one, when the FDA's own regulations provide that, except where evidence is submitted that justifies use of a different safety factor, a food additive for human use will not be granted a tolerance that will exceed 1/100th of the maximum amount demonstrated to be without harm to experimental animals.

B. Is there scientific evidence that saccharin justifies a lesser safety factor?

5. FDA in-house studies, and those done at the Wisconsin Alumni Research Foundation (WARF) show that saccharin produced "a statistically significant incidence of bladder tumors" in mice. The FDA's Director, Bureau of Foods (see page 9, GAO Report, "Need to Resolve Safety Questions on Saccharin") stated: "Dose-related carcinogenicity of administered saccharin for the urinary bladder of rats has been clearly demonstrated."

In light of the Delaney clause in the Food, Drug & Cosmetic Act, prohibiting any substances in food which cause cancer in man or animals, is continued approval of saccharin a violation of law?

6. With respect to "interim status": It is our understanding that the FDA first proposed "interim food additive status" regulations for brominated vegetable oil on July 28, 1970. Since that time, four additives, including saccharin, have been granted "interim status, including manitol and acrylonitrile (June 14, 1976), and several additives have been proposed for such status, including butylated hydroxytoluene (BHT).

Sherwin Gardner, Acting Commissioner
 Food and Drug Administration
 March 2, 1977
 Page 3

A. Please identify: I) all additives under or proposed for "interim status" regulation; II) what they are used for; III) how extensive their use is; IV) what their previous regulatory status was.

B. On what grounds and under what statutory authority does the FDA justify "interim status" regulations?

C. Is it correct that the FDA requires the same safety justifications for additives under "interim status" as for additives with "permanent status"? Please elaborate.

D. It is our understanding that the FDA's authority to issue such "interim status" regulations was challenged in court in 1971 by the Center for Science in the Public Interest and Mr. James S. Turner, Attorney. Please briefly describe the resolution of the litigation, with appropriate citations.

7. The FDA testimony respecting the GRAS list review states that the Review Committee has recommended action on two additives for which there are insufficient data to establish conditions of safe use.

What are the two additives and what are they used for?

8. Respond to all allegations in the testimony of Dr. Sidney Weiss, Health Research Group, respecting coal tar color additives. What actions is the FDA taking with respect to these additives?

9. What is the FDA's view on the merits of requiring that food additives' approval expire at regular intervals, requiring renewed approval based on current safety testing methods?

Your assistance in providing this additional information will contribute to making the hearing record current and complete with respect to the marketing, regulation and safety of food additives.

Thank you for your help.

Sincerely,

GAYLORD NELSON
 U.S. Senator

GN:jk



DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
ROCKVILLE, MARYLAND 20852

MAY 0 6 1977

Honorable Gaylord Nelson
United States Senate
Washington, D.C. 20510

Dear Senator Nelson:

Thank you for your letter of March 1, 1977, requesting additional information for the record to supplement our testimony before the Senate Select Committee on Small Business on January 13, 1977. Our response follows the order of the questions in your letter.

1. The breakdown of additives used in food by category (i.e., food additives, substances generally recognized as safe (GRAS), prior sanctions, permanently listed color additives, and provisionally listed color additives) and the functional category of those additives (e.g., preservatives, emulsifiers, stabilizers, flavorings, etc.) is found generally in part 21 of the Code of Federal Regulations (CFR). In particular, we direct your attention to 21 CFR parts 170-189 (these regulations were recodified in the Federal Register of March 15, 1977 (42 FR 14302)). Thus, for example, part 172 lists the food additives approved by the Food and Drug Administration (FDA) for use as food preservatives, coatings, films and related substances, special dietary and nutritional additives, anticaking agents, etc. The list of substances that are GRAS can be found in part 182. Color additive listings are found in parts 70-82 of Title 21 of the CFR (recodified in the Federal Register of March 22, 1977 (42 FR 15553)).

Additionally, the National Academy of Sciences (NAS), on contract with FDA, is preparing computer printouts of all ingredients that are GRAS, direct food additives, or color additives used in food. Next to each ingredient name will be listed the technical effect and where the ingredient is listed in the CFR. We have enclosed the most recent lists available within the FDA. (Tabs A-F)

2. Tab G contains the Bureau of Foods' comments on the 176 chemicals permitted for use by FDA that are part of the National Cancer Institute (NCI) carcinogen bioassay program. Where appropriate, an indication is given to the possible impact upon FDA and the users of the additives if certain of these chemicals were deemed unsuitable for specific uses.

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Of the 36 substances on the list (Tab H), as discussed in the GAO report, only vinyl chloride, asbestos and betel nuts can clearly be identified as being regulated as food additives within the meaning of section 201(s) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321(s)). Betel nuts are not approved for use in this country. Proposals are pending relative to asbestos and vinyl chloride.

In the Federal Register of September 3, 1975 (40 FR 40529), the FDA proposed regulations to restrict the uses of vinyl chloride polymers in contact with food. The proposal permits the continued use of vinyl chloride polymers in food packaging and other food-contact articles where the potential for migration of vinyl chloride is diminished to the extent that it may not reasonably be expected to become a component of food. The proposal includes an interim food additive regulation for the use of water pipes made from vinyl chloride polymers. The interim regulation would be in effect pending development of additional data to determine if vinyl chloride may reasonably be expected to be in potable water that is drawn from the tap. The proposed regulation would prohibit all other uses of vinyl chloride polymers in food contact articles, including semirigid and rigid articles such as bottles and sheets, because in those uses vinyl chloride may reasonably be expected to become a component of food.

In the Federal Register of September 28, 1973 (38 FR 27076), the Commissioner proposed that any food containing talc that is not free from asbestos fibers shall be deemed adulterated in violation of section 402(a)(1) of the Federal Food, Drug, and Cosmetic Act. As announced in the Federal Register of March 14, 1975 (40 FR 11866), a final order on this proposal had to be delayed until an acceptable method for determining the presence of asbestos particles in talc can be developed. The issues involved include the fact that there is no demonstrable evidence that ingested talc containing asbestos is a health hazard and that a suitable methodology for determining asbestos fibers has not been developed. The future state of talc as a food ingredient, and particularly as a component of a coating for rice, will be consistent with whatever regulation is promulgated when these issues have been resolved.

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3. In response to your request concerning FD&C Yellow No. 5, we are enclosing in Tab I:

1. A copy of the press release of February 3, 1977.
2. A copy of the proposed regulations published in the Federal Register on February 4, 1977.
3. A copy of the supporting material that is on file at the Hearing Clerk's Office (5600 Fishers Lane, Rockville, Maryland) which forms the basis for the proposed action.

4 and 5. In June 1972, FDA signed a contract with the National Academy of Sciences (NAS) to review the results of all experiments on the possible carcinogenicity of saccharin. They concluded that the application of a 100:1 safety factor was unduly conservative; as a consequence, a 30:1 safety factor was recommended. This action was in accord with the report of a World Health Organization (WHO) scientific group in which various justifications for decreasing the margin of safety were set forth. Hence, the Ad-Hoc Subcommittee concluded that a total intake of 1 g/day, or 15 to 20 mg/kg in adults in the 50 to 70 kg weight range was safe. The FDA accepted this recommendation.

Continued approval of saccharin was not a violation of the Delaney clause to the Federal Food, Drug, and Cosmetic Act because, until recently, saccharin had not been conclusively shown to be carcinogenic.

As noted above, the FDA accepted the recommendations of the NAS. The NAS report's primary conclusion was that the data then available, have "not established conclusively whether saccharin is or is not carcinogenic when administered orally to test animals." The NAS further recommended that several additional studies be undertaken to generate data that would permit a reasonably conclusive scientific judgment on the question of whether saccharin is a carcinogen, and that the question be reconsidered when a substantial portion of the additional data became available.

During FDA's review of the report, several studies on saccharin were undertaken by the Health Protection Branch of the Canadian Department of National Health and Welfare. At that time, these studies were

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expected to provide data that would enable FDA to make a final determination regarding the use of saccharin in food. On March 9, 1977, following careful consultation with the Canadian Government's Health Protection Branch and review of the available studies, FDA announced its intention to prohibit use of saccharin in foods and beverages because the artificial sweetener had been shown conclusively to cause bladder tumors in test animals. A copy of FDA's proposal on saccharin is enclosed. (Tab J)

6. Substances currently regulated under "interim food additive regulations" are identified in Tab K along with the additional information you requested on these ingredients. A substance soon to be proposed for interim regulation is butylated hydroxytoluene (BHT) which is used as an antioxidant or preservative. In 1970, the NAS survey on GRAS substances showed that 994,600 pounds were produced for food use. The average consumption was 28 milligrams per day. BHT is currently listed as a GRAS substance, a regulated direct food additive, a regulated indirect food additive and a prior sanctioned ingredient depending on its intended use.

An interim food additive regulation is based on the express authority of the Commissioner to establish the conditions under which a food additive may be safely used (21 U.S.C. 348(c) and (d)). New questions about the safety of old and well-established food ingredients will frequently be raised. Where the new evidence merely raises questions, and authoritative scientific judgment concludes with reasonable certainty that the ingredient may safely be used, an interim food additive regulation may be issued to allow continued use of the ingredient for a limited time while further study is undertaken. In the case of both an interim food additive regulation and an ordinary food additive regulation, the Commissioner must be "reasonably certain" that use of the additive is safe before he may approve or permit continued use of the additive.

The Commissioner's authority to promulgate interim food additive regulations was upheld in Jacobson v. Edwards D.D.C., Civil Action No. 445-71, July 6, 1971, aff'd, per curiam, D.C. Cir. No. 71-2046, December 15, 1972.

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7. Japan wax and carnauba wax are the two substances reported by the Select Committee On GRAS Substances (SCOGS) as having insufficient data available to establish conditions of safe use. Japan wax is generally recognized as safe as a substance migrating to food from cotton and cotton fabrics used in dry food packaging. Japan wax also is permitted for use as a component of paper and paperboard intended for use in producing, manufacturing, treating, packaging, transporting, or holding aqueous or fatty foods. Carnauba wax is generally recognized as safe as a miscellaneous and/or general purpose food additive.

8. Dr. Sidney Wolfe's allegations concerning color additives do not raise any new questions or present any new data that have not been thoroughly considered previously by FDA. Perhaps the greatest problem suggested by Dr. Wolfe is that surrounding the time when a decision has been made, and the data available at that time to make the decision. A decision to approve a food or color additive can, quite obviously, be based only on the data that is available and the scientific techniques to analyze those data. But, new data do become available and scientific techniques improve which make it necessary to review those decisions.

Toxicology, like all other scientific disciplines, is not static. There have been tremendous advances and changes in the views of scientists toward toxicological testing. This has led the Agency to initiate a cyclic review of all permitted additives including "permanently" listed color additives. A complete discussion of this "Cyclic Review" was presented to you on January 13, 1977. It should not be thought, however, that the Agency is viewing the Cyclic Review as a "cure-all." Prompt action is taken and will continue to be taken to end the use of any additive when the Agency determines, as was done for FD&C Red No. 2, FD&C Red No. 4, carbon black, and most recently saccharin, that there are sufficient scientific data to demonstrate that the additives should no longer be permitted.

The Agency likewise takes action when advised by consumers or other interested parties that there are reasons for viewing with concern the continued use of the additive. For example, on the basis of a comment to a proposal, the Agency stayed the effectiveness of an order permanently listing D&C Red No. 34 for use in externally applied drugs and cosmetics. The color has been continued on a provisional

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listing while we are considering whether there is a possibility that this color, as well as D&C Red Nos. 10-13, might contain B-naphthylamine, a known carcinogen. The Agency insists in all cases, however, that actions to terminate the approval of any substance be based on sound scientific principles and not merely suspicion of potential problems. To do otherwise, would be irresponsible.

The Health Research Group has submitted a petition to the Agency proposing the delisting of the six certifiable colors, which they identify as coal-tar dyes. The testimony on color additives given by Dr. Wolfe is essentially a condensation of this petition. Scientists at the Agency are examining each of the points of the petition.

9. The Federal Food, Drug, and Cosmetic Act does not require FDA to review periodically its past regulatory decisions nor does it require that approval be terminated at a regular interval. However, the Agency realizes that the data that were the basis of the initial decision can become obsolete in terms of current safety standards. Therefore, with the advent of the GRAS review program, the Agency began the cyclic review of all ingredients added to food. Periodically, each ingredient will be reevaluated in terms of how closely it is judged to meet the current set of evaluation criteria. In reality, there is no "final" FDA decision since if at any time an additive is found to be no longer safe for its intended use the Agency will take the necessary action to remove it from the marketplace.

If I can be of any further assistance, please let me know.

Sincerely yours



Sherwin Gardner
Deputy Commissioner

Enclosures

TABS

Tab A	Classification of ingredients added to food
Tab B	Alphabetized list of Generally Recognized As Safe GRAS/Non-Flavors Partial list of flavors/spices Generally Recognized as Safe (GRAS)
Tab C	Alphabetized list of direct food additives (non flavors)
Tab D	Listing of direct food additives (non flavors) by technical effect
Tab E	Listing of colors used in foods and/or drugs and/or cosmetics
Tab F	Partial list of prior sanctioned substances
Tab G	Memorandum to Associate Commissioner for Science from Acting Director Bureau of Foods: Bureau of Foods comments on backlog list of chemicals in the NCI carcinogen bioassay program
Tab H	NCI List of known Human Carcinogens
Tab I	Packet of information on FD&C Yellow No. 5
Tab J	Saccharin
Tab K	Interim Regulated Substances

CLASSIFICATION OF INGREDIENTS ADDED TO FOOD

TAB A

Classification of Substances	Number of Ingredients 3/16/77
Generally Recognized As Safe Substances (GRAS)	
Non-Flavors	439
Flavors and Spices Regarded As Generally Recognized As Safe by FDA	268
Flavors and Spices Considered Generally Recognized As Safe by Manufacturers	735
Prior Sanctioned Substances (Includes direct food additives, indirect food additives, and generally recognized as safe substances (GRAS))	Total Number of Ingredients Not Known
Direct Food Additives	
Non-Flavors	332
Flavors and Spices	946
Indirect Food Additives	Approximately 10,000
Color Additives	
I. Color Additives Permitted For Use In Food	
A. Subject to Certification	6
B. Exempt From Certification	25
C. Provisionally Regulated and Subject to Certification	3
D. Provisionally Regulated and Not Subject To Certification	0
II. Color Additives Permitted For Use In Drugs	
A. Subject to Certification	21
B. Exempt From Certification	16
C. Provisionally Regulated and Subject to Certification	34
D. Provisionally Regulated and Not Subject to Certification	1

Classification of Substances**Number of Ingredients**
3/16/77**III. Color Additives Permitted For Use
In Cosmetics**

A. Subject to Certification	13
B. Exempt From Certification	14
C. Provisionally Regulated and Subject to Certification	33
D. Provisionally Regulated and Not Subject to Certification	17

Interim Regulated Ingredients**3**

ALPHABETIZED LIST OF GENERALLY RECOGNIZED AS SAFE SUBSTANCES
GRAS/NONFLAVORS

PARTIAL LIST OF FLAVORS/SPICES GENERALLY RECOGNIZED AS SAFE

TAB B

THIS LIST IS ALPHABETIZED BY FIRST TWO LETTERS OF NAME. SUBSTANCES WITH IDENTICAL
FIRST TWO LETTERS ARE LISTED IN ORDER OF INCREASING NUMBER OF LETTERS.

ALPHABETIZED BY COMPANY STARTING WITH FIRST TWO LETTERS NOT PRECEDED BY STRAIGHTEN BY ONLY A PREFIX
IF THE FIRST LETTERS ARE IDENTICAL. IF THE FIRST TWO LETTERS ARE IDENTICAL, THE COMPANY NAME
ALPHABETIZED TO THE PRECEDING PARAGRAPH IS UNDER P

ALPHA - PHASE II

NAS + FEMA

UNAS PHAS II COMBINED FEMA & CAS SUBSTANCE LIST - ALPHABETIC
 (SUBSTANCE WITH DIFFERENT SPELLING IN FEMA & CAS LISTS APPEARS TWICE)

UBIT-1-2		FEMA	3536
UBIT-1-10-DIPE (NO		FEMA	3253E
UBIT-1	NAS 0271	FEMA	2773
UBIT-1-CUM	NAS 0001	FEMA	2041
UBIT-1		FEMA	23020
UBIT-1-10-DIPE	NAS 0448	FEMA	20014
UBIT-1-10-DIPE PHENETHYL DR		FEMA	22140
UBIT-1-10-DIPE		FEMA	23050
UBIT-1-10-DIPE	NAS 0302	FEMA	20048
UBIT-1-10-DIPE	NAS 0483	FEMA	25100
UBIT-1-10-DIPE		FEMA	20011
UBIT-1-10-DIPE		FEMA	20090
UBIT-1-10-DIPE		FEMA	35075
UBIT-1-10-DIPE		FEMA	3004
UBIT-1-10-DIPE		FEMA	3126
UBIT-1-10-DIPE		FEMA	22130
UBIT-1-10-DIPE		FEMA	36130
UBIT-1-10-DIPE	NAS 0303	FEMA	2011
UBIT-1-10-DIPE	NAS 0204	FEMA	2112
UBIT-1-10-DIPE	NAS 0306	FEMA	2012
UBIT-1-10-DIPE	NAS 0335	FEMA	2011
UBIT-1-10-DIPE	NAS 0205	FEMA	3576
UBIT-1-10-DIPE	NAS 0036	FEMA	0201
UBIT-1-10-DIPE	NAS 0413		
UBIT-1-10-DIPE EXTRACT		FEMA	23130
UBIT-1-10-DIPE CAS ANION	NAS 0177	FEMA	2015
UBIT-1-10-DIPE	NAS 0414		
UBIT-1-10-DIPE		FEMA	21170
UBIT-1-10-DIPE		FEMA	20102
UBIT-1-10-DIPE		FEMA	20190
UBIT-1-10-DIPE		FEMA	23340
UBIT-1-10-DIPE		FEMA	20470
UBIT-1-10-DIPE		FEMA	23010
UBIT-1-10-DIPE		FEMA	20011
UBIT-1-10-DIPE		FEMA	20020
UBIT-1-10-DIPE		FEMA	36110
UBIT-1-10-DIPE		FEMA	20730
UBIT-1-10-DIPE		FEMA	23460
UBIT-1-10-DIPE		FEMA	20740
UBIT-1-10-DIPE		FEMA	20360
UBIT-1-10-DIPE		FEMA	22770
UBIT-1-10-DIPE		FEMA	20210
UBIT-1-10-DIPE		FEMA	20170
UBIT-1-10-DIPE		FEMA	36120
UBIT-1-10-DIPE		FEMA	20340
UBIT-1-10-DIPE		FEMA	21400
UBIT-1-10-DIPE		FEMA	21300
UBIT-1-10-DIPE		FEMA	21270
UBIT-1-10-DIPE		FEMA	3500
UBIT-1-10-DIPE		FEMA	21400
UBIT-1-10-DIPE		FEMA	21170
UBIT-1-10-DIPE		FEMA	23000

GAS PHASE II CONTAINED PHASE I HAS SUBSTANCE LIST - ALPHABETIC
 (SUBSTANCE WITH DIFFERENT SPELLING ON PHASE I HAS LIST APPEARS TWICE)

LVL PHENYLACETATE	FFMA 20390
LVL PIPERIDATE	FFMA 20475
LVL SODIUM	FFMA 20419
LVL TIGLATE	FFMA 20430
LVL ZINC ACETATE	FFMA 20469
LVL ZINC SULFATE	FFMA 20370
LVL ZINC OXIDE	FFMA 20336
MAKING OF BITTER, FFMA	FFMA 20468
MAKING OF BITTER, FFMA	NAS 0522
MAKING OF BITTER	FFMA 20470
MAKING OF BITTER	FFMA 20440
MAGNESIUM ZINC SULFATE	NAS 0038
MAGNESIUM ZINC SULFATE	NAS 0037
MAGNESIUM SULFATE	NAS 0009
MAGNESIUM SULFATE	NAS 0013
MAGNESIUM SULFATE	NAS 0039
MAGNESIUM SULFATE	NAS 0011
MAGNESIUM SULFATE	FFMA 20497
MAGNETIC SEED, OIL	FFMA 20514
MAGNETIC SEED, OIL	FFMA 20503
MAGNETIC SEED, OIL	FFMA 20524
MAGNETIC SEED, OIL	NAS 0252
MAGNETIC SEED, OIL	NAS 0253
MAGNETIC SEED, OIL	NAS 0010
MAGNETIC SEED, OIL	NAS 0012
MAGNETIC SEED, OIL	NAS 0013
MAGNETIC SEED, OIL	NAS 0016
MAGNETIC SEED, OIL	NAS 0344
MAGNETIC SEED, OIL	NAS 0012
MAGNETIC SEED, OIL	NAS 0018
MAGNETIC SEED, OIL	NAS 0019
MAGNETIC SEED, OIL	FFMA 20530
MAGNETIC SEED, OIL	FFMA 20540
MAGNETIC SEED, OIL	FFMA 20590
MAGNETIC SEED, OIL	FFMA 3414F
MAGNETIC SEED, OIL	FFMA 20480
MAGNETIC SEED, OIL	FFMA 20730
MAGNETIC SEED, OIL	FFMA 20740
MAGNETIC SEED, OIL	FFMA 3417F
MAGNETIC SEED, OIL	FFMA 23740
MAGNETIC SEED, OIL	FFMA 3415F
MAGNETIC SEED, OIL	FFMA 3416F
MAGNETIC SEED, OIL	FFMA 2372F
MAGNETIC SEED, OIL	FFMA 2076F
MAGNETIC SEED, OIL	FFMA 2076F
MAGNETIC SEED, OIL	NAS 0050
MAGNETIC SEED, OIL	FFMA 20425
MAGNETIC SEED, OIL	FFMA 20430
MAGNETIC SEED, OIL	FFMA 20440

ALYL SALICYLATE		FEMA 3558
AMYL SALICYLATE		FEMA 22140
AMYL SALICYLATE		FEMA 22170
AMYL SALICYLATE		FEMA 22180
AMYL SALICYLATE		FEMA 21090
AMYL SALICYLATE		FEMA 22040
AMYL SALICYLATE		FEMA 31100
AMYL SALICYLATE	NAS 0032	FEMA 2103
AMYL SALICYLATE	NAS 0033	FEMA 2104
AMYL SALICYLATE		FEMA 28910
AMYL SALICYLATE	NAS 0031	FEMA 3000
AMYL SALICYLATE	NAS 0031	FEMA 3516
AMYL SALICYLATE		FEMA 22100
AMYL SALICYLATE	NAS 0037	FEMA 22710
AMYL SALICYLATE		FEMA 3503
AMYL SALICYLATE	NAS 0037	FEMA 2271
AMYL SALICYLATE		FEMA 3001E
AMYL SALICYLATE		FEMA 3522
AMYL SALICYLATE		FEMA 32030
AMYL SALICYLATE	NAS 0034	FEMA 2274
AMYL SALICYLATE		FEMA 22250
AMYL SALICYLATE		FEMA 2278
AMYL SALICYLATE	NAS 0034	
AMYL SALICYLATE	NAS 0037	
AMYL SALICYLATE	NAS 0038	
AMYL SALICYLATE	NAS 0038	
AMYL SALICYLATE	NAS 0036	
AMYL SALICYLATE	NAS 0039	
AMYL SALICYLATE	NAS 0040	
AMYL SALICYLATE	NAS 0033	
AMYL SALICYLATE	NAS 0032	FEMA 3510
AMYL SALICYLATE	NAS 0041	
AMYL SALICYLATE	NAS 0042	
AMYL SALICYLATE	NAS 0043	
AMYL SALICYLATE	NAS 0044	
AMYL SALICYLATE	NAS 0049	FEMA 3535
AMYL SALICYLATE	NAS 0046	
AMYL SALICYLATE	NAS 0047	
AMYL SALICYLATE	NAS 0040	
AMYL SALICYLATE	NAS 0048	FEMA 3656
AMYL SALICYLATE	NAS 0048	FEMA 3656
AMYL SALICYLATE	NAS 0049	
AMYL SALICYLATE	NAS 0050	FEMA 3001
AMYL SALICYLATE	NAS 0051	
AMYL SALICYLATE	NAS 0052	
AMYL SALICYLATE	NAS 0053	
AMYL SALICYLATE	NAS 0054	FEMA 3520
AMYL SALICYLATE	NAS 0055	
AMYL SALICYLATE	NAS 0056	
AMYL SALICYLATE	NAS 0260	FEMA 3572
AMYL SALICYLATE	NAS 0030	

76777 CAS FEMAS II COMBINED FEMAS & HAS SUBSTANCE LIST - ALPHABETIC
 (SUBSTANCE WITH DIFFERENT SPELLING OR FEMAS & HAS LIST'S APPEARS TWICE)

CINN SULFATE	NAS 0057	
CLAY		FEMA 2744A
COCAINE		FEMA 2265F
COCAINE		FEMA 2229D
COCAINE		FEMA 2733D
COCAINE, JAP. WHITE, OIL		FEMA 2231C
COCAINE, PIL		FEMA 2232A
COCAINE, WAX	NAS 0261	
COCAINE, WAX	NAS 0058	FEMA 2749
COCAINE, WAX		FEMA 2237A
COCAINE, WAX		FEMA 2234A
COCAINE, WAX	NAS 0059	FEMA 2235
COCAINE, WAX	NAS 0359	FEMA 2235
COCAINE, WAX	NAS 0421	FEMA 2236A
COCAINE, WAX		FEMA 2237A
COCAINE, WAX		FEMA 2238A
COCAINE	NAS 0262	
COCAINE	NAS 0060	
COCAINE	NAS 0533	
COCAINE, WAX	NAS 0223	FEMA 2239
COCAINE, WAX	NAS 0266	
COCAINE, WAX	NAS 0269	FEMA 2239
COCAINE, WAX		FEMA 2240A
COCAINE, WAX		FEMA 2241A
COCAINE, WAX		FEMA 3591
COCAINE, WAX		FEMA 2242E
COCAINE, WAX	NAS 0061	FEMA 3514
COCAINE, WAX	NAS 0262	FEMA 2263
COCAINE, WAX	NAS 0062	FEMA 2263
COCAINE, WAX	NAS 0063	FEMA 3548
COCAINE, WAX		FEMA 2744A
COCAINE, WAX		FEMA 2745D
COCAINE, WAX		FEMA 2746D
COCAINE, WAX		FEMA 2257D
COCAINE, WAX	FEMA	3623D
COCAINE, WAX		FEMA 2748D
COCAINE, WAX		FEMA 3427F
COCAINE, WAX		FEMA 2749C
COCAINE, WAX		FEMA 3487F
COCAINE, WAX		FEMA 2250D
COCAINE, WAX		FEMA 2251D
COCAINE, WAX		FEMA 3268D
COCAINE, WAX		FEMA 3269D
COCAINE, WAX		FEMA 3269D
COCAINE, WAX		FEMA 2252D
COCAINE, WAX		FEMA 3270D
COCAINE, WAX		FEMA 3488F
COCAINE, WAX		FEMA 2259C
COCAINE, WAX		FEMA 2259A
COCAINE, WAX		FEMA 2259A
COCAINE, WAX	NAS 0422	FEMA 2744A
COCAINE		FEMA 2744A

76/77 QAS PHAS II COMBINED FEMA & NAS SUBSTANCE LIST - - - ALPHABETIC
 (SUBSTANCE WITH DIFFERENT SPELLING ON FEMA & NAS LISTS APPEARS TWICE)

TA BARK, EXTRACT		FEMA 2257A
TA BARK, OIL		FEMA 2258A
TA MUDS		FEMA 2259F
TA RESIN, OIL		FEMA 2260C
TA SILE		FEMA 2261K
TANIN, EXTRACT		FEMA 2261A
TANIN, LIQUID		FEMA 2262A
TANIN	NAS 0406	
TANIN, EXTRACT		FEMA 2264C
TANIN, POWDER		FEMA 2265C
TANIN		FEMA 2266A
TANIN, OIL		FEMA 2267C
TANIN, ALC/PROP		FEMA 2270D
TANIN, JUICE		FEMA 2271D
TANIN, SEED		FEMA 2276A
TANIN, EXT. SOLID		FEMA 2270S
TANIN, EXTRACT		FEMA 2269A
TANIN, OIL		FEMA 2271A
TANIN, ALCOHOL		FEMA 2262A
TANIN	NAS 0265	
TANIN, MICROCRYSTALLINE		FEMA 2265
TANIN		FEMA 2272E
TANIN, HYDRALYZED	NAS 0423	
TANIN FLOWER EXT. ROM		FEMA 2274A
TANIN FLOWER OIL, ROM		FEMA 2272A
TANIN FLOWER OIL, ROM		FEMA 2273A
TANIN FLOWER OIL, ROM		FEMA 2275A
TANIN FLOWER	NAS 0450	
TANIN CHEESE	NAS 0517	FEMA 2277
TANIN BARK, WILD, EXT		FEMA 2276A
TANIN JUICE, FINE		FEMA 2278
TANIN LAMBL OIL, FINE		FEMA 2277C
TANIN PITS, EXTRACT		FEMA 2278C
TANIN		FEMA 2279A
TANIN LEAVES EXT.		FEMA 2282
TANIN, LEAVES EXT.		FEMA 2273C
TANIN, EXTRACT		FEMA 2280S
TANIN CAPSICUM		FEMA 2282
TANIN SEED		FEMA 2273
TANIN		FEMA 2276C
TANIN		FEMA 2278
TANIN	NAS 0424	
TANIN	NAS 0425	
TANIN		FEMA 2284F
TANIN	NAS 0266	
TANIN CHIPS	NAS 0510	
TANIN LIQUOR	NAS 0370	
TANIN ACID	NAS 0064	
TANIN TARTRATE	NAS 0065	
TANIN CHLORIDE	NAS 0066	
TANIN EXTRACT	NAS 0067	FEMA 2296
TANIN, BARK, RED		FEMA 2281C

(SUBSTANCE WITH DIFFERENT SPELLING ON FEMA & HAS LIT. \$ APPEARS TWICE)

YCLLEFF, EXTRACT		FEMA 2125A
YCLLEFF, EXTRACT		FEMA 2125B
YCLLEFF, EXTRACT	NAS 0371	FEMA 1518
YCLLEFF, EXTRACT	NAS 0372	
YCLLEFF, EXTRACT		FEMA 1778A
YCLLEFF, EXTRACT	NAS 0373	FEMA 3659
YCLLEFF, EXTRACT	NAS 0373	FEMA 3659
YCLLEFF, EXTRACT		FEMA 1778B
YCLLEFF, EXTRACT		FEMA 1779A
YCLLEFF, EXTRACT		FEMA 2391A
YCLLEFF, EXTRACT		FEMA 2392A
YCLLEFF, EXTRACT	NAS 0280	
YCLLEFF, EXTRACT	NAS 1426	
YCLLEFF, EXTRACT		FEMA 3280C
YCLLEFF, EXTRACT	NAS 0069	
YCLLEFF, EXTRACT	NAS 3-27	
YCLLEFF, EXTRACT		FEMA 2339A
YCLLEFF, EXTRACT		FEMA 2318A
YCLLEFF, EXTRACT		FEMA 3281C
YCLLEFF, EXTRACT	NAS 0540	
YCLLEFF, EXTRACT	NAS 0534	
YCLLEFF, EXTRACT	NAS 0249	
YCLLEFF, EXTRACT	NAS 0374	FEMA 3675
YCLLEFF, EXTRACT		FEMA 2335A
YCLLEFF, EXTRACT	NAS 0411	
YCLLEFF, EXTRACT	NAS 0270	
YCLLEFF, EXTRACT	NAS 0375	
YCLLEFF, EXTRACT		FEMA 3282C
YCLLEFF, EXTRACT		FEMA 2336C
YCLLEFF, EXTRACT	NAS 0376	FEMA 3680
YCLLEFF, EXTRACT	NAS 0376	FEMA 3680
YCLLEFF, EXTRACT		FEMA 3629F
YCLLEFF, EXTRACT		FEMA 3629F
YCLLEFF, EXTRACT		FEMA 3629F
YCLLEFF, EXTRACT		FEMA 2337D
YCLLEFF, EXTRACT		FEMA 2338C
YCLLEFF, EXTRACT		FEMA 2338C
YCLLEFF, EXTRACT		FEMA 2340A
YCLLEFF, EXTRACT		FEMA 2347A
YCLLEFF, EXTRACT		FEMA 2361E
YCLLEFF, EXTRACT		FEMA 2361D
YCLLEFF, EXTRACT	NAS 0070	
YCLLEFF, EXTRACT		FEMA 2345A
YCLLEFF, EXTRACT		FEMA 2344A
YCLLEFF, EXTRACT		FEMA 2344F
YCLLEFF, EXTRACT		FEMA 3601
YCLLEFF, EXTRACT		FEMA 2201A
YCLLEFF, EXTRACT		FEMA 2348D
YCLLEFF, EXTRACT		FEMA 2347D
YCLLEFF, EXTRACT		FEMA 3594
YCLLEFF, EXTRACT		FEMA 2346D
YCLLEFF, EXTRACT		FEMA 2350D
YCLLEFF, EXTRACT		FEMA 2351D
YCLLEFF, EXTRACT		FEMA 2352D

1-THYL TARTRATE		FEMA 2378D
1-DIETHYLPIPERAZINE		FEMA 3136E
5-DIETHYLTHIOURACILIN		FEMA 3243D
4-DIETHYLAMINE		FEMA 366Z
DIETHYLSELENITE		FEMA 2379D
DIETHYLACRYLATE		FEMA 2380D
DIETHYLAMINE		FEMA 2381F
DIETHYLIMIDAZOLE	NAS 0077	
DIETHYLIMINE		FEMA 2382E
DIETHYLIMINE		FEMA 2384L
DIETHYLIMINE		FEMA 2385L
DIETHYL CYCLOHEXANONE		FEMA 3427L
DIETHYL ETHANEDIAMINE		FEMA 3478F
DIETHYLTHIOURACILIN		FEMA 3136E
DIETHYLUREA		FEMA 2395D
DIETHYLENEGLYCOL		FEMA 2386D
DIETHYLENEGLYCOL		FEMA 3137E
DIETHYLENEGLYCOL-1		FEMA 2391D
DIETHYLENEGLYCOL		FEMA 3596E
DIETHYLENEGLYCOL		FEMA 3597F
DIETHYLENEGLYCOL		FEMA 3616E
DIETHYLENEGLYCOL		FEMA 2396D
DIETHYLENEGLYCOL		FEMA 2746D
DIETHYLENEGLYCOL		FEMA 3596E
DIETHYLENEGLYCOL		FEMA 3143E
DIETHYLENEGLYCOL		FEMA 3143E
DIETHYLENEGLYCOL		FEMA 3291F
DIETHYLENEGLYCOL		FEMA 2389D
DIETHYLENEGLYCOL		FEMA 2145E
DIETHYLENEGLYCOL		FEMA 2387D
DIETHYLENEGLYCOL		FEMA 3137E
DIETHYLENEGLYCOL		FEMA 2388D
DIETHYLENEGLYCOL		FEMA 2390D
DIETHYLENEGLYCOL		FEMA 2392D
DIETHYLENEGLYCOL		FEMA 2393D
DIETHYLENEGLYCOL		FEMA 2394D
DIETHYLENEGLYCOL		FEMA 2395D
DIETHYLENEGLYCOL		FEMA 3144E
DIETHYLENEGLYCOL		FEMA 3670F
DIETHYLENEGLYCOL		FEMA 3579E
DIETHYLENEGLYCOL		FEMA 2397D
DIETHYLENEGLYCOL	NAS 0537	
DIETHYLENEGLYCOL	NAS 0541	FEMA 3292E
DIETHYLENEGLYCOL	NAS 0543	FEMA 3293E
DIETHYLENEGLYCOL	NAS 0197	FEMA 2399E
DIETHYLENEGLYCOL	NAS 0541	FEMA 3292E
DIETHYLENEGLYCOL	NAS 0543	FEMA 3293E
DIETHYLENEGLYCOL		FEMA 2451D
DIETHYLENEGLYCOL		FEMA 2450D
DIETHYLENEGLYCOL		FEMA 2482D
DIETHYLENEGLYCOL		FEMA 3611E
DIETHYLENEGLYCOL		FEMA 2432E

1/12/77 UNK PMS II COMMENT PMS & HAS SOME ML LIST - (SUBSTANCE WITH DIFFERENT SPELLING ON PMS & HAS LI'S APPEARS TWICE)

ALYENE	NAS 0356	FEMA 3110F
ALYEN HYPOCHLORIDE		FEMA 3576
ALYCHOLIC ACID	NAS 0091	
ALYCHOLIC AMMONIATED		FEMA 2528A
ALYCHOLIC SALT GUM		FEMA 3490F
ALYCHOLIC MANGROSE		FEMA 2520A
ALYCHOLIC NATURAL		FEMA 3538
ALYCHOLIC ESSENCE, NET		FEMA 3549
ALYCHOLIC OIL CMC		FEMA 3631
ALYCHOLIC OIL		FEMA 2540F
ALYCHOLIC GRANULS		FEMA 3567
ALYCHOLIC PHOSPHATE		FEMA 3570
ALYCHOLIC EXTRACT	NAS 0093	FEMA 2531
ALYCHOLIC EXTRACT		FEMA 2517C
ALYCHOLIC OIL		FEMA 2540C
ALYCHOLIC		FEMA 2542D
ALYCHOLIC POLYMER		FEMA 3311D
ALYCHOLIC PHENYLACETATE		FEMA 2535D
ALYCHOLIC		FEMA 3312D
ALYCHOLIC ACETATE		FEMA 3313D
ALYCHOLIC GUM	NAS 0092	FEMA 2537
ALYCHOLIC GUM	NAS 0392	FEMA 2537
ALYCHOLIC GUM		FEMA 2536C
ALYCHOLIC GUM	NAS 0393	FEMA 2531
ALYCHOLIC VEGETABLE	NAS 0282	
ALYCHOLIC MANGROSE, EXT		FEMA 2510C
ALYCHOLIC PIN		FEMA 3622
ALYCHOLIC	NAS 0094	
ALYCHOLIC DIENE-OL		FEMA 3629
ALYCHOLIC DIENE-OL-TR		FEMA 3164E
ALYCHOLIC LACTONE		FEMA 2539D
ALYCHOLIC		FEMA 2540D
ALYCHOLIC DIMETHYL ACETAL		FEMA 2541D
ALYCHOLIC DIMETHYL ACETAL		FEMA 2542D
ALYCHOLIC 1,2-DIACETAL		FEMA 3314D
ALYCHOLIC 1,2-DIACETAL		FEMA 2543D
ALYCHOLIC		FEMA 3564
ALYCHOLIC		FEMA 3315D
ALYCHOLIC		FEMA 2544D
ALYCHOLIC		FEMA 2545D
ALYCHOLIC		FEMA 2546D
ALYCHOLIC		FEMA 3165E
ALYCHOLIC		FEMA 3490E
ALYCHOLIC ACETATE		FEMA 2547D
ALYCHOLIC ACETAL		FEMA 2548D
ALYCHOLIC BUTYRATE		FEMA 2549D
ALYCHOLIC INANATE		FEMA 2551D
ALYCHOLIC FORMATE		FEMA 2552D
ALYCHOLIC ISOBUTYRATE		FEMA 2550D
ALYCHOLIC OCTANOATE		FEMA 2553D
ALYCHOLIC	NAS 0383	
ALYCHOLIC COMPLEX	NAS 0243	
ALYCHOLIC		FEMA 2554D

SUBSTANCE WITH DIFFERENT SPELLING ON FEMA & HAS LIST# APPEARS TWICE

ISOMYL ALCOHOL	FEMA 2057D
ISOMYL BENZOATE	FEMA 2058D
ISOMYL BUTYRATE	FEMA 2060D
ISOMYL CINNAMATE	FEMA 2063D
ISOMYL FORMATE	FEMA 2069D
ISOMYL FUSIL OIL	FEMA 2497D
ISOMYL HYDROATE	FEMA 2075D
ISOMYL ISOBUTYRATE	FEMA 2084D
ISOMYL LAMATE	FEMA 2077D
ISOMYL LACTATE	FEMA 2078D
ISOMYL OCTANOATE	FEMA 2090D
ISOMYL PHENYL ACETATE	FEMA 2081D
ISOMYL PICOINATE	FEMA 2082D
ISOMYL PYMULATE	FEMA 2081D
ISOMYL SALICYLATE	FEMA 2044D
ISOMYL 2-HYDROXYBUTYRATE	FEMA 2074D
ISOMYL 2-HYDROXYPROPANOATE	FEMA 2071D
ISOMYL 2-HYDROXYPROPANOATE	FEMA 2073D
ISOMYROL	FEMA 2158D
ISOMYROL ACETATE	FEMA 2160D
ISOMYROL FORMATE	FEMA 2162D
ISOMYROL ISOBUTYRATE	FEMA 2166D
ISOMYROL PROPANOATE	FEMA 2163D
ISOMYROL 2-HYDROXYPROPANOATE	FEMA 2167D
ISOMYROL	NAS 0435
ISOMYROL ACETATE	FEMA 2179D
ISOMYROL ACETOACETATE	FEMA 2177D
ISOMYROL ALCOHOL	FEMA 2179D
ISOMYROL ACETATE	FEMA 2183D
ISOMYROL ANTHRANILATE	FEMA 2182D
ISOMYROL BENZOATE	FEMA 2185D
ISOMYROL BUTYRATE	FEMA 2187D
ISOMYROL CACONATE	FEMA 2193D
ISOMYROL CECYLATE	FEMA 2197D
ISOMYROL CECYLSOAP	FEMA 2224D
ISOMYROL DECANOATE	FEMA 2202D
ISOMYROL ISOBUTYRATE	FEMA 2189D
ISOMYROL PHENYL ACETATE	FEMA 2210D
ISOMYROL PROPANOATE	FEMA 2212D
ISOMYROL SALICYLATE	FEMA 2213D
ISOMYROL 2-FURANPROPIONATE	FEMA 2199D
2-ISOMYRYL-3-METHOXYPIRAZOL	FEMA 3132F
2-ISOMYRYL-3-METHOXYPIRAZOL	FEMA 3133E
2-ISOMYRYL-3-METHOXYPIRAZOL	FEMA 2208D
2-ISOMYRYL-3-METHOXYPIRAZOL	FEMA 3134E
ISOMYRYL OXIDE	FEMA 2223D
ISOMYRYL ACID	FEMA 2222D
ISOMYRYL ETHYL	FEMA 2563
ISOMYRYL	FEMA 2468D
ISOMYRYL ACETATE	FEMA 2470D
ISOMYRYL ETHYL ETHER	FEMA 2472D
ISOMYRYL FORMATE	FEMA 2474D
ISOMYRYL ETHYL ETHER	FEMA 2476D

(SUBSTANCE WITH DIFFERENT SPELLING ON FEMA & NAS LISTS APPEARS IN ITALIC>

EUGENYL PHENYLACETATE		FEMA 2477D
ISOFURIF	NAS 0101	FEMA 3310
SILICIC ACID	NAS 0102	
ETHYL ISOBUTYR. AC-		FEMA 2714D
ETHYL FURIF		FEMA 3215C
ETHYL ACETATE		FEMA 2926D
ETHYL ALCOHOL		FEMA 2929D
ETHYL FOSPHATE		FEMA 2477D
ETHYL MYRISTATE		FEMA 2615D
ETHYL CINNAMATE		FEMA 2929D
ETHYL CITRATE (S)	NAS 0205	
ETHYL FORMATE		FEMA 2944D
ETHYL HEXANOATE		FEMA 2952D
ETHYL ISOBUTYRATE		FEMA 2970D
ETHYL ISOBUTYRATE		FEMA 2967D
ETHYL PHENYLACETATE		FEMA 2956D
ETHYL PHOSPHATE		FEMA 2959D
ETHYL THIOATE		FEMA 3229E
ETHYL PHTHALATE		FEMA 2937D
ETHYL PHTHALATE		FEMA 2927D
ETHYL PHTHALATE		FEMA 2937D
ETHYL PHTHALATE		FEMA 2956D
ETHYL PHTHALATE		FEMA 2952D
ETHYL PHTHALATE		FEMA 2965D
ETHYL PHTHALATE		FEMA 2965D
ETHYL PHTHALATE		FEMA 2965D
ETHYL PHTHALATE		FEMA 2978D
ETHYL PHTHALATE		FEMA 3107D
ETHYL PHTHALATE		FEMA 3124C
ETHYL PHTHALATE		FEMA 2590A
ETHYL PHTHALATE		FEMA 2590A
ETHYL PHTHALATE		FEMA 2630A
ETHYL PHTHALATE		FEMA 2628A
ETHYL PHTHALATE		FEMA 3104E
ETHYL PHTHALATE		FEMA 3494E
ETHYL PHTHALATE	NAS 0205	FEMA 2612E
ETHYL PHTHALATE		FEMA 2603A
ETHYL PHTHALATE		FEMA 2604A
ETHYL PHTHALATE	NAS 0215	FEMA 2605
ETHYL PHTHALATE		FEMA 2608A
ETHYL PHTHALATE		FEMA 2607A
ETHYL PHTHALATE		FEMA 2609C
ETHYL PHTHALATE		FEMA 2609C
ETHYL PHTHALATE		FEMA 2617C
ETHYL PHTHALATE	NAS 0470	
ETHYL PHTHALATE	NAS 0385	
ETHYL PHTHALATE	NAS 0103	FEMA 2611
ETHYL PHTHALATE	NAS 0404	FEMA 2667
ETHYL PHTHALATE	NAS 0566	
ETHYL PHTHALATE		FEMA 2612A
ETHYL PHTHALATE		FEMA 2617E
ETHYL PHTHALATE		FEMA 2616E
ETHYL PHTHALATE		FEMA 2615D
ETHYL PHTHALATE		FEMA 2616D

UNYL ALCOHOL		FEMA 2617D
ZVINDIA OIL		FEMA 2618A
ZVINDIA		FEMA 2619A
ZVINDIA ABSOLUTE		FEMA 2620A
ZVINDIA CONCRETE		FEMA 2621A
ZVINDER OIL		FEMA 2622A
ZITHIN	NAS 0104	FEMA 3919
ZITHIN, MPO M/BENZ PER	NAS 0246	
ZITHIN, MPO M/NO PEP	NAS 0287	
ZITHIN		FEMA 3326F
ZITHIN, MPO M/NO PEP		FEMA 2626A
ZITHIN, MPO M/NO PEP		FEMA 3573
ZITHIN, MPO M/NO PEP		FEMA 2624A
ZITHIN, MPO M/NO PEP		FEMA 2627A
ZITHIN, MPO M/NO PEP		FEMA 2629A
ZITHIN, MPO M/NO PEP		FEMA 3377C
ZITHIN, MPO M/NO PEP	NAS 010A	FEMA 39A3
ZITHIN, MPO M/NO PEP	NAS 0105	FEMA 3329
ZITHIN, MPO M/NO PEP	NAS 0105	FEMA 3329
ZITHIN, MPO M/NO PEP		FEMA 2627D
ZITHIN, MPO M/NO PEP	NAS 0697	
ZITHIN, MPO M/NO PEP	NAS 0564	FEMA 2630A
ZITHIN, MPO M/NO PEP		FEMA 2628A
ZITHIN, MPO M/NO PEP		FEMA 2628A
ZITHIN, MPO M/NO PEP	NAS 0280	
ZITHIN, MPO M/NO PEP		FEMA 3688
ZITHIN, MPO M/NO PEP		FEMA 2632A
ZITHIN, MPO M/NO PEP		FEMA 3648
ZITHIN, MPO M/NO PEP		FEMA 2611A
ZITHIN, MPO M/NO PEP		FEMA 3689
ZITHIN, MPO M/NO PEP		FEMA 3690
ZITHIN, MPO M/NO PEP		FEMA 2613E
ZITHIN, MPO M/NO PEP		FEMA 3649
ZITHIN, MPO M/NO PEP		FEMA 2634C
ZITHIN, MPO M/NO PEP		FEMA 2635E
ZITHIN, MPO M/NO PEP		FEMA 3328D
ZITHIN, MPO M/NO PEP		FEMA 2636B
ZITHIN, MPO M/NO PEP		FEMA 2637D
ZITHIN, MPO M/NO PEP		FEMA 2638D
ZITHIN, MPO M/NO PEP		FEMA 2639D
ZITHIN, MPO M/NO PEP		FEMA 2640D
ZITHIN, MPO M/NO PEP		FEMA 2641D
ZITHIN, MPO M/NO PEP		FEMA 2642D
ZITHIN, MPO M/NO PEP		FEMA 2643D
ZITHIN, MPO M/NO PEP		FEMA 2644D
ZITHIN, MPO M/NO PEP		FEMA 2645D
ZITHIN, MPO M/NO PEP		FEMA 2646D
ZITHIN, MPO M/NO PEP		FEMA 2647A
ZITHIN, MPO M/NO PEP		FEMA 2647A
ZITHIN, MPO M/NO PEP	NAS 0107	
ZITHIN, MPO M/NO PEP	NAS 2386	
ZITHIN, MPO M/NO PEP	NAS 0672	
ZITHIN, MPO M/NO PEP	NAS 0971	

(SUBSTANCE WITH DIFFERENT SPELLING ON FEMA & HAS LIST, APPEARS TWICE)

BISPHENOL A	FEMA 33510
BISPHENOL B	FEMA 33530
BIPHENYL	FEMA 30557
BIPHENYL ALCOHOL	FEMA 30569
BIPYRIDINE	FEMA 27373
L-ASCORBIC ACID	FEMA 27399
L-ASCORBIC ACID-2	FEMA 3617
L-ASCORBIC ACID-3	FEMA 3617
L-ASCORBIC ACID-4	FEMA 26147
L-ASCORBIC ACID-5	FEMA 27350
L-ASCORBIC ACID-6	FEMA 27359
L-ASCORBIC ACID-7	FEMA 27369
L-ASCORBIC ACID-8	FEMA 27370
L-ASCORBIC ACID-9	FEMA 27380
L-ASCORBIC ACID-10	FEMA 27390
L-ASCORBIC ACID-11	FEMA 27390
L-ASCORBIC ACID-12	FEMA 27390
L-ASCORBIC ACID-13	FEMA 27390
L-ASCORBIC ACID-14	FEMA 27390
L-ASCORBIC ACID-15	FEMA 27390
L-ASCORBIC ACID-16	FEMA 27390
L-ASCORBIC ACID-17	FEMA 27390
L-ASCORBIC ACID-18	FEMA 27390
L-ASCORBIC ACID-19	FEMA 27390
L-ASCORBIC ACID-20	FEMA 27390
L-ASCORBIC ACID-21	FEMA 27390
L-ASCORBIC ACID-22	FEMA 27390
L-ASCORBIC ACID-23	FEMA 27390
L-ASCORBIC ACID-24	FEMA 27390
L-ASCORBIC ACID-25	FEMA 27390
L-ASCORBIC ACID-26	FEMA 27390
L-ASCORBIC ACID-27	FEMA 27390
L-ASCORBIC ACID-28	FEMA 27390
L-ASCORBIC ACID-29	FEMA 27390
L-ASCORBIC ACID-30	FEMA 27390
L-ASCORBIC ACID-31	FEMA 27390
L-ASCORBIC ACID-32	FEMA 27390
L-ASCORBIC ACID-33	FEMA 27390
L-ASCORBIC ACID-34	FEMA 27390
L-ASCORBIC ACID-35	FEMA 27390
L-ASCORBIC ACID-36	FEMA 27390
L-ASCORBIC ACID-37	FEMA 27390
L-ASCORBIC ACID-38	FEMA 27390
L-ASCORBIC ACID-39	FEMA 27390
L-ASCORBIC ACID-40	FEMA 27390
L-ASCORBIC ACID-41	FEMA 27390
L-ASCORBIC ACID-42	FEMA 27390
L-ASCORBIC ACID-43	FEMA 27390
L-ASCORBIC ACID-44	FEMA 27390
L-ASCORBIC ACID-45	FEMA 27390
L-ASCORBIC ACID-46	FEMA 27390
L-ASCORBIC ACID-47	FEMA 27390
L-ASCORBIC ACID-48	FEMA 27390
L-ASCORBIC ACID-49	FEMA 27390
L-ASCORBIC ACID-50	FEMA 27390
L-ASCORBIC ACID-51	FEMA 27390
L-ASCORBIC ACID-52	FEMA 27390
L-ASCORBIC ACID-53	FEMA 27390
L-ASCORBIC ACID-54	FEMA 27390
L-ASCORBIC ACID-55	FEMA 27390
L-ASCORBIC ACID-56	FEMA 27390
L-ASCORBIC ACID-57	FEMA 27390
L-ASCORBIC ACID-58	FEMA 27390
L-ASCORBIC ACID-59	FEMA 27390
L-ASCORBIC ACID-60	FEMA 27390
L-ASCORBIC ACID-61	FEMA 27390
L-ASCORBIC ACID-62	FEMA 27390
L-ASCORBIC ACID-63	FEMA 27390
L-ASCORBIC ACID-64	FEMA 27390
L-ASCORBIC ACID-65	FEMA 27390
L-ASCORBIC ACID-66	FEMA 27390
L-ASCORBIC ACID-67	FEMA 27390
L-ASCORBIC ACID-68	FEMA 27390
L-ASCORBIC ACID-69	FEMA 27390
L-ASCORBIC ACID-70	FEMA 27390
L-ASCORBIC ACID-71	FEMA 27390
L-ASCORBIC ACID-72	FEMA 27390
L-ASCORBIC ACID-73	FEMA 27390
L-ASCORBIC ACID-74	FEMA 27390
L-ASCORBIC ACID-75	FEMA 27390
L-ASCORBIC ACID-76	FEMA 27390
L-ASCORBIC ACID-77	FEMA 27390
L-ASCORBIC ACID-78	FEMA 27390
L-ASCORBIC ACID-79	FEMA 27390
L-ASCORBIC ACID-80	FEMA 27390
L-ASCORBIC ACID-81	FEMA 27390
L-ASCORBIC ACID-82	FEMA 27390
L-ASCORBIC ACID-83	FEMA 27390
L-ASCORBIC ACID-84	FEMA 27390
L-ASCORBIC ACID-85	FEMA 27390
L-ASCORBIC ACID-86	FEMA 27390
L-ASCORBIC ACID-87	FEMA 27390
L-ASCORBIC ACID-88	FEMA 27390
L-ASCORBIC ACID-89	FEMA 27390
L-ASCORBIC ACID-90	FEMA 27390
L-ASCORBIC ACID-91	FEMA 27390
L-ASCORBIC ACID-92	FEMA 27390
L-ASCORBIC ACID-93	FEMA 27390
L-ASCORBIC ACID-94	FEMA 27390
L-ASCORBIC ACID-95	FEMA 27390
L-ASCORBIC ACID-96	FEMA 27390
L-ASCORBIC ACID-97	FEMA 27390
L-ASCORBIC ACID-98	FEMA 27390
L-ASCORBIC ACID-99	FEMA 27390
L-ASCORBIC ACID-100	FEMA 27390

EXTRACTS WITH DIFFERENT SPELLING ON FEMA & CAS LISTS APPROVED 1972

HYTHIOPHOSPHAZIDENE		FEMA 27670
HYTHIOPHOSPHAZINE		FEMA 27670
HYVALERIC ACID		FEMA 27670
HYDROXY-ETHYL-BENZEN		FEMA 27670
HYDROXYMETHYL		FEMA 27670
HYDROXYMETHYL	NAS 0443	
HYDROXYMETHYL	NAS 0293	
HYDROXYMETHYL	NAS 0458	
HYDROXYMETHYL	NAS 0458	
HYDROXYMETHYL	NAS 0316	
HYDROXYMETHYL	NAS 0374	
HYDROXYMETHYL	NAS 0471	
HYDROXYMETHYL		FEMA 2755C
HYDROXYMETHYL	NAS 0471	
HYDROXYMETHYL	NAS 0471	
HYDROXYMETHYL	NAS 0471	FEMA 2679
HYDROXYMETHYL	NAS 0471	FEMA 2671
HYDROXYMETHYL	NAS 0129	FEMA 2668
HYDROXYMETHYL	NAS 0130	FEMA 2611
HYDROXYMETHYL	NAS 0294	
HYDROXYMETHYL	NAS 0130	FEMA 2611
HYDROXYMETHYL	NAS 0129	FEMA 2640
HYDROXYMETHYL	NAS 0131	
HYDROXYMETHYL	NAS 0030	
HYDROXYMETHYL	NAS 0132	
HYDROXYMETHYL	NAS 0133	FEMA 2623
HYDROXYMETHYL	NAS 0134	FEMA 2754
HYDROXYMETHYL	NAS 0161	
HYDROXYMETHYL	NAS 0135	
HYDROXYMETHYL	NAS 0140	FEMA 2614
HYDROXYMETHYL		FEMA 2757C
HYDROXYMETHYL		FEMA 2656C
HYDROXYMETHYL		FEMA 2758C
HYDROXYMETHYL		FEMA 2619
HYDROXYMETHYL		FEMA 2759A
HYDROXYMETHYL		FEMA 2677
HYDROXYMETHYL		FEMA 2760A
HYDROXYMETHYL		FEMA 2645
HYDROXYMETHYL		FEMA 2761A
HYDROXYMETHYL		FEMA 2717D
HYDROXYMETHYL		FEMA 2762D
HYDROXYMETHYL		FEMA 2764E
HYDROXYMETHYL		FEMA 2765C
HYDROXYMETHYL		FEMA 2766C
HYDROXYMETHYL		FEMA 2657C
HYDROXYMETHYL		FEMA 2674T
HYDROXYMETHYL		FEMA 2767C
HYDROXYMETHYL		FEMA 2769E
HYDROXYMETHYL	NAS 0485	FEMA 2769E
HYDROXYMETHYL	NAS 0485	FEMA 2769
HYDROXYMETHYL		FEMA 2670D
HYDROXYMETHYL		FEMA 2770D
HYDROXYMETHYL		FEMA 2771A
HYDROXYMETHYL		FEMA 2772D

SUBSTANCE WITH DIFFERENT SPELLING OR FORM & HAS LIS APPEARS TWICE

ACETATE		FEMA	27730
ACETATE		FEMA	27749
ACETATE		FEMA	27753
ACETATE		FEMA	27750
ACETATE		FEMA	27780
ACETATE		FEMA	27770
ACETATE	NAS 0136		
ACETATE	NAS 0137		
ACETATE	NAS 0138		
ACETATE	NAS 0139	FEMA	27794
ACETATE		FEMA	28175
ACETATE		FEMA	3465F
ACETATE		FEMA	3466F
ACETATE		FEMA	27409
ACETATE		FEMA	27410
ACETATE		FEMA	27420
ACETATE		FEMA	27840
ACETATE		FEMA	3467F
ACETATE		FEMA	27840
ACETATE		FEMA	356A
ACETATE		FEMA	27850
ACETATE		FEMA	2786C
ACETATE		FEMA	3212E
ACETATE		FEMA	3468F
ACETATE		FEMA	27880
ACETATE		FEMA	27890
ACETATE		FEMA	27910
ACETATE		FEMA	27909
ACETATE		FEMA	26099
ACETATE	NAS 0431	FEMA	2792A
ACETATE		FEMA	2794A
ACETATE		FEMA	3529
ACETATE		FEMA	2794C
ACETATE		FEMA	2795C
ACETATE		FEMA	2799C
ACETATE	NAS 0910		
ACETATE		FEMA	33590
ACETATE		FEMA	2714F
ACETATE		FEMA	27950
ACETATE		FEMA	27950
ACETATE		FEMA	27950
ACETATE	NAS 0058	FEMA	27990
ACETATE		FEMA	27990
ACETATE		FEMA	28310
ACETATE		FEMA	3362D
ACETATE		FEMA	29040
ACETATE		FEMA	20220
ACETATE		FEMA	24060
ACETATE		FEMA	3362D
ACETATE		FEMA	3425E
ACETATE		FEMA	3469F
ACETATE		FEMA	28350
ACETATE		FEMA	20960

INCORRECT WITH DIFFERENT SPELLING ON FEPA & HAS LISTED APPROX INDEX

BYE LACAPSIN	FEPA 2837A
ICH FLOWED EXT	FEPA 3494
ICA FLOWED NEAR EXT	FEPA 3367C
ICBY OIL	FEPA 2829C
IC LEAVES	FEPA 3168C
IT OIL	NAS 0391
IN	NAS 0335
IR, UNREFINED	NAS 0437
IRISH	NAS 0392
IRONYL OIL	FEPA 2419C
IRONYL LACTONE	FEPA 2050D
IRONYL DIENE	FEPA 3217E
IRONYL DIONE	FEPA 2841D
IRONYL	FEPA 28-2D
IRONYL-1-OL	FEPA 3470F
IRONYL-2-OL	FEPA 3471F
IRONYL-3-OL	FEPA 3467D
IRONYL	FEPA 3218E
IRONYL ACID	FEPA 2842D
IR, BLACK	FEPA 2844A
IR, BLACK, OIL	FEPA 2845A
IR, BLACK, GORES	FEPA 2846A
IR, RED	FEPA 2849A
IR, RED, GORES SIN	FEPA 3537
IR, WHITE	FEPA 2850A
IR, WHITE, OIL	FEPA 2851A
IR, WHITE, GORES	FEPA 2852A
IRONYL LEAVES	FEPA 2867A
IRONYL OIL	FEPA 2963A
IR	NAS 0296
IR	NAS 0297
IR, UNREFINED	FEPA 3440D
IRONYL ACETATE	FEPA 3370D
IRONYL LEMON OIL	FEPA 2853A
IRONYL MIND OIL	FEPA 2854A
IRONYL OIL	FEPA 2855A
IRONYL	NAS 0528
IRONYL O, O'HEPVL ACET	FEPA 2877D
IRONYL ACETATE	FEPA 2869D
IRONYL-1-PROP PYRAZOL	FEPA 3474F
IRONYL ACETATE	FEPA 2897D
IRONYL ALCOHOL	FEPA 2898D
IRONYL ANTHRANILATE	FEPA 2899D
IRONYL ANIMATE	FEPA 2903D
IRONYL BUTYRATE	FEPA 2861D
IRONYL CINNAMATE	FEPA 2863D
IRONYL CINNAMATE	FEPA 2864D
IRONYL CROCIDATE	FEPA 3221C
IRONYL ISOBUTYRATE	FEPA 2862D
IRONYL ISOVALERATE	FEPA 2871D
IRONYL OCTANOATE	FEPA 3222E
IRONYL OXYVALERATE	FEPA 2866D
IRONYL PROPIONATE	FEPA 2865D

2677 GRAS PHAS II CONTAINED FEMA & NAS SUBSTANCE LIST - ALPHABETIC
 (SUBSTANCE WITH DIFFERENT SPELLING ON FEMA & NAS LISTS APPEARS TWICE)

ACETONE		FEMA 2408D
ACETIC ACID		FEMA 1374F
ACID		FEMA 2930D
ACRYLAMIDE		FEMA 3497F
ACRYLONITRILE		FEMA 3440F
ACRYLONITRILE		FEMA 2910D
ACRYLONITRILE		FEMA 2011D
ACRYLONITRILE		FEMA 2912D
ACRYLONITRILE		FEMA 2913D
ACRYLONITRILE		FEMA 2701F
ACRYLONITRILE		FEMA 2915A
ACRYLONITRILE		FEMA 3375D
ACRYLONITRILE		FEMA 2919D
ACRYLONITRILE	NAS 0393	FEMA 2914D
ACRYLONITRILE	NAS 0535	
ACRYLONITRILE	NAS 0394	FEMA 2917D
ACRYLONITRILE		FEMA 2918A
ACRYLONITRILE		FEMA 3376C
ACRYLONITRILE		FEMA 2919A
ACRYLONITRILE		FEMA 2920D
ACRYLONITRILE	NAS 0395	
ACRYLONITRILE	NAS 0140	
ACRYLONITRILE	NAS 0147	
ACRYLONITRILE	NAS 0148	
ACRYLONITRILE	NAS 0149	
ACRYLONITRILE	NAS 0900	
ACRYLONITRILE	NAS 0150	
ACRYLONITRILE	NAS 0151	
ACRYLONITRILE	NAS 0152	
ACRYLONITRILE	NAS 0300	
ACRYLONITRILE	NAS 0153	
ACRYLONITRILE	NAS 0154	
ACRYLONITRILE	NAS 0301	
ACRYLONITRILE	NAS 0302	
ACRYLONITRILE	NAS 0346	
ACRYLONITRILE	NAS 0155	
ACRYLONITRILE	NAS 0156	
ACRYLONITRILE	NAS 0303	
ACRYLONITRILE	NAS 0536	
ACRYLONITRILE	NAS 0157	
ACRYLONITRILE	NAS 0304	
ACRYLONITRILE	NAS 0305	
ACRYLONITRILE	NAS 0307	
ACRYLONITRILE	NAS 0158	FEMA 2921
ACRYLONITRILE	NAS 0159	
ACRYLONITRILE	NAS 0308	
ACRYLONITRILE	NAS 0538	
ACRYLONITRILE	NAS 0539	
ACRYLONITRILE		FEMA 3411
ACRYLONITRILE	NAS 0161	FEMA 3377
ACRYLONITRILE	NAS 0161	FEMA 3377
ACRYLONITRILE	NAS 0162	
ACRYLONITRILE	NAS 0160	

7/20/77 GRAS FEMA II LISTING FEMA & CAS NUMBERS (SEE "REVISIONS")
 (SUBSTANCE WITH DIFFERENT SPELLING ON FEMA & CAS LISTS APPEARS TWICE)

ACRYLIC ANHYDRIDE	FEMA 3277E
ACRYLONITRILE	FEMA 2922D
ACRYLAMIDE	FEMA 2923D
ACRYLIC ACID	FEMA 2924
ACRYLIC ACETATE	FEMA 2925D
ACRYLIC ALCOHOL	FEMA 2926D
ACRYLIC ANISOLE	FEMA 2927D
ACRYLIC ANTHRACENE	FEMA 2928D
ACRYLIC BENZYLATE	FEMA 2929D
ACRYLIC BUTYLATE	FEMA 2930D
ACRYLIC CAPRYLATE	FEMA 2931D
ACRYLIC CECYLATE	FEMA 2932D
ACRYLIC CHLORIDE	FEMA 2933D
ACRYLIC COCAINE	FEMA 2934D
ACRYLIC DODECYLATE	FEMA 2935D
ACRYLIC DODECYLENE	FEMA 2936D
ACRYLIC DODECYLENE	FEMA 2937D
ACRYLIC DODECYLENE	FEMA 2938D
ACRYLIC DODECYLENE	FEMA 2939D
ACRYLIC DODECYLENE	FEMA 2940D
ACRYLIC DODECYLENE	FEMA 2941D
ACRYLIC DODECYLENE	FEMA 2942D
ACRYLIC DODECYLENE	FEMA 2943D
ACRYLIC DODECYLENE	FEMA 2944D
ACRYLIC DODECYLENE	FEMA 2945D
ACRYLIC DODECYLENE	FEMA 2946D
ACRYLIC DODECYLENE	FEMA 2947D
ACRYLIC DODECYLENE	FEMA 2948D
ACRYLIC DODECYLENE	FEMA 2949D
ACRYLIC DODECYLENE	FEMA 2950D
ACRYLIC DODECYLENE	FEMA 2951D
ACRYLIC DODECYLENE	FEMA 2952D
ACRYLIC DODECYLENE	FEMA 2953D
ACRYLIC DODECYLENE	FEMA 2954D
ACRYLIC DODECYLENE	FEMA 2955D
ACRYLIC DODECYLENE	FEMA 2956D
ACRYLIC DODECYLENE	FEMA 2957D
ACRYLIC DODECYLENE	FEMA 2958D
ACRYLIC DODECYLENE	FEMA 2959D
ACRYLIC DODECYLENE	FEMA 2960D
ACRYLIC DODECYLENE	FEMA 2961D
ACRYLIC DODECYLENE	FEMA 2962D
ACRYLIC DODECYLENE	FEMA 2963D
ACRYLIC DODECYLENE	FEMA 2964D
ACRYLIC DODECYLENE	FEMA 2965D
ACRYLIC DODECYLENE	FEMA 2966D
ACRYLIC DODECYLENE	FEMA 2967D
ACRYLIC DODECYLENE	FEMA 2968D
ACRYLIC DODECYLENE	FEMA 2969D
ACRYLIC DODECYLENE	FEMA 2970D
ACRYLIC DODECYLENE	FEMA 2971D
ACRYLIC DODECYLENE	FEMA 2972D
ACRYLIC DODECYLENE	FEMA 2973D
ACRYLIC DODECYLENE	FEMA 2974D
ACRYLIC DODECYLENE	FEMA 2975D
ACRYLIC DODECYLENE	FEMA 2976D
ACRYLIC DODECYLENE	FEMA 2977D
ACRYLIC DODECYLENE	FEMA 2978D
ACRYLIC DODECYLENE	FEMA 2979D
ACRYLIC DODECYLENE	FEMA 2980D
ACRYLIC DODECYLENE	FEMA 2981D
ACRYLIC DODECYLENE	FEMA 2982D
ACRYLIC DODECYLENE	FEMA 2983D
ACRYLIC DODECYLENE	FEMA 2984D
ACRYLIC DODECYLENE	FEMA 2985D
ACRYLIC DODECYLENE	FEMA 2986D
ACRYLIC DODECYLENE	FEMA 2987D
ACRYLIC DODECYLENE	FEMA 2988D
ACRYLIC DODECYLENE	FEMA 2989D
ACRYLIC DODECYLENE	FEMA 2990D
ACRYLIC DODECYLENE	FEMA 2991D
ACRYLIC DODECYLENE	FEMA 2992D
ACRYLIC DODECYLENE	FEMA 2993D
ACRYLIC DODECYLENE	FEMA 2994D
ACRYLIC DODECYLENE	FEMA 2995D
ACRYLIC DODECYLENE	FEMA 2996D
ACRYLIC DODECYLENE	FEMA 2997D
ACRYLIC DODECYLENE	FEMA 2998D
ACRYLIC DODECYLENE	FEMA 2999D
ACRYLIC DODECYLENE	FEMA 3000D

72677

UNOS FROM 11 COMBINED FROM 4 AND 5 UNDER 11
 ESUBSTANCE WITH DIFFERENT SPELLING ON FEMA & NAS LISTS APPEARS TWICE

IRINE SULFATE		FEMA 29756
IRINE HYDROCHLORIDE		FEMA 29756
IRINE SULFATE		FEMA 29756
IRINE - MANGNE	NAS 0506	
IRINE - PINE	NAS 0505	
IRINE STEARIC HYDROGENAT	NAS 0637	
IRINE SODIUM	NAS 0628	FEMA 3361C
IRINE		
IRINE EXTRACT		FEMA 2979C
IRINE		FEMA 2980D
IRINE SULFATE		FEMA 2981D
IRINE NITRATE		FEMA 2982D
IRINE FORMATE		FEMA 2984D
IRINE ISOSULFATE		FEMA 2983D
IRINE ISOSULFATE		FEMA 2987D
IRINE ISOSULFATE		FEMA 2985D
IRINE ISOSULFATE		FEMA 2986D
IRINE ACET		FEMA 3362C
IRINE	NAS 0169	
IRINE 5-PHOSPHATE	NAS 0170	
IRINE	NAS 0676	
IRINE	NAS 0687	
IRINE	NAS 0512	
IRINE OIL		FEMA 2969A
IRINE EXTRACT		FEMA 2990A
IRINE		FEMA 3236C
IRINE STYDGEN		FEMA 2997A
IRINE		FEMA 2988A
IRINE		FEMA 3383C
IRINE		FEMA 2991A
IRINE OIL		FEMA 2992A
IRINE STYDGEN		FEMA 3003
IRINE		FEMA 3364C
IRINE		FEMA 2994A
IRINE		FEMA 2995A
IRINE	NAS 0675	
IRINE	NAS 0379	FEMA 2996D
IRINE	NAS 0512	
IRINE WHITE	NAS 0554	
IRINE	NAS 0676	
IRINE	NAS 0511	
IRINE	NAS 0171	FEMA 3672
IRINE CALCIUM	NAS 0054	FEMA 3528
IRINE SODIUM SALT	NAS 0203	FEMA 3487
IRINE		FEMA 2998A
IRINE EXTRACT		FEMA 2999A
IRINE		FEMA 3002A
IRINE		FEMA 3001A
IRINE		FEMA 3092A
IRINE		FEMA 3307A
IRINE OIL		FEMA 3004D
IRINE		FEMA 3366C
IRINE		FEMA 3005C

(SUBSTANCE WITH DIFFERENT SPELLING OR FEMA & NAS LETTERS APPEARS TWICE)

ACID CITRATE	NAS 0149	FEMA 1026
ACID CRYST	NAS 0156	
ACID DIACETATE	NAS 0190	FEMA 3512
ACID FORTHINATE	NAS 0317	
ACID FORTHINATE	NAS 0318	
ACID FORTHINATE	NAS 0191	
ACID HYDROPHOSPHATE	NAS 0194	FEMA 1027
ACID HYDROPHOSPHATE	NAS 0207	
ACID HYDROPHOSPHATE	NAS 0119	
ACID HYDROPHOSPHATE	NAS 0211	
ACID LACTIC	NAS 0155	
ACID LACTATE	NAS 0403	
ACID LACTYL SULFATE	NAS 2347	
ACID LACTYL SULFATE	NAS 0243	
ACID LACTYL SULFATE	NAS 3144	FEMA 3027
ACID LACTYL SULFATE	NAS 1121	
ACID LACTYL SULFATE	NAS 0342	FEMA 3551
ACID LACTYL SULFATE	NAS 0343	FEMA 3552
ACID LACTYL SULFATE	NAS 0322	
ACID LACTYL SULFATE	NAS 0156	
ACID LACTYL SULFATE	NAS 0195	
ACID LACTYL SULFATE DI	NAS 0157	FEMA 2304
ACID LACTYL SULFATE MONO	NAS 0132	FEMA 3554
ACID LACTYL SULFATE TRI	NAS 0199	
ACID POTASSIUM TARTRATE	NAS 0240	
ACID PHTHALATE	NAS 0291	FEMA 3513
ACID PHTHALATE THREA	NAS 0702	FEMA 3625
ACID PHTHALATE THREA	NAS 0702	FEMA 3625
ACID PHTHALATE THREA	NAS 0234	FEMA 2307
ACID PHTHALATE THREA	NAS 0704	
ACID PHTHALATE	NAS 0205	
ACID PHTHALATE	NAS 0256	
ACID PHTHALATE	NAS 0237	
ACID PHTHALATE	NAS 0238	
ACID PHTHALATE	NAS 0239	
ACID PHTHALATE	NAS 0210	FEMA 3553
ACID PHTHALATE METASILICATE	NAS 0323	
ACID PHTHALATE	NAS 0211	FEMA 3551
ACID PHTHALATE	NAS 0211	FEMA 3624
ACID PHTHALATE	NAS 0211	FEMA 3391F
ACID PHTHALATE	NAS 0401	FEMA 3392D
ACID PHTHALATE	NAS 0401	FEMA 3028D
ACID PHTHALATE	NAS 0401	FEMA 3028
ACID PHTHALATE	NAS 0212	FEMA 3029
ACID PHTHALATE	NAS 0212	FEMA 3029
ACID PHTHALATE	NAS 0434	
ACID PHTHALATE	NAS 0324	
ACID PHTHALATE ISOLATE	NAS 0446	
ACID PHTHALATE	NAS 0486	
ACID PHTHALATE	NAS 0407	FEMA 3695
ACID PHTHALATE	NAS 0407	FEMA 3030
ACID PHTHALATE	NAS 0407	FEMA 3031A
ACID PHTHALATE	NAS 0407	FEMA 3032A

(SUBSTANCE WITH DIFFERENT SPELLING ON FEMA & NAS LISTS APPEARS TWICE)

IME LAVENDER OIL		FEMA 3033A
IMMUNO PXT.		FEMA 3053
IMMUNO EXTRACT		FEMA 3312F
IMMUNO OIL		FEMA 3034C
JOHNS PEPP SOLID.EXT.		FEMA 3594
JOHNSWAT		FEMA 3385C
K-NIC CALORIDE	NAS 0496	
K-NIC CALORIDE	NAS 0213	
K-CM	N/S 0602	
K-CM MODIFIER	NAS 0325	
KRBA DISTILLATE	NAS 0326	
KRBA ACID	NAS 0479	FEMA 3035E
KRBA ALCOHOL. +RESWAR	NAS 0327	
KRBA CITRATE	NAS 0214	
KRBA GUM	NAS 0215	FEMA 2635
KRBA		FEMA 3036C
KRBA JUICE		FEMA 3696
KRBA EXTRACT		FEMA 3337C
KRBA OIL		FEMA 3697
KRBA		FEMA 3233D
KRBA ACID	NAS 0216	FEMA 3569
KRBA AMYRIDE	NAS 0439	
KRBA	NAS 0328	
KRBA ACET ISOMYRATE		FEMA 3394F
KRBA LIQUID	NAS 0480	
KRBA ACETACETATE		FEMA 3039D
KRBA-INVERT SUGAR BLND	NAS 0492	
KRBA SOLID. EXT.		FEMA 3698
KRBA STIMUL. ALKAL.	NAS 0329	
KRBA DICRYDE	NAS 0217	FEMA 3039
KRBA ACID	NAS 0218	
KRBA-CADIPRAL		FEMA 3135E
KRBA OIL		FEMA 3340C
KRBA	NAS 0567	
KRBA HYDROGENATED	NAS 0298	
KRBA EXTRACT		FEMA 3395A
KRBA		FEMA 3645
KRBA ESSENCE. MAT		FEMA 3341
KRBA OIL		FEMA 3041A
KRBA ACID	NAS 0330	FEMA 3042A
KRBA		FEMA 3326E
KRBA STARCH	NAS 0444	
KRBA		FEMA 3043A
KRBA ACID	NAS 0219	FEMA 3045
KRBA-LIC ACID	NAS 0220	
KRBA OIL		FEMA 3349F
KRBA EXTRACT		FEMA 3357A
KRBA-TERPENE		FEMA 3341D
KRBA-TERPENE		FEMA 3673
KRBA-TERPENE		FEMA 3342D
KRBA-TERPENE		FEMA 3345D
KRBA-TERPENE		FEMA 3399D
KRBA-TERPENE		FEMA 3046D

776777 GRAS PLUS II COMBINED FEMA & NAS SUBSTANCE LIST - - ALPHABETIC
 (SUBSTANCE WITH DIFFERENT SPELLING ON FEMA & NAS LISTS APPEARS TWICE)

LILLY ALCOHOL		FEMA 3463F
LILLY	NAS 0236	
LILLY	NAS 0433	FEMA 3106A
LILLY EXTRACT	NAS 0442	FEMA 3102A
LILLY CLEFRESIN		FEMA 3106A
LILLY	NAS 0336	FEMA 3107E
LILLY ACETATE		FEMA 3106D
LILLY ACETONE		FEMA 3464F
LILLY OIL	NAS 0476	FEMA 3722
LILLY OIL, HYDROGENAT	NAS 0438	
LILLY OIL		FEMA 3106D
LILLY		FEMA 3470F
LILLY		FEMA 3474C
LILLY		FEMA 3405C
LILLY		FEMA 3472C
LILLY		FEMA 3476
LILLY ACETATE		FEMA 3465F
LILLY	NAS 0403	
LILLY		FEMA 3748E
LILLY		FEMA 2749D
LILLY ABSOL		FEMA 3102A
LILLY		FEMA 3407C
LILLY A	NAS 0241	
LILLY ACETATE	NAS 0242	
LILLY, B, PALMITATE	NAS 0243	
LILLY B COMPLEX & SYRUP	NAS 0331	
LILLY B12	NAS 0244	
LILLY B2	NAS 0245	
LILLY B3	NAS 0246	
LILLY B	NAS 0447	
LILLY EXTRACT		FEMA 3112C
LILLY	NAS 0346	
LILLY SHELLAC	NAS 0332	
LILLY STARCH	NAS 0404	
LILLY	NAS 0352	
LILLY	NAS 0462	
LILLY VITAL	NAS 0465	
LILLY	NAS 0406	
LILLY, RICH, MILLED	NAS 0513	
LILLY, DEMINERALIZED	NAS 0483	
LILLY, DRIED	NAS 0463	
LILLY, ELCTRODIALYZED	NAS 0491	
LILLY, OVER DRYING & DELACT	NAS 0486	
LILLY, EXTRACT		FEMA 3112B
LILLY, OIL		FEMA 3113C
LILLY, SHEET		FEMA 3428C
LILLY		FEMA 3114C
LILLY, EXTRACT		FEMA 3115C
LILLY, OIL		FEMA 3116C
LILLY	NAS 0464	
LILLY	NAS 0544	
LILLY		FEMA 3249E

7777 FPMAS PHASE II CONDENSED FPMAS & CAS SUBSTANCE LIST - ALPHABETIC
 (SUBSTANCE WITH DIFFERENT SPELLING IN FPMAS & CAS LISTS APPEARS TWICE)

1 VATA		FEMA 3405F
2 VATA		FEMA 3117C
ST WATSON	PAS 0481	FEMA 3521
ST P. STAN	PAS 0405	
ST. INDICIVE (LAV)	PAS 0409	
STG.	PAS 0333	
STG. AUTOLYSED	PAS 0305	
ST. SUTTA, PLO EXTP.		FEMA 3119C
ST. SUTTA, SIL EXTRACT		FEMA 3119B
ST. J. SUTTA T-1-E	PAS 0339	FEMA 3120C
ST. J. SUTTA, EXTRACT		FEMA 3121C
ST. J. SUTTA, EXTRACT		FEMA 3122A
ST. J. SUTTA, EXTRACT		FEMA 3122B
ST. J. SUTTA, EXTRACT	PAS 0334	
ST. J. SUTTA, EXTRACT	PAS 0247	
ST. J. SUTTA, EXTRACT	PAS 0248	
ST. J. SUTTA, EXTRACT	PAS 0249	
ST. J. SUTTA, EXTRACT	PAS 0250	
ST. J. SUTTA, EXTRACT	PAS 0251	
ST. J. SUTTA, EXTRACT		FEMA 3124D

ALPHABETIZED LIST OF DIRECT FOOD ADDITIVES (NONFLAVORS)

TAB C

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ALPHABETIC LISTING -		CFR		
FDG OR PART	NAME	SECTION NO	TECHNICAL EFFECTS	ADN
1326	C ACETONE	121.1062	25	0023
5302	C ACETONE PEROXIDES	121.1022	12 05	0056
5001	A ACETYLATED MONOLYCERIDES	121.1010	(SEE NOTE 5A)	0069
5326	C ACRYLAMID-ACRYLIC ACID RESIN (HYDROLYSED POLYACRYLAMIDE)	121.1092 A	22	0075
5301	C ACRYLAMIDE-BOOTHEN ACRYLATE RESIN	121.1080	02	0081
5307	C ACRYLATE ACRYLAMIDE RESINS	121.1092	22	0092
0004	A L-ALANINE	(SEE NOTE 1A)	(SEE NOTE 5A)	0115
		121.1002	15	0130
		(SEE NOTE 2A)	(SEE NOTE 5A)	0141
		(SEE NOTE 3A)	(SEE NOTE 5A)	0207
		(SEE NOTE 3A)	(SEE NOTE 5A)	0230
		(SEE NOTE 3A)	(SEE NOTE 5A)	0253
5306	C ALIPHATIC ACID MIXTURE	121.1091	30	0276
5003	A ALUMINUM CAPRYLATE	121.1071	13 07 01	0299
5304	C ALUMINUM OXIDE ADSORBS OF ALKYL ALCOHOLS/PHOSPHATE SALTS OF ALUMINUM OXIDE ADSORBS OF ALKYL ALCOHOLS, MIXTURE OF	121.1077	20	0311
5004	A ALUMINUM CAPRYLATE	121.1071	13 07 01	0322
5005	A ALUMINUM CAPRYLATE	121.1071	13 07 01	0305
5006	A ALUMINUM SEBACATE	121.1071	13 07 01	0308
5007	A ALUMINUM NICOTINATE	121.1101	15	0301

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2. ALPHABETIC LISTING - GENERAL PARTS A-C AND D

SUB NR	PART NAME	REFUG NR	TECHNICAL EFFECTS	ASN
5008	A ALUMINUM OLEATE	121.1071	13 07 01	0418
5009	A ALUMINUM PALMITATE	121.1071	13 07 01	0437
		123.20	14	0460
0539	A ALUMINUM STEARATE	121.1099 A 3 121.1071	27 13 07 01	0506
		121.1002	19	0529
0012	C ANIONIC ALGINATE	121.1083	82	0552
		(SEE NOTE 2A)	(SEE NOTE 5A)	0575
		(SEE NOTE 4A)	(SEE NOTE 5A)	0598
5394	C ANTI-OXIDIZ. COAL, SULFONATED	121.1188 A 2	43	0656
3254	A ARABINOGALACTAN	121.1174	07 26 13	0713
3254	C ARABINOGALACTAN	121.1219 A 2	13	0736
0021	A L-ARGININE	121.1002	19	0759
0352	A L-ASPARAGINE	121.1002	19	0782
5016	A ASPARTAME	121.1256	40 10	0805
0025	A L-ASPARTIC ACID	121.1002	19	0828
0496	A AZODICARBONAMIDE	121.1085	12 05	0851
5019	A BAKER'S YEAST GLYCAN	121.1262	07 26 30	0874
5020	A BAKER'S YEAST PROTEIN	121.1261	19	0897
		(SEE NOTE 3A)	(SEE NOTE 5A)	0920
		(SEE NOTE 3A)	(SEE NOTE 5A)	0943
5021	A BHA	121.1094 A 3 121.1035	27 02	0966

2. ADJUVANTS LISTING - OVERALL, PARTS A, C, AND D

SUB NR	PART	NAME	SECTION NR	TECHNICAL EFFECTS	AMP
5022	A	BHT	121.1099 A 3 121.1034	27 02	0800
					1012
5023	A	REFINED VEGETABLE OIL	121.1098	02	1050
5026	A	N-BUTOXYPOLY(ETHYLENE POLY- OXYPROPYLENE GLYCOL)	121.1099 A 4	27	1001
5027	A	BUTYL STEARATE	121.1099 A 3	27	1104
5506	A	1,3-BUTYLENE GLYCOL	121.1176	25 11	1127
5026	A	TEET-NUTLYHYDROQUINONE	121.1259	02	1150
			(SEE NOTE 4A)	(SEE NOTE 5A)	1173
5020	A	CALCIUM CAPRATE	121.1071	13 07 01	1196
5029	A	CALCIUM CAPRYLATE	121.1071	13 07 01	1219
0532	A	CALCIUM DIBOHOIN EDTA	121.1017	03 11 20 32 21 26	1242
5031	A	CALCIUM FIRMATE	121.1130	19	1263
			(SEE NOTE 4A)	(SEE NOTE 5A)	1270
5032	A	CALCIUM LACTOIONATE	121.1162	09	1311
5033	A	CALCIUM LAURATE	121.1071	13 07 01	1334
5026	A	CALCIUM MYRISTATE	121.1071	13 07 01	1357
5035	A	CALCIUM OLEATE	121.1071	13 07 01	1380
5036	A	CALCIUM PALMITATE	121.1071	13 07 01	1403
5037	A	CALCIUM PANTOTHENATE, CALCIUM CHLORIDE DOUBLE SALT	121.1027	10	1426
			(SEE NOTE 4A)	(SEE NOTE 5A)	1449

2. ALPHANUMERIC LISTING - OVERALL FACTS A-C, AND D

DSB NR	PART	NAME	SECTION NR	TECHNICAL EFFECTS	ASN
5055	A	CARRAGEENAN, CALCIUM SALT OF, WITH POLYSORBATE 80	121.1193	07 26	1932
5058	A	CARRAGEENAN, POTASSIUM SALT OF	121.1067	07 26	1955
5056	A	CARRAGEENAN, POTASSIUM SALT OF, WITH POLYSORBATE 80	121.1193	07 26	1978
			121.1067	07 26	2001
5053	A	CARRAGEENAN SALTS WITH POLY- SILICATE 89	121.1193	07 26	2024
5051	A	CARRAGEENAN, SODIUM SALT OF	121.1067	07 26	2047
5057	A	CARRAGEENAN, SODIUM SALT OF, WITH POLYSORBATE 80	121.1193	07 26	2070
5052	A	CARRAGEENAN WITH POLYSORBATE 80	121.1193	07 26	2073
5050	A	CAROTEN OIL	121.1079	17 20	2116
5060	C	CETYL ALCOHOL, SYNTHETIC	121.1238	33	2162
			121.1021	34	2185
5061	A	CINDING GUM BASE	121.1059	(SEE NOTE 6A)	2208
			(SEE NOTE 2A)	(SEE NOTE 3A)	2211
			(SEE NOTE 2A)	(SEE NOTE 5A)	2254
5110	C	CHLOROPENTALIMBOLTHANE	121.1101	21	2277
			121.1172	28	2300
5116	C	CODALY SULFATE	121.1000	02	2321
			121.1224	11	2344
5118	D	COMBUSTION PRODUCT GAS	121.1060	02	2364

2. ALPHABETIC LISTING - CHEMICAL, INACTIVE, MSR, M

MSR NO.	PART	NAME	SECTION No	TECHNICAL EFFECTS	ASH
5076	A	ETHYLATED FOOD- AND DIGLYCERIDES (POLYETHYLENE (20) MONO- AND DIGLYCERIDES OF FATTY ACIDS)	121.1221	05 07	2690
5077	A	ETHOXYGEN	121.1001	02 01	2921
5078	A	ETHYL CELLULOSE	121.1007	11 13 20	2900
			121.210	10	2967
5320	C	ETHYLENE DICHLORIDE	121.1000	25	2790
			121.200	10	3013
5060	A	ETHYLENE OXIDE AND PROPYLENE OXIDE, COPOLYMER CONDENSATES OF	121.1275	11	3075
5079	A	ETHYLENE OXIDE POLYMER	141.1161	26	3076
			121.1070	17 13 27	3050
			121.1099 A 3	27	
			121.1179 B 2	20	
5081	A	FATTY ACIDS, SALTS OF	121.1071	13 07 01	3002
5002	C	FATTY ALCOHOLS, SYNTHETIC	121.1230	33	3105
			(SEE NOTE 2A)	(SEE NOTE 5A)	3120
			(SEE NOTE 6A)	(SEE NOTE 5A)	3151
			(SEE NOTE 6A)	(SEE NOTE 5A)	3170
			(SEE NOTE 6A)	(SEE NOTE 5A)	3197
5003	A	FERRIC FUMARATE	121.1120	77	3220
5004	A	FISH PROTEIN CONCENTRATE, WHOLE	121.1202	79	3266
0410	A	FOLIC ACID (POLACIN)	121.1130	70	3200
5006	A	FOOD STARCH-MODIFIED	121.1021	(SEE NOTE 5A)	3212

7. ALPHABETIC LISTING - OVERALL PARTS A-C, AND D

GEN NR	PART	NAME	SECTION NR	TECHNICAL EFFECTS*	ASH
5117	A	FOMALDEHYDE	121.1099 A 2 121.1099 A 3	27 27	3335
0302	A	FUMARIC ACID	121.1130	19	3350
5111	A	FURFURAL	121.1130	19	3391
5327	C	FUNGICIDS FOR PROCESSED GRAINS U.S.D. IN PRODUCTION OF FLOCCULATED MALT BEVERAGES	123.230	10	3404
0479	A	FURCELLARAN	121.1060	07 26	3527
5122	A	FURCELLARAN, AMMONIUM SALT OF	121.1069	07 26	3450
5123	A	FURCELLARAN, CALCIUM SALT OF	121.1069	07 26	3473
5124	A	FURCELLARAN, POTASSIUM SALT OF	121.1069	07 26	3496
5125	A	FURCELLARAN, SODIUM SALT OF	121.1069	07 26	3519
5125	A	FURCELLARAN, SODIUM SALT OF	121.1069	07 26	3542
5328	A	GIPPERTIC ACID	121.1010	35	3589
3295	A	L-GLUTAMIC ACID	121.1002	19	3611
0355	A	L-GLUTAMINE	121.1002	19	3634
5329	C	GUAFALONIDE	121.1219 A 3	13	3657
5129	A	GLYCERIN, SYNTHETIC	123.1111	(SEE NOTE 5A)	3680
5130	A	GLYCEROL ESTER OF WOOD ROBIN	121.1004	11	3703
5131	A	GLYCERYL-LACTO ESTERS OF FATTY ACIDS	121.1004	07 13	3726
3207	A	GLYCINE	121.1002	19	3749
5132	A	HEXYLAMINE	121.1257	10 26	3772
5133	A	HEXYLAMINE	121.1106	21	3772

7. ALPHABETIC LISTING - CURSILL, PARTS A, C, AND D

SUB NO	PART NAME	SECTION NO	TECHNICAL EFFECTS	ACT
		(SEE NOTE 2A)	(SEE NOTE 5A)	3795
5120	C. BILAMT	121.1045	25	3810
5134	C HEXYL ALCOHOL, SYNTHETIC	121.1238	33	3841
0896	A L-HISTIDINE	121.1062	19	3884
		121.1052	11	3887
5332	C HYDRAZINE	121.1090	02	3910
5133	A α -HYDRO- 1,2,3,4 -HYDROXY-POLY- (OXYETHYLENE) POLY (OXYPROPYLENE) - (51-63 MOLES) POLY (OXYETHYLENE) BLOCK COPOLYMER, AVG. MOL. WT. 9,760-13,290	121.1235 A 1	11	3933
5138	C α -HYDRO- 1,2,3,4 -HYDROXY-POLY- (OXYETHYLENE) POLY (OXYPROPYLENE) - (31-53 MOLES) POLY (OXYETHYLENE) - (10-16 MOLES) BLOCK COPOLYMER, AVG. MOL. WT. 3,500-8,125	121.1235 A 2	22 27	3956
5142	C α -HYDRO- 1,2,3,4 -HYDROXY-POLY (OXYETHYLENE) / POLY- (OXYPROPYLENE) (MIN. 1% MOLES) / POLY (OXYETHYLENE) BLOCK COPOLYMER, MIN. AVG. MOL. WT. 1,500	121.1235 A 3	27	3964
5145	A α -HYDRO- 1,2,3,4 -HYDROXY-POLY (OXYETHYLENE) POLY- (OXYPROPYLENE) (MIN. 5% MOLES) / POLY (OXYETHYLENE) BLOCK COPOLYMER, AVG. MOL. WT. 14,600	121.1235 A 4	05	3972
		121.200	19	3779
5139	A HYDROXYLATED LECITHIN	121.1099 A 3	27	4002
		121.1027	07	
5148	A α -HYDROXYETHYL-2,6-DI-TERT- BUTYLPEROXIDE	121.1200	02	4025
5141	A HYDROXYPROPYL CELLULOSE	121.1160	07 13 26	4048
6534	A HYDROXYPROPYL METHYLCELLULOSE	121.1021	03 13 26	4071

2. ALPHANUMERIC LISTING - OVERALL PAGES A-C, AND D

608 NR	PART	NAME	SECTION NR	TECHNICAL EFFECTS	ADN
5333	C	ION-EXCHANGE MEMBRANE	121.1180	22	8082
			121.1180	47	8090
5143	A	IRON AMMONIUM CITRATE	121.1190	01	8117
5149	A	IRON-CHOLINE CITRATE COMPLEX	121.1180	19	8160
8102	A	L-CITROLOXINE	121.1002	19	8163
5146	A	ISOPROPYL ALCOHOL	121.1099 A	3 27	8209
5146	C	ISOPROPYL ALCOHOL	121.1043	25	8233
			(SEE NOTE 3A)	(SEE NOTE 5A)	8255
5147	A	HELP	121.1149	19	8278
			(SEE NOTE 2A)	(SEE NOTE 5A)	8301
			(SEE NOTE 2A)	(SEE NOTE 5A)	8324
5148	A	LACTYLATED FATTY ACID ESTERS OF GLYCEROL AND PROPYLENE GLYCOL	121.1122	07 13 27	8347
5149	A	LACTYLIC ESTERS OF FATTY ACIDS	121.1048	07 13 27	8370
5150	A	LAMBIC ACID	121.1070	17 13 27	8393
5150	C	LAMBIC ACID	121.1070	31	8505
5151	C	LAMBYL ALCOHOL, SYNTHETIC	121.1230	33	8416
5277	A	L-LYSINE	121.1062	19	8439
5317	C	LIGNOSULFONIC ACID	121.1088	62	8462
			(SEE NOTE 8A)	(SEE NOTE 5A)	8520
8169	A	L-LYSINE	121.1062	19	8531
5154	A	MAGNESIUM CAPRATE	121.1071	13 87 91	8554

2. ALPHABETIC LISTING - OVERALL, PD-13 A,C, AND D

DBS OR PART NAME	SECTION NO	TECHNICAL EFFECTS	AGE
	(SEE NOTE 4A)	(SEE NOTE 5A)	5440
0121 A HOMOGLYCERIDE CITRATE	121.1026	02	5451
	(SEE NOTE 4A)	(SEE NOTE 5A)	5468
5350 C NORTHOLOINE	121.1088	02	5474
5176 A NORTHOLOINE SALTS OF FATTY ACIDS	121.1105	20	5497
5177 A HYBRISTIC ACID	121.1070	17 13 27	5520
5177 C HYBRISTIC ACID	121.1070	33	5531
5178 A HYBRISTIC ALCOHOL, SYNTHETIC	121.1238	33	5563
5179 A NICOTINAMIDE-ARABIC ACID COBLENCE	121.1095	19	5566
5351 C OCTADECYLAMINE	121.1088	02	5589
5352 C OCTYFLUOROCYCLOHEXANE	121.1065	23	5612
5180 C N-OCTYL ALCOHOL	121.1219 A 3	13	5625
5181 C OCTYL ALCOHOL, SYNTHETIC	121.1238	33	5658
5182 A OLEIC ACID	121.1070	17 13 27	5681
5182 C OLEIC ACID	121.1070	33	5693
5183 A OLEIC ACID, FROM TALL OIL FATTY ACIDS	121.1237 121.1179 B 2 121.1099 A 3	17 13 27 20 27	5704
5184 A OXYTETRAIN	121.1016 121.1099 A 3	17 17 27	5727
5185 A PALMITIC ACID	121.1070	17 13 27	5750
5185 C PALMITIC ACID	121.1070	33	5761
5186 A D-PALMITOERANOLIDE	121.1123	19	5796

2. ALPHABETIC LISTING - OVSALL, PARTS A.C. AND D

SUB NR	PART NAME	SECTION NR	TECHNICAL EFFECTS	ASV
5187	A PARAFFIN AND SUCCINIC DERIVATIVES, SYNTHETIC	121.1220	28	5819
		123.330	21	5842
		(SEE NOTE 2A)	(SEE NOTE 5A)	5965
		(SEE NOTE 2A)	(SEE NOTE 5A)	5888
5188	A PENTAERYTHRITOL ESTER OF MALIC AMHYDIDE-EMANIFIED WOOD ROBIN (ACID NO. 138-185; EMOP-GOSTER- INC POINT 1279-1379)	121.1175 B 2	28	5934
5189	A PENTAERYTHRITOL ESTER OF MALIC AMHYDIDE-EMANIFIED WOOD ROBIN (ACID NO. 176-184; EMOP-GOSTER- INC POINT 1100-1180)	121.1179 B 2	28	5957
0528	A PETROLATUM	121.1099 A 3 121.1166	27 17 28 27	5980
5335	C PETROLEUM HYDROCARBONS, ISOPARAFFINIC, SYNTHETIC	121.1154 121.1099 A 3	34 28 35 04 27	5993
5191	A PETROLEUM HYDROCARBONS, OLEFINIC, LIGHT	121.1099 A 3 121.1182	27 28 27 34 35	6003
5192	A PETROLEUM NAPHTHA	121.1179 B 4 121.1201	28 28	6026
5193	A PETROLEUM WAX	121.1099 A 3 121.1156	27 11 28 27	6049
5194	A PETROLEUM WAX, SYNTHETIC	121.1099 A 3 121.1209	27 11 28 27	6072
5354	C PHENOL-FORMALDEHYDE, CROSS-LINKED, ACTIVATED WITH TETRAETHYLENE- PYRAMINE	121.1168 A 7	43	6095
5355	C PHENOL-FORMALDEHYDE, CROSS-LINKED, ACTIVATED WITH TRIETHYLENE- TETRAMINE	121.1168 B 7	43	6118

2. ALIPHATIC LIGERS - OVERALL PARTS 5, C, AND D

SDS NR	PART	NAME	SECTION NR	TECHNICAL EFFECTS	ASH
5354	C	PHENOL-FORMALDEHYDE, CLAS-LINKED, ACTIVATED WITH TRIMETHYLENE- TETRAMINE AND TETRAETHYLENE- TETRAMINE	121.1108 A 7	43	6181
5383	C	PHENOL-FORMALDEHYDE, SULFITE- PAPERED CROSS LINKED	121.1108 A 3	43	6153
8100	A	L-PHENYLALANINE	121.1002	10	6160
8400	A	PERFORMIC ACID	(SEE NOTE 4A)	(SEE NOTE 5A)	6187
5357	C	POLYACRYLAMIDE	121.1091 121.1119	30 30	6210
5358	C	POLYACRYLAMIDE FIBER, MODIFIED	121.1192	22	6233
5196	A	POLYMETHYLIC ACID, SODIUM SALT	121.1092 A 2	27	6256
5198	A	POLYETHYLENE GLYCOL (D.W. 200-9,500)	121.1185 121.1092 A 7 121.1179 B 2 121.1179 B 3	13 26 11 26 13 17 27 24 28	6279
5190	C	POLYETHYLENE GLYCOL (D.W. 200-9,500)	121.1088	42	6302
5197	A	POLYETHYLENE, ORIMESAD	121.1142	28	6325
5199	A	POLYGLYCOL ESTERS OF FATTY ACIDS	121.1120	07 37	6308
5200	A	POLYETHYLENE 00 MONOMERATE	121.1099 A 2	27	6371
5201	A	POLYETHYLENE (000) DIOLATE	121.1099 A 3	27	6390
5202	A	POLYETHYLENE (000) MONO- DICUMULATE	121.1099 A 3	27	6417
5360	C	POLYPROPYLENE GLYCOL	121.1088	42	6440
5203	A	POLYPROPYLENE GLYCOL	121.1099 A 3	27	6463

2. ALPHABETIC LISTING - OVERALL, PARTS A, C, AND D

ENS IN	PART	NAME	SECTION NR	TECHNICAL EFFECTS	ASH
0393	A	POLYISOPRENE 60	121.1030	07 05 27 28	6486
			121.1099 A 2	27	
0535	A	POLYISOPRENE 65	121.1092 A 2	27	6508
			121.1008	07	
0394	A	POLYISOPRENE 60	121.1099	07 27	6512
			121.1099 A 3	27	
5318	C	POLYETHYLENE, CROSS-LINKED	121.1168 A 5	01	6548
5361	C	POLYVINYLFLUORIDE	121.1110	22	6555
5207	A	POLYVINYLPIRROLIDONE	121.1126	32 11 26 27 13	6578
			121.1179 B 3	28	
			(SEE NOTE 6A)	(SEE NOTE 5A)	6601
			(SEE NOTE 3A)	(SEE NOTE 5A)	6624
			(SEE NOTE 1C)	(SEE NOTE 2C)	6635
0299	C	POTASSIUM FOSPHATE	121.1194	35	6647
5363	C	POTASSIUM NITRIDE	121.1091	34	6670
5208	A	POTASSIUM CAPRYLATE	121.1071	11 07 01	6693
5209	A	POTASSIUM CAPRYLATE	121.1071	11 07 01	6716
0150	C	POTASSIUM CARBONATE	121.1088	02	6739
5210	A	POTASSIUM FERRIC FOSPHATE	121.1130	19	6762
5364	A	POTASSIUM GLUCONATE	121.1010	35	6785
			(SEE NOTE 6A)	(SEE NOTE 5A)	6808
			(SEE NOTE 6A)	(SEE NOTE 5A)	6831
			(SEE NOTE 2A)	(SEE NOTE 5A)	6854
0195	A	POTASSIUM IODIDE	121.1072	12	6877

2. ALPHABETIC LISTING - OVERALL, PARTS A-C, APP D

SUB NR	PART	NAME	SECTION NR	TECHNICAL EFFECTS	ASN
5212	A	POTASSIUM LAURATE	121.1071	13 07 01	6900
0929	A	POTASSIUM LACTATE	(SEE NOTE 1C)	(SEE NOTE 2C)	5911
5213	A	POTASSIUM MYRISTATE	121.1071	13 07 01	6923
0536	C	POTASSIUM NITRATE (FOR COD ROX)	121.1122	04	6946
5215	A	POTASSIUM OLATE	121.1071	13 07 01	6969
5216	A	POTASSIUM PALMITATE	121.1071	13 07 01	6992
5217	A	POTASSIUM PERNITRATE	121.1179 B 3 28		7013
0930	A	POTASSIUM PERMANGANATE	(SEE NOTE 5A)	(SEE NOTE 5A)	7038
5219	A	POTASSIUM STEARATE	121.1071 121.1099 A 3 27	13 07 01	7004
0388	C	POTASSIUM TRIPOLYPHOSPHATE	121.1098	02	7096
0931	A	POTASSIUM TRISULFATE	(SEE NOTE 6A)	(SEE NOTE 5A)	7099
3319	A	L-PROLINE	121.1002	19	7107
0397	B	PROPYLENE GLYCOL ALGINATE	121.1015 121.1099 A 3 27 121.1179 B 3 28	07 26	7130
5222	A	PROPYLENE GLYCOL MONO- AND DIESTERS OF FATTY ACIDS	121.1099 A 3 27 121.1113		7153
0932	A	PROPYLENE GLYCOL SEbacATE	121.100	18	7176
0933	A	PROPYLENE GLYCOL SEbacATE	(SEE NOTE 1A)	(SEE NOTE 5A)	7199
4373	A	PROPYLENE GLYCOL SEbacATE	121.1001	11	7268
4374	A	PROPYLENE GLYCOL SEbacATE	121.1001	11	7281
5366	C	RESIN, REACTION OF FORMALDEHYDE, ACETONE, AND TETRAHYDRO- PYRIMIDINE	121.1108 A 0 43		7360

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2. ALPHABETIC LISTING - WYDALL PAT'S, INC. AND P.

SYN NO	PART	NAME	SECTION NO	TECHNICAL EFFECTS	ASH
5226	A	RICE SHAW WAX	121.1998	28 13	7406
5038	A	ROSIN, PARTIALLY DIMERIZED, CALCIUM SALT OF	121.1179 D A 28		7420
5227	A	ROSIN, PARTIALLY HYDROGENATED	121.1179 D 2 28		7428
5209		SODIUM POLYACRYLATE OR SACCHARIN	121.1097	11	7452
0173	A	L-TRYPTINE	121.1002	19	7475
5230	A	SILICON DIOXIDE	121.1058 01 13 121.1099 A 2 27		7488
5230	C	SILICON DIOXIDE	121.1219 A 2 11 121.1658 26		7521
0175	C	SODIUM ACETATE	121.1082	42	7548
0176		SODIUM ALGINATE	(SEE NOTE 8A)	(SEE NOTE 5A)	7567
0177	C	SODIUM ALGINATE	121.1088	42	7590
5369	C	SODIUM N-ALYLBIPHENYLSULFONATE	121.1091	38	7613
5369	C	SODIUM ALUMINATE	121.1084	42	7636
5370		SODIUM ALUMINATE	(SEE NOTE 8A)	(SEE NOTE 5A)	7659
5371		SODIUM ALUMINATE	(SEE NOTE 8A)	(SEE NOTE 5A)	7682
5372		SODIUM ALUMINATE	(SEE NOTE 1C)	(SEE NOTE 2C)	7694
5231	A	SODIUM CAPRYLATE	121.1071	13 07 01	7705
5232	A	SODIUM CAPRYLATE	121.1071	13 07 01	7728
0185	C	SODIUM CARBONATE	121.1086	42	7751
0186	C	SODIUM CARBOXYMETHYLCELLULOSE	121.1089	42	7774
5233	A	SODIUM DECYLBIPHENYLSULFONATE	121.1179 D 3 28		7797

2. ALPHABETIC LISTING - OVERALL, PARTS A, C, AND D

SUB NR	PART	NAME	SECTION NO	TECHNICAL EFFECTS	ASFI
5236	P	SODIUM DIBROMOACETATE	121.1099	21	7820
5370	C	SODIUM DOSECYLIMETHANE SULFONATE	121.1091	38	7891
5371	C	SODIUM 2-ETHYLHEXYL SULFATE	121.1091	38	7866
5235	A	SODIUM FIFANATE	121.1130	19	7897
5372	C	SODIUM GLUCONATES	121.1088	42	7912
0194	C	SODIUM HEXAFLUOROPHOSPHATE	121.1088	42	7935
5373	C	SODIUM HUMATE	121.1088	42	7958
0192	C	SODIUM HYPOPHOSPHITE	121.1088	42	7991
0319	C	SODIUM HYPOCHLORITE	121.1091	38	8004
			(SEE NOTE 2A)	(SEE NOTE 5A)	8027
5234	A	SODIUM LAURATE	121.1071	13 07 01	8050
0347	A	SODIUM LAURYL SULFATE	121.1012	07 27	8073
			121.1179 B 2	28	
5374	C	SODIUM LIGNOSULFONATE	121.1088	42	8096
0193	C	SODIUM METABISULFITE	121.1088	42	8120
			(SEE NOTE 1C)	(SEE NOTE 2C)	
			(SEE NOTE 4A)	(SEE NOTE 5A)	8142
0321	C	SODIUM METASILICATE	121.1088	42	8165
5238	C	SODIUM MONO- AND DINETHYL NAPHTHALENE SULFONATES	121.1091	38	8211
			121.1198	38 22 01	
5239	A	SODIUM MYRISTATE	121.1071	13 07 01	8234
0342	C	SODIUM NITRATE	121.1088	21 03	8257
0342	C	SODIUM NITRATE	121.1088	42	8280

2. ALPHABETIC LISTING - OVERALL, PARTS A, C, AND D

ROW NR	PART	NAME	SECTION NR	TECHNICAL EFFECTS	ASN
8343	C	SODIUM NITRITE	121.1068 121.1230	21 03 21	8303
8322	A	SODIUM OLEATE	121.1071	13 07 01	8326
8243	A	SODIUM PALMITATE	121.1071	13 07 01	8349
8197	A	SODIUM PHOSPHATE, DIBASIC	(SEE NOTE 4A)	(SEE NOTE 5A)	8372
8197	C	SODIUM PHOSPHATE, DIBASIC	121.1088	02	8395
8198	A	SODIUM PHOSPHATE, MONOBASIC	(SEE NOTE 5A)	(SEE NOTE 5A)	8418
8198	C	SODIUM PHOSPHATE, MONOBASIC	121.1088	02	8441
8199	A	SODIUM PHOSPHATE, TRIBASIC	(SEE NOTE 4A)	(SEE NOTE 5A)	8464
8199	C	SODIUM PHOSPHATE, TRIBASIC	121.1088	02	8487
8377	C	SODIUM POLYACRYLATE	121.1088	02	8510
8375	C	SODIUM POLYACRYLATE-ACRYLAMIDE GELUM	121.1092 A 2	22	8522
8378	C	SODIUM POLYMETHACRYLATE	121.1088	02	8533
8564	C	SODIUM STYRATE	(SEE NOTE 4A)	(SEE NOTE 5A)	8544
8556	C	SODIUM STYRATE	(SEE NOTE 4A)	(SEE NOTE 5A)	8556
8570	C	SODIUM SILICATE	121.1088	02	8602
8245	A	SODIUM STYRATE	121.1071	13 07 01	8625
8247	A	SODIUM STYRYL-2-LACTYLATE	121.1211	07 05 27	8648
8246	A	SODIUM STYRYL PHOSPHATE	121.1183	05 27 26	8671
8380	C	SODIUM SULFATE	121.1088	02	8694
8286	C	SODIUM SULFITE, ALKALINE	121.1088 (SEE NOTE 1C)	02 (SEE NOTE 2C)	8718
8281	C	SODIUM SULFITE, NEUTRAL	121.1088	02	8740

2. ALPHABETIC LISTING - U'FALL, PARIS A.C. AND D

SUP NR	PART	NAME	SECTION NR	TECHNICAL EFFECTS	ASH
0210	A	SODIUM TRIPOLYPHOSPHATE	(SEE NOTE 6A)	(SEE NOTE 5A)	8763
0210	C	SODIUM TRIPOLYPHOSPHATE	121.1008	82	8786
0401	A	SORBITAN MONOSTEARATE	121.1029 121.1099 A 2	07 27 27	8809
5249	A	SOYBEAN OIL FATTY ACIDS, HYDROGENATED	121.1099 A 3	27	8832
5750	A	SPEARM OIL	121.1179 B 4	28	8855
5251	A	SPEARM OIL, HYDROGENATED	121.1101	17	8878
0213	A	STANNOUS CHLORIDE	121.1211	03	8901
0479	A	STEARIC ACID	121.1070	17 13 27	8924
0479	C	STEARIC ACID	121.1070	33	8935
5254	C	STEARYL ALCOHOL, SYNTHETIC	121.1230	33	8987
5255	A	STEARYL MONOGLYCERIDYL CITRATE	121.1080	26	8970
5385	C	STYRENE AND DIVINYLBENZENE, SULFONATED COPOLYMERS	121.1148 A 1	43	8982
5386	C	STYRENE, DIVINYLBENZENE, AND ACRYLONITRILE, SULFONATED TERPOLYMERS	121.1148 A 11	43	8985
5388	C	STYRENE, DIVINYLBENZENE, ACRYLONITRILE, AND METHYL ACRYLATE, SULFONATED TERPOLYMERS	121.1148 A 15	43	8988
5387	C	STYRENE, DIVINYLBENZENE, AND METHYL ACRYLATE, SULFONATED TERPOLYMERS	121.1148 A 11	43	8991
5382	C	SUCCINYLATED GELATIN	121.1219 A 2	13	8993
5256	A	SUCCINYLATED MONOGLYCERIDES	121.1195	07 05	9016
5257	A	SUCCINATEIN (STEARYL PROPYLENE- GLYCOL HYDROGEN SUCCINATE)	121.1197	07	9039

2. ALPHABETIC LISTING - OVERALL PARTS A, C, AND D

SDS NR	PART	NAME	FRACTION NR	TECHNICAL EFFECTS	AGN
			121.1086	11	9062
			(SEE NOTE 1C)	(SEE NOTE 2C)	9211
5259	A	TALLOW ALCOHOL, HYDROGENATED	121.1099 A 3	27	9223
5260	A	TALLOW, HYDROGENATED, OXIDIZED OR SULFATED	121.1099 A 3	27	9206
			(SEE NOTE 3A)	(SEE NOTE 5A)	9269
5389	C	TANNIN (INCLUDING QUERCETIN EXTRACT)	121.1088	42	9292
			(SEE NOTE 1A)	(SEE NOTE 5A)	9315
5261	A	TEMPERIN	121.1077	13	9338
5351	C	TETRAETHYLENEPENTAMINE CROSS-LINKED WITH EPICHLOROHYDRIN	121.1108 A 6	43	9304
5392	C	TETRAEOSIN EDTA	121.1088	42	9407
0202	C	TETRAPOTASSIUM PYROPHOSPHATE	121.1088	42	9430
5262	A	THDP (2,4,5-TRIHYDROXYDIHYDRO-PIRONE)	121.1116	02	9453
0225	A	L-THREONINE	121.1002	19	9476
5584	C	TRICHLOROETHYLENE	121.1061	25	9499
5394	C	TRIMETHYLENEDIETHANAMINE CROSS-LINKED WITH EPICHLOROHYDRIN	121.1108 A 6	43	9522
5395	C	TRISODIUM DITHIOTRIMETATE	121.1088	42	9545
0236	A	L-TRYPTOPHAN	121.1002	19	9568
0234	A	L-TYROSINE	121.1002	19	9591
5266	A	L-VALINE	121.1002	19	9618

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		Technical effect
121.1155	Chemical for controlling micro-organisms in cane sugar and beet sugar mills	anti-microbial agent
121.1165	Rhizopus oryzae	Enzyme
121.1170	Bacterial Catalase	Enzyme
121.1171	Sodium methyl sulfate	firming agent
121.1102	Calcium lignosulfonate	stabilizer and thickner
121.1225	Adjuvants for pesticide use dilution. (7 individual listings)	surface active agents
121.1199	a(1) Endothia panasitica classified as follows: Class Ascomycetes; order, Sphaeriales; family, Disportheceae; genus, Endothia; species, parasitica.	Enzyme
	a(2) Bacillus cereus classified as follows: Class, Schizomycetes; order Eubacteriales; family, Bacillaceae; genus, Bacillus; species, cereus	Enzyme
	a(3) Mucor pusillus Lindt classified as follows: Class, Phycomycetes; subclass Zygomycetes; order, Mucorales; family, Mucoraceae; genus, Mucor; species, pusillus; variety, Lindt.	Enzyme
	a(4) Mucor miehei Cooney et Emerson classified as follows: Class, Phycomycetes; subclass, Zygomycetes; order, Mucorales; family, Mucoraceae; genus, Mucor; species miehi, variety, Conney et Emerson.	Enzyme
121.1233	Carbohydrase and cellulase enzyme preparation	Enzyme
121.1259	Candida lipolytica	Enzyme
121.1260	Alpha-galactosidase from Mortierella vinacea var. raffinoseutilizer	Enzyme
121.1265	Amyloglucosidase enzyme product	Enzyme
121.1267	Solvent extraction process for citric acid	Processing aid
121.1244	Tertiary butylhydroquinone (TBHQ)	Antioxidants

LISTING OF DIRECT FOOD ADDITIVES (NONFLAVORS) BY TECHNICAL EFFECT

TAB D

Listing by Technical Effect of Regulated Food Additives and GRAS Substances to be Included in NAS Appendix 1, Part A (Regular Direct) and Part C (Incidental Direct) -- NOTE: Not listed here (but to be included in the Final Appendix 1) are the color additives (Part B) and the group of flavoring ingredients being prepared by FDA.

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9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL					
TECH EFFECT	ADD NR	PART	NAME	SECTION NR	
	01	5003	A ALUMINUM CAPRATE	121.1071	0299
(Anticaking Agents)		5004	A ALUMINUM CAPRYLATE	121.1071	0322
		5005	A ALUMINUM LAURATE	121.1071	0345
		5006	A ALUMINUM MYRISTATE	121.1071	0368
		5008	A ALUMINUM OLEATE	121.1071	0418
		5009	A ALUMINUM PALMITATE	121.1071	0437
		0539	A ALUMINUM STEARATE	121.1071	0506
		5028	A CALCIUM CAPRATE	121.1071	1144
		5029	A CALCIUM CAPRYLATE	121.1071	1219
		5033	A CALCIUM LAURATE	121.1071	1324
		5034	A CALCIUM MYRISTATE	121.1071	1357
		5035	A CALCIUM OLEATE	121.1071	1380
		5036	A CALCIUM PALMITATE	121.1071	1403
		0055	A CALCIUM SILICATE	121.1135	1564
		0260	A CALCIUM STEARATE	121.1071	1587
		5081	A FATTY ACIDS, SALTS OF	121.1071	3082
		5183	A IRON AMMONIUM CITRATE	121.1190	4117
		5154	A MAGNESIUM CAPRATE	121.1071	4554
		5155	A MAGNESIUM CAPRYLATE	121.1071	4577
		5157	A MAGNESIUM LAURATE	121.1071	4646
		5158	A MAGNESIUM MYRISTATE	121.1071	4669
		5159	A MAGNESIUM OLEATE	121.1071	4692
		5160	A MAGNESIUM PALMITATE	121.1071	4715
		0116	A MAGNESIUM STEARATE	121.1071	4784
		5208	A POTASSIUM CAPRATE	121.1071	6693
		5209	A POTASSIUM CAPRYLATE	121.1071	6716

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASN
01 (Anticaking Agents)	5212	A	POTASSIUM LACTATE	121.1071	6900
	5213	A	POTASSIUM HYDRATE	121.1071	6923
	5215	A	POTASSIUM OLEATE	121.1071	6969
	5216	A	POTASSIUM PALMITATE	121.1071	6992
	5219	A	POTASSIUM STEARATE	121.1071	7088
	5230	A	SILICON DIOXIDE	121.1058	7498
	5231	A	SODIUM CAPRATE	121.1071	7705
	5232	A	SODIUM CAPRYLATE	121.1071	7728
	5236	A	SODIUM LACTATE	121.1071	8050
	5258	C	SODIUM MONO- AND DIMETHYL NAPHTHALENE SULFONATES	121.1198	8211
5239	A	SODIUM MYRISTATE	121.1071	8234	
0322	A	SODIUM OLEATE	121.1071	8326	
5243	A	SODIUM PALMITATE	121.1071	8349	
5245	A	SODIUM STEARATE	121.1071	8425	
5256	C	YELLOW PRUSSIAN OF SODA	121.1032	9275	
02 (Antioxidants)	5021	A	BHA	121.1035	0966
	5022	A	BHT	121.1034	0989
	5026	A	2,2,6,6-TETRAHYDRO-1,3,5-TRIMETHYLBENZENE	121.1244	1150
	5316	C	COMBUSTION PRODUCT GAS	121.1060	2369
	5077	A	ETHOXYQUIN	121.1001	2921
	5148	A	4-HYDROXYBENZOIC-2,6-DI-TERT-BUTYLPHENOL	121.1268	4025
	0131	A	HEMOGLYCERIDE CITRATE	121.1036	5451
	5252	A	THP (2,3,5-TRIHYDROXYBUTYRO-PHENONE)	121.1116	9453
03 (Colors / Additives)	0532	A	CALCIUM DIBODIUM EDTA	121.1017	1242

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL					
TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASR
03 (Colors & Pigments)	5073	A	DIMETHYL DIALKYL AMMONIUM CHLORIDE	121.1168	2751
	0537	A	DISODIUM EDTA	121.1056	2806
	5077	A	ETHOXYQUIN	121.1001	2921
	0342	C	SODIUM NITRATE	121.1053	9252
	0343	C	SODIUM NITRITE	121.1064	8303
0213	A	STANNOUS CHLORIDE	121.1213	8901	
04 (Curing, Peeling Agents)	0537	A	DISODIUM EDTA	121.1056	2806
	0529	A	MINERAL OIL, WHITE	121.1146	5336
	5335	C	PETROLEUM HYDROCARBONS, ISOPARAFFINIC, SYNTHETIC	121.1154	5993
	0536	C	POTASSIUM NITRATE (FOR COD NO2)	121.1132	6945
05 (Dough Conditioners)	5302	C	ACETONE PEROXIDES	121.1023	0046
	0496	A	AZODICARBONAMIDE	121.1085	0851
	0538	A	CALCIUM STEAROYL-2-LACTYLATE	121.1047	1610
	5076	A	ETHOXYLATED MONO- AND DIGLYCERIDES (POLYOXYETHYLENE (20) MONO- AND DIGLYCERIDES OF FATTY ACIDS)	121.1221	2898
	5185	A	ϵ -HYDRO-O ¹ -[2]-HYDROXY-POLY(OXYETHYLENE) POLY-(OXYPROPYLENE) - (51-57 MOLES) POLY(OXYETHYLENE) BLOCK COPOLYMER, AVG. MOL. WT. 14,000	121.1235 A 4	3972
	0393	A	POLYCARBATE 60	121.1030	6486
	5247	A	SODIUM STEAROYL-2-LACTYLATE	121.1211	8688
5246	A	SODIUM STEARYL PHTHALATE	121.1183	8671	
5254	A	SUCCINYLATED MONOGLYCERIDES	121.1195	9074	
06 (Dough Additive)	5359	A	METHYL ESTERS OF FATTY ACIDS (EDIBLE)	121.1266	5072
07 (Emulsifiers)	5003	A	ALUMINUM CAPRATE	121.1071	0259
	5004	A	ALUMINUM CAPRYLATE	121.1071	0322

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASN
07 (Complexions)	5005	A	ALUMINUM LAURATE	121.1071	0345
	5006	A	ALUMINUM MYRISTATE	121.1071	0369
	5008	A	ALUMINUM OLEATE	121.1071	0414
	5009	A	ALUMINUM PALMITATE	121.1071	0437
	0539	A	ALUMINUM STEARATE	121.1071	0506
	3254	A	APABINOGALACTAN	121.1174	0713
	5019	A	BAKER'S YEAST GLYCAN	121.1262	0874
	5028	A	CALCIUM CAPRATE	121.1071	1199
	5029	A	CALCIUM CAPRYLATE	121.1071	1219
	5033	A	CALCIUM LAURATE	121.1071	1334
	5034	A	CALCIUM MYRISTATE	121.1071	1357
	5035	A	CALCIUM OLEATE	121.1071	1380
	5036	A	CALCIUM PALMITATE	121.1071	1403
	0290	A	CALCIUM STEARATE	121.1071	1587
	5046	A	CARRAGEENAN	121.1066	1840
	5048	A	CARRAGEENAN, AMMONIUM SALT OF	121.1067	1863
	5050	A	CARRAGEENAN, AMMONIUM SALT OF, WITH POLYSORBATE 80	121.1193	1896
	5049	A	CARRAGEENAN, CALCIUM SALT OF	121.1067	1909
	5055	A	CARRAGEENAN, CALCIUM SALT OF, WITH POLYSORBATE 80	121.1193	1932
	5050	A	CARRAGEENAN, POTASSIUM SALT OF	121.1067	1955
	5056	A	CARRAGEENAN, POTASSIUM SALT OF, WITH POLYSORBATE 80	121.1193	1978
	5047	A	CARRAGEENAN SALTS	121.1067	2001
	5053	A	CARRAGEENAN SALTS WITH POLY- SORBATE 80	121.1193	2024
	5051	A	CARRAGEENAN, SODIUM SALT OF	121.1067	2047

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASN
07 (Emulsifier)	5027	A	CAPPAGESEAN, SODIUM SALT OF, WITH POLYCORRATE 80	121.1193	2070
	5052	A	CAPRAGESEAN WITH POLYCORRATE 80	121.1193	2093
	5072	A	DIOCTYL SODIUM SULFOSUCCINATE	121.1137	2783
	5076	A	ETHOXYLATED MONO- AND DIGLYCERIDES (POLYETHYLENE (20) MONO- AND DIGLYCERIDES OF FATTY ACIDS)	121.1221	2898
	5081	A	FATTY ACIDS, SALTS OF	121.1071	3082
	0499	A	FURCELLERAN	121.1068	3427
	5122	A	FURCELLERAN, AMMONIUM SALT OF	121.1069	3450
	5121	A	FURCELLERAN, CALCIUM SALT OF	121.1069	3473
	5124	A	FURCELLERAN, POTASSIUM SALT OF	121.1069	3496
	5121	A	FURCELLERAN, SALTS OF	121.1069	3519
	5125	A	FURCELLERAN, SODIUM SALT OF	121.1069	3542
	5131	A	GLYCERYL-LACTO ESTERS OF FATTY ACIDS	121.1004	3726
	5139	A	HYDROXYLATED LECITHIN	121.1027	4002
	5141	A	HYDROXYPROPYL CELLULOSE	121.1160	4088
	0534	A	HYDROXYPROPYL METHYLCELLULOSE	121.1021	4071
	5168	A	LACTYLATED FATTY ACID ESTERS OF GLYCEROL AND PROPYLENE GLYCOL	121.1122	4347
	5149	A	LACTYLIC ESTERS OF FATTY ACIDS	121.1048	4370
	5154	A	MAGNESIUM CAPRATE	121.1071	4554
	5155	A	MAGNESIUM CAPRYLATE	121.1071	4577
	5157	A	MAGNESIUM LAURATE	121.1071	4646
	5158	A	MAGNESIUM MYRISTATE	121.1071	4669
	5159	A	MAGNESIUM OLEATE	121.1071	4692
	5160	A	MAGNESIUM PALMITATE	121.1071	4715
	0516	A	MAGNESIUM STEARATE	121.1071	4784

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASN
07 (Enols/Prost)	5166	A	METHYL ETHYL CELLULOSE	121.1112	5083
	5199	A	POLYGLYCEROL ESTERS OF FATTY ACIDS	121.1120	6388
	0393	A	POLYSORBATE 60	121.1030	6986
	0535	A	POLYSORBATE 65	121.1008	6509
	0394	A	POLYSORBATE 80	121.1009	6532
	5208	A	POTASSIUM CAPRATE	121.1071	6693
	5209	A	POTASSIUM CAPRYLATE	121.1071	6716
	5212	A	POTASSIUM LAURATE	121.1071	6900
	5213	A	POTASSIUM MYRISTATE	121.1071	6923
	5215	A	POTASSIUM OLEATE	121.1071	6969
	5216	A	POTASSIUM PALMITATE	121.1071	6992
	5219	A	POTASSIUM STEARATE	121.1071	7088
	0397	A	PROPYLENE GLYCOL ALGINATE	121.1015	7130
	5231	A	SODIUM CAPRATE	121.1071	7705
	5232	A	SODIUM CAPRYLATE	121.1071	7728
	5236	A	SODIUM LAURATE	121.1071	8050
	0347	A	SODIUM LAURYL SULFATE	121.1012	8073
	5239	A	SODIUM MYRISTATE	121.1071	8238
	0322	A	SODIUM OLEATE	121.1071	8326
	5243	A	SODIUM PALMITATE	121.1071	8349
	5245	A	SODIUM STEARATE	121.1071	8625
	5247	A	SODIUM STEAROYL-2-LACTYLATE	121.1211	8648
	0401	A	SORBITAN MONOSTEARATE	121.1029	8809
	5256	A	SUCCINYLATED MONOGLYCERIDES	121.1195	9016
	5257	A	SUCCINTEARIN (STEAROYL PROPYLENE-GLYCOL HYDROGEN SUCCINATE)	121.1197	9039

08 (Enzymes) (Enols/Prost)

9. INDEXIC LISTING OF TECHNICAL EFFECTS - OVERALL					
TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASB
09 (Flavoring Agents)	5032	A	CALCIUM LACTOGLUCONATE	121.1162	1311
10 (Flavor Guaranties)	5016	A	ASPARTAME	121.1258	0905
	0541	A	DIOIODIN GUANYLATE	121.1109	2829
	3287	A	GLUCINE	121.1257	3749
	5272	A	YEAST-NALT SPROUT EXTRACT	121.1250	9729
11 (Flavoring Agents & Additives)	5508	A	1,3-BUTYLENE GLYCOL	121.1176	1127
	0532	A	CALCIUM DIOIODIN EDTA	121.1017	1242
	5063	A	COCOA WITH DIOCTYL SODIUM SULFO-SUCCINATE FOR MANUFACTURING	121.1229	2346
	0540	A	DIOIODIN INOSINATE	121.1096	2852
	0078	A	ETHYL CELLULOSE	121.1097	2994
	5064	A	ETHYLENE OXIDE AND PROPYLENE OXIDE, COPOLYMER, CONDENSATE OF	121.1235	3025
	5130	A	GLYCEROL ESTER OF WOOD ROBIN	121.1084	3703
	5136	A	HOP EXTRACT, MODIFIED	121.1082	3807
	5137	A	α -HYDRO- ω -POLY-HYDROXY-POLY-(OXYETHYLENE) POLY (OXYPROPYLENE) - (55-61 MOLES) POLY (OXYETHYLENE) BLOCK COPOLYMER, AVG. MOL. WT. 9,760-13,200	121.1235 A 1	3933
	5198	A	POLYETHYLENE GLYCOL (M.W. 200-9,500)	121.1185	6279
	5207	A	POLYVINYLPIRROLIDONE	121.1139	6578
	5223	A	QUININE HYDROCHLORIDE	121.1081	7268
	5224	A	QUININE SULFATE	121.1081	7291
	5228	A	SAPROLE-FREE EXTRACT OF SASSAFRAS	121.1097	7652
	5258	A	SUGAR BEET EXTRACT FLAVOR BASE	121.1086	9062
12 (Flavoring Agents & Additives)	5302	C	ACETONE PEROXIDE	121.1023	0046

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUP NR	PART	NAME	SECTION NR	ASN
12	0496	A	ASODICARBONAMIDE	121.1065	0851
*Plant-Treatment Accept					
13	5003	A	ALUMINUM CAPRATE	121.1071	0299
(FORMULATED A.102)					
	5004	A	ALUMINUM CAPRYLATE	121.1071	0322
	5005	A	ALUMINUM LAURATE	121.1071	0345
	5006	A	ALUMINUM MYRISTATE	121.1071	0368
	5008	A	ALUMINUM OLEATE	121.1071	0418
	5009	A	ALUMINUM PALMITATE	121.1071	0437
	0539	A	ALUMINUM STEARATE	121.1071	0506
	3254	A	ARABINOGALACTAN	121.1179	0713
	3254	C	ARABINOGALACTAN	121.1219 A 2	0736
	5028	A	CALCIUM CAPRATE	121.1071	1196
	5029	A	CALCIUM CAPRYLATE	121.1071	1219
	5033	A	CALCIUM LAURATE	121.1071	1334
	5034	A	CALCIUM MYRISTATE	121.1071	1357
	5035	A	CALCIUM OLEATE	121.1071	1380
	5036	A	CALCIUM PALMITATE	121.1071	1403
	0240	A	CALCIUM STEARATE	121.1071	1567
	5043	A	CAPRIC ACID	121.1070	1656
	0058	A	CAPRYLIC ACID	121.1070	1679
	5076	A	ETHYL CELLULOSE	121.1087	2644
	5080	A	FATTY ACIDS	121.1070	2059
	5081	A	FATTY ACIDS, SALTS OF	121.1071	3082
	5329	C	GLUTARALDEHYDE	121.1219 A 3	3657
	5131	A	GLYCERYL-LACTO ESTERS OF FATTY ACIDS	121.1004	3726
	5103	A	HYDROXYPROPYL CELLULOSE	121.1164	4048

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	STD NR	PART	NAME	SECTION NR	ASN
13 (Formulation 479)	0538	A	HYDROXYPROPYL METHYLCELLULOSE	121.1021	4071
	5188	A	LACTYLATED FATTY ACID ESTERS OF GLYCEROL AND PROPYLENE GLYCOL	121.1122	4347
	5149	A	LACTYLIC ESTERS OF FATTY ACIDS	121.1048	4370
	5150	A	LACTIC ACID	121.1070	4393
	5158	A	MAGNESIUM CAPRYLATE	121.1071	4554
	5155	A	MAGNESIUM CAPRYLATE	121.1071	4577
	5157	A	MAGNESIUM LAURATE	121.1071	4644
	5158	A	MAGNESIUM MYRISTATE	121.1071	4669
	5159	A	MAGNESIUM OLATE	121.1071	4692
	5160	A	MAGNESIUM PALMITATE	121.1071	4715
	0116	A	MAGNESIUM STEARATE	121.1071	4784
	5163	A	METHACRYLIC ACID-DIVINYLBENZENE COPOLYMER	121.1136	4876
	5386	C	MICROCAPSULES FOR FLAVORING OILS	121.1219	5198
	0529	A	MINERAL OIL, WHITE	121.1148	5336
	5177	A	MYRISTIC ACID	121.1070	5520
	5180	C	n-OCTYL ALCOHOL	121.1219 A 3	5635
	5182	A	OLEIC ACID	121.1070	5681
	5183	A	OLEIC ACID, FROM TALL OIL FATTY ACIDS	121.1237	5704
	5185	A	PALMITIC ACID	121.1070	5750
	5198	A	POLYETHYLENE GLYCOL (M.W. 200-9,500)	121.1185	6279
	5198	A	POLYETHYLENE GLYCOL (M.W. 200-9,500)	121.1185	6279
	5207	A	POLYVINYLPIRROLIDONE	121.1139	6578
	5208	A	POTASSIUM CAPRYLATE	121.1071	6693
	5209	A	POTASSIUM CAPRYLATE	121.1071	6716

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9. NINERIC LISTING OF TECHNICAL EFFECTS - OVERALL					
TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASN
13 (Formulation Aids)	5212	A	POTASSIUM LAURATE	121,1071	6900
	5213	A	POTASSIUM MYRISTATE	121,1071	6923
	5215	A	POTASSIUM OLEATE	121,1071	6969
	5216	A	POTASSIUM PALMITATE	121,1071	6992
	5219	A	POTASSIUM STEARATE	121,1071	709a
	5226	A	RICE BRAN WAX	121,1098	7406
	5236	A	SILICON DIOXIDE	121,1058	7498
	5239	C	SILICON DIOXIDE	121,1219 A, 2	7521
	5231	A	SODIUM CAPRYLATE	121,1071	7705
	5232	A	SODIUM CAPRYLATE	121,1071	7728
	5234	A	SODIUM LAURATE	121,1071	8050
	5239	A	SODIUM MYRISTATE	121,1071	823a
	5242	A	SODIUM OLEATE	121,1071	8326
5243	B	SODIUM PALMITATE	121,1071	8399	
5245	A	SODIUM STEARATE	121,1071	8625	
0479	A	STEARIC ACID	121,1070	8924	
5302	C	SUCCINYLATED GELATIN	121,1219 A, 2	8992	
5261	A	TERPENE RESIN	121,1077	9338	
18 (Formulants)	5305	C	ALUMINUM PHOSPHIDE	123,20	0460
	5309	C	CARBON TETRACHLORIDE AND CARBON DISULFIDE	123,230 A, 1	1725
	5310	C	CARBON TETRACHLORIDE AND CARBON DISULFIDE WITH PENTANE	123,230 A, 1	1748
	5311	C	CARBON TETRACHLORIDE AND ETHYLENE DICHLORIDE	123,230 A, 1	1771
	5312	C	CARBON TETRACHLORIDE AND ETHYLENE DICHLORIDE WITH PENTANE	123,230 A, 1	1790
	0079	C	BUTYL FORMATE	123,210	2567

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL					
TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASH
10 (PRESERVATIVES)	5325	C	ETHYLENE OXIDE	123.200	3013
	5327	C	FUNGICIDE FOR PROCESSED GRAINS USED IN PRODUCTION OF FERMENTED MALT BEVERAGES	123.230	2404
	5331	C	HYDROGEN CYANIDE	123.240	3979
	5343	C	METHYL BROMIDE AND ETHYLENE DI- BROMIDE	123.230 A	5060
	5344	C	METHYL FORMATE	123.310	5106
5345	C	PROPYLENE OXIDE	123.300	7176	
15 (CONDICITORS)	0529	A	MINERAL OIL, WHITE	121.1146	5336
17 (LUBRICANTS)	5003	A	CAPRIC ACID	121.1070	1654
	0050	A	CAPRYLIC ACID	121.1070	1670
	5050	A	CASTOR OIL	121.1020	2116
	5060	A	FATTY ACIDS	121.1070	3053
	5150	A	LINOLIC ACID	121.1070	3322
	0529	A	MINERAL OIL, WHITE	121.1146	5336
	5177	A	MYRISTIC ACID	121.1070	5520
	5182	A	OLEIC ACID	121.1070	5481
	5183	A	OLEIC ACID, FROM TALL OIL FATTY ACIDS	121.1237	5704
	5184	A	OSTEADIN	121.1016	5727
	5185	A	PALMITIC ACID	121.1070	5750
	0520	A	PETROLIUM	121.1146	5980
	5190	A	POLYETHYLENE GLYCOL (M.W. 700-3,500)	121.1185	6279
	5251	A	SPERM OIL, HYDROGENATED	121.1101	6470
0475	A	STEARIC ACID	121.1070	6524	
18 (POLYMERIZING AGENTS) (CATIONIC) (CATIONIC)					

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASN
15	9066	A	L-ALANINE	121.1002	0138
(continued-subsequent)	9067	A	ALONININ NICOTINATE	121.1101	0391
	9012	A	AMINO ACIDS	121.1002	0529
	0021	A	L-ARGININE	121.1002	0759
	0352	A	L-ASPARAGINE	121.1002	0782
	0025	A	L-ASPARTIC ACID	121.1002	0820
	9026	A	BAKED'S YEAST PROTEIN	121.1263	0897
	9021	A	CALCIUM FUMARATE	121.1130	1245
	9037	A	CALCIUM PANTOTHENATE, CALCIUM CHLORIDE DOUBLE SALT	121.1037	1426
	3263	A	L-CYSTEINE	121.1002	2574
	0073	A	L-CYSTEINE	121.1002	2599
	9003	A	FERRIC FUMARATE	121.1130	3220
	9004	A	FISH PROTEIN CONCENTRATE, WHOLE	121.1202	3266
	0010	A	FOLIC ACID (POLACIN)	121.1134	3289
	0302	A	FUMARIC ACID	121.1130	3358
	5119	A	FUMARIC ACID, SALES OF	121.1130	3381
	3205	A	L-GLUTAMIC ACID	121.1002	3611
	0355	A	L-GLUTAMINE	121.1002	3625
	3207	A	GLYCINE	121.1002	3709
	0096	A	L-HISTIDINE	121.1002	3864
	5100	A	IRON-CHOLINE CITRATE COMPLEX	121.1100	0300
	0902	A	L-ISOLEUCINE	121.1002	0163
	5107	A	ISOP	121.1109	0270
	3297	A	L-LEUCINE	121.1002	0539
	0309	A	L-LYSINE	121.1002	0531
	3156	A	MAGNESIUM FUMARATE	121.1130	0623

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASH
19	3301	A	DL-METHIONINE	121.1002	4922
<i>(Methionine Sulfonamide)</i>					
	0360	A	L-METHIONINE	121.1002	4945
	5179	A	NICOTINAMIDE-ASCORBIC ACID COMPLEX	121.1095	5566
	5186	A	D-PANTOTHICAMIDE	121.1123	5796
	0148	A	L-PHENYLALANINE	121.1002	6166
	5210	A	POTASSIUM FUMARATE	121.1130	6762
	0155	A	POTASSIUM IODIDE	121.1071	6877
	2319	A	L-PROLINE	121.1002	7107
	0173	A	L-GLUTINE	121.1002	7475
	5235	A	SODIUM FUMARATE	121.1130	7883
	0225	A	L-THREONINE	121.1002	9076
	0236	A	L-TRYPTOPHAN	121.1002	9568
	0238	A	L-TYROSINE	121.1002	9551
	5266	A	L-VALINE	121.1002	9610
	5278	A	XYLITOL	121.1110	9706
<i>(Xylitol Ascorbyl) (Xylitol-L-ASCORBATE)</i>					
2C	0169	C	POTASSIUM BISULFITE	(SEE NOTE 1C)	6635
<i>(Sulfonamide ester of Ascorbyl, Part C) ADD TO 2100000 THE FOLLOWING)</i>					
	0158	C	POTASSIUM METABISULFITE	(SEE NOTE 1C)	6911
	0183	C	SODIUM BISULFITE	(SEE NOTE 1C)	7494
	0193	C	SODIUM METABISULFITE	(SEE NOTE 1C)	8120
	0206	C	SODIUM SULFITE, ALKALINE	(SEE NOTE 1C)	8718
	0217	C	SULFUR DIOXIDE	(SEE NOTE 1C)	9211
21	0532	A	CALCIUM DIOSMITH EDTA	121.1017	1282
<i>(Diosmith EDTA)</i>					
	5070	A	DEHYDROACETIC ACID	121.1009	2648
	0537	A	DIOSMITH EDTA	121.1056	2806
	5133	B	HEPTYL PARABEN	121.1186	3772

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASN
(Preservatives)	21	5353	C PARAFORMALDEHYDE	121.1330	5842
		5238	A SODIUM BENTHOACETATE	121.1089	7820
		0342	C SODIUM NITRATE	121.1063	8257
		0343	C SODIUM NITRITE	121.1064	8303
	0343	C SODIUM NITRATE	121.1230	8303	
(Preservatives and)	22	5326	C ACRYLAMIDE-ACRYLIC ACID RESIN (HYDROLYZED POLYACRYLAMIDE)	121.1092 A 1	0075
		5303	C ACRYLATE ACRYLAMIDE RESIN	121.1092	0092
		5072	A DIOCTYL SODIUM SULFOSECTATE	121.1137	2783
		5138	C 4-HYDRO-OHHEA-HYDROXY-POLY-(OXYETHYLENE) POLY (OXYPROPYLENE) - (53-59 MOLES) POLY (OXYETHYLENE) - (14-16 MOLES) BLOCK COPOLYMER, AVG. MOL. WT. 3,500-4,125	121.1235 A 2	3956
		5133	C ION-EXCHANGE MEMBRANES	121.1180	6042
		5197	C MOLECULAR SIEVE RESIN	121.1206	5355
		5358	C POLYACRYLAMIDE RESIN, MODIFIED	121.1192	6233
		5361	C POLYVINYLPIRROLIDONE	121.1110	6555
		5207	A POLYVINYLPIRROLIDONE	121.1139	6578
		5238	C SODIUM MONO- AND DIMETHYL NAPHTHALENE SULFONATES	121.1198	8211
	5375	C SODIUM POLYACRYLATE-ACRYLAMIDE RESIN	121.1092 A 2	0522	
(Preservatives - Artificially Acquired)	23	5314	C CHLORO-PENTAFLUOROBENZENE	121.1181	2277
		5164	A METHYL ETHYL CELLULOSE	121.1112	5083
		5352	C OCTAFLUORO-CYCLOHEXANE	121.1055	5412
(Preservatives - Artificially Acquired)	24	0532	A CALCIUM DISODIUM EDTA	121.1017	1202
		0537	A DISODIUM EDTA	121.1054	2006

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	ADD NR	PART NAME	SECTION NR	ACT
	25	3276 C ACETONE	121.1002	0023
(Carbohydrate-Extractable)	5508 A 1,3-BUTYLENE GLYCOL	121.1176	1127	
	5326 C ETHYLENE DICHLORIDE	121.1000	2990	
	5330 C HEXANE	121.1005	3018	
	5106 C ISOPROPYL ALCOHOL	121.1003	0232	
	5302 C METHYL ALCOHOL	121.1000	5037	
	5305 C METHYLENE CHLORIDE	121.1039	5152	
	5500 C TRICHLOROETHYLENE	121.1001	9099	
	26	3250 A ARABINGALACTAN	121.1170	0713
(Carbohydrate-Extractable)	5019 A BAKER'S YEAST GLYCAN	121.1202	0070	
	5023 A BROMINATED VEGETABLE OIL	121.0000	1050	
	5006 A CARRAGEENAN	121.1000	1000	
	5008 A CARRAGEENAN, AMMONIUM SALT OF	121.1007	1063	
	5020 A CARRAGEENAN, AMMONIUM SALT OF, WITH POLYSORBATE 80	121.1193	1086	
	5009 A CARRAGEENAN, CALCIUM SALT OF	121.1007	1009	
	5055 A CARRAGEENAN, CALCIUM SALT OF, WITH POLYSORBATE 80	121.1193	1032	
	5050 A CARRAGEENAN, POTASSIUM SALT OF	121.1007	1955	
	5056 A CARRAGEENAN, POTASSIUM SALT OF, WITH POLYSORBATE 80	121.1193	1070	
	5007 A CARRAGEENAN SALTS	121.1007	2001	
	5053 A CARRAGEENAN SALTS WITH POLY- SORBATE 80	121.1193	2020	
	5051 A CARRAGEENAN, SODIUM SALT OF	121.1007	2007	
	5057 A CARRAGEENAN, SODIUM SALT OF, WITH POLYSORBATE 80	121.1193	2070	
	5052 A CARRAGEENAN WITH POLYSORBATE 80	121.1193	2093	

9. SURGICAL LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ACT
26 (Pharmaceutical-Preparations)	0517	A	DISODIUM EDTA	121.1056	2806
	5079	A	ETHYLENE OXIDE POLYMER	121.1161	3036
	0499	A	FURCELLEFAN	121.1068	3427
	5122	A	FURCELLEFAN, AMMONIUM SALT OF	121.1069	3450
	5123	A	FURCELLEFAN, CALCIUM SALT OF	121.1069	3473
	5124	A	FURCELLEFAN, POTASSIUM SALT OF	121.1069	3496
	5121	A	FURCELLEFAN, SALTS OF	121.1069	3519
	5125	A	FURCELLEFAN, SODIUM SALT OF	121.1069	3542
	3287	A	GLYCINE	121.1257	3749
	5141	A	HYDROXYPROPYL CELLULOSE	121.1160	4043
	0534	A	HYDROXYPROPYL METHYLCELLULOSE	121.1021	4071
	5198	A	POLYETHYLENE GLYCOL (M.W. 200-9,500)	121.1185	6279
	5207	A	POLYVINYLPIRROLIDONE	121.1139	6578
	0397	A	PROPYLENE GLYCOL ALGINATE	121.1015	7130
	5230	C	SILICON DIOXIDE	121.1058	7521
5246	A	SODIUM STEARYL FUMARATE	121.1183	8671	
5255	A	STEARYL MONOGLYCERIOYL CITRATE	121.1080	8970	
0544	A	XANTHAN GUM	121.1224	9441	
27 (Surfactants)	0539	A	ALUMINUM STEARATE	121.1099 A 3	0504
	5021	A	BHA	121.1099 A 1	0964
	5022	A	BHT	121.1099 A 3	0969
	5026	A	N-BUTOXYPOLYOXYETHYLENE POLY- OXYPROPYLENE GLYCOL	121.1099 A 4	1081
	5027	A	BUTYL STEARATE	121.1099 A 3	1104
	0260	A	CALCIUM STEARATE	121.1099 A 3	1587
	0538	A	CALCIUM STEAROYL-2-LACTYLATE	121.1047	1610

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART NAME	SECTION NR	ASH
77 CORROSION	5063	A CAPRIC ACID	121.1070	1656
	0056	A CAPRIC ACID	121.1070	1679
	5069	A DEFOAMING AGENTS (AND COMPONENTS)	121.1099	2645
	5071	A DIMETHYLPOLYSILOXANE	121.1099 A 2	2740
	5072	A DIOCTYL ROSEIN SULFOSUCCINATE	121.1137	2783
	5080	A FATTY ACIDS	121.1099 A 3	3059
	5080	A FATTY ACIDS	121.1070	3059
	5117	A FORMALDEHYDE	121.1099 A 2	3335
	5117	A FORMALDEHYDE	121.1099 A 3	3335
	5120	C e-HYDRO-OXIDO-HYDROXY-POLY-(OXYTETYLENE) POLY (OXYHEPTYLENE) - (53-59 MOLES) POLY (OXYTETYLENE) - (10-16 MOLES) BLOCK COPOLYMER, AVG. MOL. WT. 3,500-8,125	121.1235 A 2	3956
	5102	C e-HYDRO-OXIDO-HYDROXY-POLY (OXYTETYLENE) /POLY-(OXYHEPTYLENE) (MOL. 15 MOLES) /POLY (OXYHEPTYLENE) BLOCK COPOLYMER, M.W. AVG. MOL. WT. 1,900	121.1235 A 3	3966
	5150	A HYDROLYZED LECITHIN	121.1099 A 3	4002
	5166	A ISOPROPYL ALCOHOL	121.1099 A 3	4209
	5108	A LACTYLATED FATTY ACID ESTERS OF GLYCEROL AND PROPYLENE GLYCOL	121.1122	4347
	5109	A LACTYLIC ESTERS OF FATTY ACIDS	121.1040	4370
	5150	A LARIC ACID	121.1070	4393
	0116	A MAGNESIUM STEARATE	121.1099 A 3	4784
	5166	A METHYL ETHYL CELLULOSE	121.1112	5083
	5167	A METHYL GLUCOSIDES-COCONUT OIL ESTER	121.1151	5129
	0529	A MINERAL OIL, WHITE	121.1106	5336
	0529	A MINERAL OIL, WHITE	121.1099 A 2	5336
	0529	A MINERAL OIL, WHITE	121.1099 A 3	5336

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASN
27 (RETRACTED)	5177	A	MYRISTIC ACID	121.1070	5520
	5182	A	OLEIC ACID	121.1070	5581
	5183	A	OLEIC ACID, FROM TALL OIL FATTY ACIDS	121.1237	5704
	5183	A	OLEIC ACID, FROM TALL OIL FATTY ACIDS	121.1099 A 3	5704
	5184	A	OXYSTEARIN	121.1099 A 3	5727
	5185	A	PALMITIC ACID	121.1070	5750
	0528	A	PETROLATUM	121.1166	5980
	0528	A	PETROLATUM	121.1099 A 3	5980
	4135	C	PETROLEUM HYDROCARBONS, INOPARAFFINIC, SYNTHETIC	121.1099 A 3	5993
	5191	A	PETROLEUM HYDROCARBONS, ODOORLESS, LIGHT	121.1099 A 3	6003
	5191	A	PETROLEUM HYDROCARBONS, ODOORLESS, LIGHT	121.1182	6003
	5193	A	PETROLEUM WAX	121.1099 A 3	6049
	5193	A	PETROLEUM WAX	121.1154	6049
	5194	A	PETROLEUM WAX, SYNTHETIC	121.1099 A 3	6072
	5194	A	PETROLEUM WAX, SYNTHETIC	121.1239	6072
	5196	A	POLYACRYLIC ACID, SODIUM SALT	121.1099 A 2	6256
	5198	A	POLYETHYLENE GLYCOL (M.W. 200-9,500)	121.1099 A 2	6279
	5200	A	POLYETHYLENE 90 MONOSTEARATE	121.1099 A 2	6371
	5201	A	POLYETHYLENE (66) DIOLSETE	121.1099 A 3	6390
	5202	A	POLYETHYLENE (669) MONO-TRICHOLENATE	121.1099 A 3	6417
	5203	A	POLYPROPYLENE GLYCOL	121.1099 A 3	6463
	0393	A	POLYPROPATE 60	121.1099 A 2	6486
	0393	A	POLYPROPATE 60	121.1030	6486

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASN
(SURFACE FINISH)	0535	A	POLYISOBUTATE 65	121.1099 A 2	6509
	0394	A	POLYISOBUTATE 80	121.1099 A 3	6532
	0394	A	POLYISOBUTATE 80	121.1009	6532
	5707	A	POLYVINYLPIRROLIDONE	121.1139	6578
	5219	A	POTASSIUM STEARATE	121.1099 A 3	7084
	0397	A	PROPYLENE GLYCOL ALGINATE	121.1099 A 2	7130
	5222	A	PROPYLENE GLYCOL MONO- AND DILESTERS OF FATTY ACIDS	121.1099 A 3	7153
	5230	A	SILICON DIOXIDE	121.1099 A 2	7498
	0347	A	SODIUM LAURYL SULFATE	121.1012	8073
	5247	A	SODIUM STEAROYL-2'-LACTYLATE	121.1211	8668
	5246	A	SODIUM STEARYL PHOSPHATE	121.1183	8671
	0401	A	SORBITAN MONOSTEARATE	121.1029	8809
	0401	A	SORBITAN MONOSTEARATE	121.1099 A 2	8809
	5249	A	SOYBEAN OIL FATTY ACIDS, HYDROGENATED	121.1099 A 3	8932
	0479	A	STEARIC ACID	121.1070	8924
	5259	A	TALLOW ALCOHOL, HYDROGENATED	121.1099 A 3	9223
	5260	A	TALLOW, HYDROGENATED, OXIDIZED OR SULFATED	121.1099 A 3	9264
	0544	A	XANTHAN GUM	121.1224	9683
7A (SURFACE FINISH)	4058	A	CARTOP OIL	121.1028	2114
	5062	A	COATINGS ON FRESH CITRUS FRUIT	121.1179	2300
	5066	A	CURCUMONE-INDIGO RESIN	121.1050	2507
	5078	A	ETHYL CELLULOSE	121.1087	2944
	5880	A	FATTY ACIDS	121.1179 B 2	3059
	0529	A	MINERAL OIL, WHITE	121.1106	5336

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART NAME	SECTION NR	ASN
28 (Suppl'd #numbers)	5176	A	121.1185	5497
	5183	A	121.1179 B 2	5704
	5187	A	121.1228	5819
	5188	A	121.1179 B 2	5938
	5189	A	121.1179 B 2	5957
	0528	A	121.1166	5980
	5335	C	121.1154	5993
	5193	A	121.1182	6003
	5192	A	121.1203	6028
	5192	A	121.1179 B 4	6026
	5193	A	121.1156	6049
	5198	A	121.1239	6072
	5198	A	121.1185	6279
	5198	A	121.1179 B 2	6279
	5198	A	121.1179 B 3	6279
	5197	A	121.1182	6325
	0393	A	121.1030	6406
	5207	A	121.1179 B 3	6578
	5217	A	121.1179 B 3	7015
	0757	A	121.1179 B 3	7330

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASN
28 (SURFACE FINISHES)	5226	A	RICE BRAN WAX	121.1098	7606
	5038	A	ROSIN, PARTIALLY DIMETHYLED, CALCIUM SALT OF	121.1179 B 4	7420
	5227	A	ROSIN, PARTIALLY HYDROGENATED	121.1179 B 2	7429
	5233	A	SODIUM DODECYLBENZENE SULFONATE	121.1179 B 3	7797
	0347	A	SODIUM LAUREL SULFATE	121.1179 B 2	8072
	5250	A	SPERM OIL	121.1179 B 4	8855
29 (SYNTHETIC CHEMICALS)	5267	A	VINYL CHLORIDE-VINYLIDENE CHLORIDE COPOLYMER (75% SOLN.)	121.1179 B 3	9637
	5268	A	WOOD ROBIN	121.1179 B 2	9640
30 (CYTROPOLYMER)	5019	A	BAKER'S YEAST GLYCAN	121.1242	0874
	0532	A	CALCIUM DISODIUM EDTA	121.1017	1242
31 (CHEMISTRIALLY SUBSTANCES - NOT ASST. MAT.)	5193	A	PETROLEUM WAX	121.1156	6049
	5194	A	PETROLEUM WAX, SYNTHETIC	121.1239	6072
	0532	A	CALCIUM DISODIUM EDTA	121.1017	1242
33 (MANUFACTURING COMPONENTS)	5043	C	CAPRIC ACID	121.1070	1647
	0058	C	CAPRYLIC ACID	121.1070	1697
	5060	C	CETYL ALCOHOL, SYNTHETIC	121.1238	2162
	5068	C	DECYL ALCOHOL, SYNTHETIC	121.1238	2422
	5082	C	PALM ALCOHOLS, SYNTHETIC	121.1238	3105
	5134	C	HEXYL ALCOHOL, SYNTHETIC	121.1238	3841
	5150	C	LAURIC ACID	121.1070	4404
	5151	C	LAURYL ALCOHOL, SYNTHETIC	121.1238	4416
	5177	C	MYRISTIC ACID	121.1070	5531
	5178	A	MYRISTYL ALCOHOL, SYNTHETIC	121.1238	5543

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASN
33 (Manufacturing Control)	5181	C	OCTYL ALCOHOL, SYNTHETIC	121.1238	5658
	5182	C	OLEIC ACID	121.1070	5692
	5185	C	PALMITIC ACID	121.1070	5761
	0479	C	STEARIC ACID	121.1070	8935
	5254	C	STEARYL ALCOHOL, SYNTHETIC	121.1238	8947
34 (Washing/Polishing Aids)	5308	C	ALIPHATIC ACID MIXTURE	121.1091	0276
	5306	C	ALKYLENE OXIDE ADDUCTS OF ALKYL ALCOHOLS/PHOSPHATE ESTERS OF ALKYLENE OXIDE ADDUCTS OF ALKYL ALCOHOLS, MIXTURE OF	121.1091	0311
	5313	C	CHEMICALS USED IN WASHING OR LYE PERLING FRUIT AND VEGETABLES	121.1091	2185
	5335	C	PETROLEUM HYDROCARBONS, ISOPARAFFINIC, SYNTHETIC	121.1158	5993
	5191	A	PETROLEUM HYDROCARBONS, ODORLESS, LIGHT	121.1182	6002
	5357	C	POLYACRYLAMIDE	121.1091	6210
	5357	C	POLYACRYLAMIDE	121.1119	6210
	5363	C	POTASSIUM BROMIDE	121.1091	6670
	5368	C	SODIUM N-ALKYLENE SULFONATE	121.1091	7613
	5370	C	SODIUM DODECYLBENZENE SULFONATE	121.1091	7843
35 (Washing/Fermentation Aids)	5371	C	SODIUM 2-ETHYLHEXYL SULFATE	121.1091	7866
	0319	C	SODIUM HYPOCHLORITE	121.1091	8004
	5238	C	SODIUM MONO- AND DIMETHYL NAPHTHALENE SULFONATES	121.1091	8211
	5238	C	SODIUM MONO- AND DIMETHYL NAPHTHALENE SULFONATES	121.1198	8211
	5328	A	GIBBERELIC ACID	121.1010	3588
	0529	A	MINERAL OIL, WHITE	121.1146	5336

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ADR	
(Methyl Parabenolates)	15	5135	C	PETROLEUM HYDROCARBONS, ISOPARAFFINIC, SYNTHETIC	121.1154	5993
		5191	A	PETROLEUM HYDROCARBONS, ODORLESS, LIGHT	121.1182	6003
		0299	C	POTASSIUM BICRATE	121.1198	6447
		5364	A	POTASSIUM GIBBERELLATE	121.1010	6785
(Propylene Glycol)	26	5120	C	DICHLORODIFLUOROMETHANE	121.1209	2491
(Soybean Lecithin)	17	5167	A	METHYL GLUCOSIDE-COCONUT OIL PHEN	121.1151	5129
		5184	A	OXYSTEARIN	121.1016	5727
(Sorbitol)		5199	A	POLYGLYCEROL ESTERS OF FATTY ACIDS	121.1120	6346
(Nutritive Sweeteners)	40	5016	A	ASPARTAME	121.1258	0805
(Boiler Water Additives)	42	5011	C	ACRYLAMIDE-SODIUM ACRYLATE RESIN	121.1088	0081
		0012	C	ANIONIC ALGINATE	121.1088	0552
		5307	C	BOILER WATER ADDITIVES	121.1088	1032
		5315	C	CORALITE SULFATE	121.1088	2323
		5319	C	CYCLOHEXYLAMINE	121.1088	2553
		5321	C	DIETHYLANHMOETHANOL	121.1088	2714
		5332	C	HYDRAKATINE	121.1088	3910
		5337	C	LIGNOSULFONIC ACID	121.1088	6462
		5347	C	HOMOGENYL STYERS OF POLYETHYLENE-POLYPROPYLENE GLYCOL	121.1088	6428
		5350	C	MORPHOLINE	121.1088	5473
		5351	C	OCTADECYLAMINE	121.1088	5589
		5398	C	POLYETHYLENE GLYCOL (H.W. 200-9,500)	121.1088	6302
		5340	C	POLYPROPYLENE GLYCOL	121.1088	6448

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL					
TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ACN
02 (SOLUBLE WATER SOLUTIONS)	5392	C	TETRASODIUM EDTA	121.1088	9407
	0202	C	TETRASODIUM PYROPHOSPHATE	121.1088	9430
	5395	C	TRISODIUM METHILOTRIACETATE	121.1088	9585
03 (NON-EXCHANGEABLE POLYMER STAGES)	5386	C	ANTHRACITE COAL, SULFONATED	121.1148 A 2	0656
	5322	C	DIPHTHYLURETHAMINE CROSS-LINKED WITH EPICHLOROHYDRIN	121.1148 A 6	2737
	5323	C	EPICHLOROHYDRIN CROSS-LINKED WITH AMMONIA	121.1148 A 14	2875
	5334	C	ION-EXCHANGE PEDIANS	121.1148	4095
	5163	C	METHACRYLIC ACID-DIVINYLBENZENE COPOLYMER	121.1148 A 4	4899
	5339	C	METHYL ACRYLATE-DIVINYLBENZENE CONDENSER, MIN. 25 W/W DIVINYLBENZENE, AMINOLYSED WITH DIMETHYLAMINOPROPYLAMINE	121.1148 A 12	4968
	5348	C	METHYL ACRYLATE-DIVINYLBENZENE CONDENSER, MIN. 1.55 W/W DIVINYLBENZENE, AMINOLYSED WITH DIMETHYLAMINOPROPYLAMINE	121.1148 A 13	4997
	5341	C	METHYL ACRYLATE-DIVINYLBENZENE-DIETHYLENE GLYCOL-DIVINYL ETHER TERPOLYMER, MIN. 3.5% W/W DIVINYLBENZENE AND MAX. 0.4% W/W DIETHYLENE GLYCOL DIVINYLETHER, AMINOLYSED WITH DIMETHYLAMINOPROPYLAMINE	121.1148 A 16	5014
	5317	C	METHYL ACRYLATE AND DIVINYLBENZENE COPOLYMERS, COMPLETELY HYDROLYSED	121.1148 A 9	5024
	5396	C	METHYL ACRYLATE, DIVINYLBENZENE, AND ACRYLONITRILE TERPOLYMERS, COMPLETELY HYDROLYSED	121.1148 A 10	5030
5354	C	PHENOL-FORMALDEHYDE, CROSS-LINKED, ACTIVATED WITH TETRATHYLENE-DIPTALAMINE	121.1148 A 7	6095	
5355	C	PHENOL-FORMALDEHYDE, CROSS-LINKED, ACTIVATED WITH TRISTYLENE-TETRAMINE	121.1148 A 7	6118	

9. MINERAL LITERATURE OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SOR NR	PART NAME	SECTION NR	ASH
5356	C	PHENOL-FORMALDEHYDE, CROSS-LINKED, ACTIVATED WITH TRIETHYLENE-TETRAAMINE AND TETRAETHYLENE-PENTAMINE	121.1140 A 7	6161
5363	C	PHENOL-FORMALDEHYDE, SULFITE-MODIFIED CROSS LINKED	121.1140 A 3	6151
5319	C	POLYTYRONE, CROSS-LINKED	121.1140 A 5	6504
5366	C	RESIN, REACTION OF FORMALDEHYDE, ACETONE, AND TETRAETHYLENE-PENTAMINE	121.1140 A 6	7360
5385	C	STYRENE AND DIVINYLBENZENE, SULFONATED COPOLYMER	121.1140 A 1	8982
5386	C	STYRENE, DIVINYLBENZENE, AND ACRYLONITRILE, SULFONATED TERPOLYMER	121.1140 A 11	8985
5388	C	STYRENE, DIVINYLBENZENE, ACRYLONITRILE, AND METHYL ACRYLATE, SULFONATED TERPOLYMER	121.1140 A 15	8988
5387	C	STYRENE, DIVINYLBENZENE, AND METHYL ACRYLATE, SULFONATED TERPOLYMER	121.1140 A 11	8991
5391	C	TETRAETHYLENEPENTAMINE CROSS-LINKED WITH EPICHLOROHYDRIN	121.1140 A 6	9384
5394	C	TRIETHYLENETETRAAMINE CROSS-LINKED WITH EPICHLOROHYDRIN	121.1140 A 6	9522
5001	A	ACETYLATED MONOGLYCERIDES	121.1018	0069
5005	A	ADIPIC ACID	(SEE NOTE 1A)	0115
5013	A	ALUMINUM	(SEE NOTE 2A)	0161
5276	A	ALGAE, BROWN--LONIDARIA SPP. AND HECOCYTIS SPP. (NATURAL EXTRACTIVE)	(SEE NOTE 3A)	0207
5277	A	ALGAE, RED--POLYCHA SPP. AND BRONKHIAHALMATA (L.) GRUY. (NATURAL SUBSTANCE)	(SEE NOTE 3A)	0230
5278	A	ALGAE, RED--POLYCHA SPP. AND BRONKHIAHALMATA (L.) GRUY. (NATURAL EXTRACTIVE)	(SEE NOTE 3A)	0253
0340	A	AMMONIUM CHLORIDE	(SEE NOTE 2A)	0575
0016	A	AMMONIUM PHOSPHATE, DIAMIC	(SEE NOTE 6A)	0590

5A
 (SUBSTANCES LISTED IN
 "A" GROUPS) PART A
 FOR WHICH RESPONSES
 ARE TO BE GIVEN THE
 PROGRAMMER'S OFFICE

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9. GENERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUN NR	PART	NAME	SECTION NR	ASN
(SUBSTANCES LISTED IN APPROVAL, PART A, FOR WHICH NO ADDITIONAL NOTES SPECIFY THE TECHNICAL EFFECT)	0027	A	RESINATE, BLEACHED	(SEE NOTE 3A)	0943
	0030	A	CAFFEINE	(SEE NOTE 6A)	1173
	0042	A	CALCIUM GLYCEROPHOSPHATE	(SEE NOTE 6A)	1288
	0048	A	CALCIUM PHOSPHATE, DI-BASIC	(SEE NOTE 6A)	1009
	0049	A	CALCIUM PHOSPHATE, MONOBASIC	(SEE NOTE 6A)	1072
	0050	A	CALCIUM PHOSPHATE, TRIBASIC	(SEE NOTE 6A)	1095
	0052	A	CALCIUM PROPIONATE	(SEE NOTE 6A)	1529
	0061	A	CANWAX WAX	(SEE NOTE 3A)	1017
	0020	A	CHLORINE	(SEE NOTE 2A)	2231
	0025	A	CHLORINE DIOXIDE	(SEE NOTE 2A)	2250
	0027	A	COPPER SULFATE	(SEE NOTE 2A)	2030
	0270	A	CORN SYRUP	(SEE NOTE 2A)	2461
	5067	A	COTTONSEED FLOUR, DEFATTED	121, 1019 A 2	2071
	5065	A	COTTONSEED FLOUR, PARTIALLY DEFATTED, COOKED	121, 1019 A 1	2000
	0020	A	FERRIC CHLORIDE	(SEE NOTE 2A)	3120
	0080	A	FERRIC PHOSPHATE	(SEE NOTE 6A)	3151
	0081	A	FERRIC PYROPHOSPHATE	(SEE NOTE 6A)	3170
	0082	A	FERRIC SODIUM PYROPHOSPHATE	(SEE NOTE 6A)	3197
	5006	A	FOOD STARCH-MODIFIED	121, 1031	5312
	5129	A	GLYCERIN, SYNTHETIC	121, 1111	3600
	0303	A	HESPERIDIN	(SEE NOTE 2A)	3795
	5201	A	JAPAN TINK	(SEE NOTE 3A)	4255
	0070	A	LACTALBUMIN	(SEE NOTE 2A)	4303
	0305	A	LACTALBUMIN PHOSPHATE	(SEE NOTE 2A)	4320
	0003	A	LOCKNET (CALSON) BEAN OIL	(SEE NOTE 6A)	4520
	0307	A	MAGNESIUM CHLORIDE	(SEE NOTE 2A)	4600

5. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	NSR NR	PART	NAME	SECTION NR	ASH
	0113	A	MANGANESE PHOSPHATE, DIBASIC	(SEE NOTE 8A)	4738
(SUBSTANCES LISTED IN APPENDIX PART A, FOR WHICH REPRESENTATIVES ARE TO SPECIFY THE TECHNICAL EFFECT)	0122	A	MANGANESE GLYCEROPHOSPHATE	(SEE NOTE 8A)	4807
	0123	A	MANGANESE HYPOPHOSPHITE	(SEE NOTE 8A)	4830
	0490	A	MENADIOL SODIUM DIPHOSPHATE	(SEE NOTE 8A)	4853
	0128	A	METHYLPARABEN	(SEE NOTE 1A)	5175
	0130	A	MONO- AND DIGLYCERIDES	(SEE NOTE 8A)	5400
	0134	A	MONOGAMMINE GLUTAMATE	(SEE NOTE 8A)	5468
	0335	A	PECTIN	(SEE NOTE 2A)	5865
	0432	A	PECTIN, MODIFIED	(SEE NOTE 2A)	5888
	0143	A	PHOSPHORIC ACID	(SEE NOTE 8A)	6187
	0392	A	POTASSIUM ACID PYROPHOSPHATE	(SEE NOTE 8A)	6591
	0144	A	POTASSIUM ACID TARTRATE	(SEE NOTE 1A)	6620
	0153	A	POTASSIUM GLYCEROPHOSPHATE	(SEE NOTE 8A)	6808
	0302	A	POTASSIUM HYPOPHOSPHITE	(SEE NOTE 8A)	6821
	0346	A	POTASSIUM IODATE	(SEE NOTE 2A)	6854
	0157	A	POTASSIUM PHOSPHATE, DIBASIC	(SEE NOTE 8A)	7038
	0308	A	POTASSIUM TRIOXYPHOSPHATE	(SEE NOTE 8A)	7099
	0146	A	PROPYLPARABEN	(SEE NOTE 1A)	7199
	0174	A	SODIUM ACID PYROPHOSPHATE	(SEE NOTE 8A)	7547
	0179	A	SODIUM ALUMINUM PHOSPHATE, ACIDIC	(SEE NOTE 8A)	7659
	0348	A	SODIUM ALUMINUM PHOSPHATE, BASIC	(SEE NOTE 8A)	7682
	0320	A	SODIUM HYPOPHOSPHITE	(SEE NOTE 2A)	8027
	0190	A	SODIUM METAPHOSPHATE	(SEE NOTE 8A)	8142
	0197	A	SODIUM PHOSPHATE, DIBASIC	(SEE NOTE 8A)	8372
	0198	A	SODIUM PHOSPHATE, MONOBASIC	(SEE NOTE 8A)	8618
	0199	A	SODIUM PHOSPHATE, TRIBASIC	(SEE NOTE 8A)	8668

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASN
(SUBSTANCES LISTED IN APPENDIX 1, PART A, FOR WHICH RESIDUES ARE TO SPECIFY THE TECHNICAL EFFECT)	0201	A	SODIUM PROPIONATE	(SEE NOTE 4A)	8544
	0202	A	SODIUM PYROPHOSPHATE	(SEE NOTE 4A)	8556
	0210	A	SODIUM TRIPOLYPHOSPHATE	(SEE NOTE 4A)	8763
	0330	A	TANNIC ACID	(SEE NOTE 3A)	9269
	0219	A	TARTARIC ACID	(SEE NOTE 1A)	9315
	5271	A	YEAST, DRIED	121, 1125	9752
(SPECIAL SUB-SUBJECT TO BE CONDUCTED ON THOSE SUBSTANCES)	5061	A	CHWING GUM BASE	121, 1059	2500

DFD:DC:FN
21 JAN 77

9. NUMERIC LISTING OF TECHNICAL EFFECTS - OVERALL

TECH EFFECT	SUB NR	PART	NAME	SECTION NR	ASN
82 (POLYMER WATER ADDITIVE)	0150	C	POTASSIUM CARBONATE	121.1088	6739
	0308	C	POTASSIUM TRIPOLYPHOSPHATE	121.1088	7096
	0175	C	SODIUM ACETATE	121.1088	7588
	0177	C	SODIUM ALUMINATE	121.1088	7590
	5169	C	SODIUM ALUMINATE	121.1088	7678
	0185	C	SODIUM CARBONATE	121.1088	7751
	0186	C	SODIUM CARBOXYMETHYLCELLULOSE	121.1088	7774
	5172	C	SODIUM GLUCONATE	121.1088	7912
	0194	C	SODIUM HEXAMETAPHOSPHATE	121.1088	7925
	5173	C	SODIUM HYDROXIDE	121.1088	7958
	0192	C	SODIUM HYDROXIDE	121.1088	7981
	5174	C	SODIUM LIGNOSULFONATE	121.1088	8096
	0193	C	SODIUM METABISULFITE	121.1088	8120
	0321	C	SODIUM METASILICATE	121.1088	8145
	0342	C	SODIUM NITRATE	121.1088	8280
	0197	C	SODIUM PHOSPHATE, DIBASIC	121.1088	8395
	0198	C	SODIUM PHOSPHATE, MONOBASIC	121.1088	8401
	0199	C	SODIUM PHOSPHATE, TRIBASIC	121.1088	8407
	5177	C	SODIUM POLYACRYLATE	121.1088	8510
	5378	C	SODIUM POLYMETHACRYLATE	121.1088	8533
	5379	C	SODIUM SILICATE	121.1088	8602
	5380	C	SODIUM SULFATE	121.1088	8694
	0206	C	SODIUM SULFITE, ALKALINE	121.1088	8718
	5381	C	SODIUM SULFITE, NEUTRAL	121.1088	8740
	0210	C	SODIUM TRIPOLYPHOSPHATE	121.1088	8784
	5189	C	TANNIN (INCLUDING QUINACHO EXTRACT)	121.1088	9292

LISTING OF COLORS USED IN FOODS AND/OR DRUGS AND/OR COSMETICS

TAB E

1. Color Additives Permitted for Use in Food

Listed Subject to Certification

Citrus Red No. 2	-- For Coloring Oranges Only
Orange B	-- For Coloring Sausages
FD & C Blue No. 1	
FD & C Red No. 3	
FD & C Red No. 40	
FD & C Yellow No. 5	

Exempt from Certification

Dried algae meal	-- Chicken Feed
β -apo-3'-Carotenol	
Caramel	
β -Carotene	
Annatto Extract	
Tagetes Meal and Extract	-- Chicken Feed
Paprika	
Paprika Oleoresin	
Turmeric	
Turmeric Oleoresin	
Saffron	
Fruit Juice	
Vegetable Juice	
Toasted Partially Defatted Cooked Cottonseed Flour	
Titanium Dioxide	
Cochineal Extract; Carmine	
Grape Skin Extract (Enocianina)	-- Beverages
Ultramarine Blue	-- Coloring Salt for Animal Feed
Ferrous Gluconate	--Ripe Olives
Dehydrated Beets(Beet Powder)	
Corn Endosperm Oil	-- Chicken feed
Riboflavin	
Carrot Oil	
Synthetic Iron Oxide	-- Dog and Cat Foods
Canthaxanthin	

Provisionally listed and Subject to Certification

FD & C Green No. 3
FD & C Yellow No. 6
FD & C Blue No. 2

2. Color Additives Permitted for Use in Drugs

Listed Subject to Certification

FD & C Blue No. 1	Ingested drugs
FD & C Blue No. 2	Sutures
[Rhthalocyaninato(2-)] copper	Sutures
D & C Blue No. 9	Sutures
D & C Green No. 5	Sutures
D & C Green No. 6	Sutures
FD & C Red No. 3	Ingested drugs
FD & C Red No. 40	
D & C Red No. 39	Germicide
D & C Violet No. 2	Sutures
FD & C Yellow No. 5	Ingested drugs
D & C Green No. 8	Externally applied drugs
D & C Yellow No. 7	Externally applied drugs
D & C Yellow No. 8	Externally applied drugs
D & C Yellow No. 11	Externally applied drugs
D & C Red No. 17	Externally applied drugs
D & C Red No. 31	Externally applied drugs
D & C Red No. 34	Externally applied drugs
D & C Violet No. 2	Externally applied drugs
Ext. D & C Yellow No. 7	Externally applied drugs
D & C Blue No. 4	Externally applied drugs

Exempt from Certification

Synthetic Iron Oxide	
Caramel	
Annatto Extract	Ingested drugs
B-Carotene	Ingested drugs
Titanium Dioxide	
Pyrophyllite	Externally applied drugs
Cochineal Extract; Carmine	
Chromium-cobalt-aluminum Oxide	Sutures
Alumina (Dried Aluminum Hydroxide)	
Calcium Carbonate	
Talc	
Potassium Sodium Copper Chlorophyllin	Dentifrices
Carthaxanthin	Ingested drugs
Dihydroxyacetone	Suntan lotions
Pyrogallol	Sutures
Ferric Ammonium Citrate	Sutures

3. Color Additives Permitted for Use in Cosmetics

Listed Subject to Certification

FD & C Red No. 40	
D & C Green No. 8	Externally applied cosmetics
D & C Yellow No. 7	Externally applied cosmetics
D & C Yellow No. 8	Externally applied cosmetics
D & C Yellow No. 11	Externally applied cosmetics
D & C Red No. 17	Externally applied cosmetics
D & C Red No. 31	Externally applied cosmetics
D & C Red No. 34	Externally applied cosmetics
D & C Violet No. 2	Externally applied cosmetics
D & C Brown No. 1	Externally applied cosmetics
Ext. D & C Yellow No. 7	Externally applied cosmetics
Ext. D & C Violet No. 2	Externally applied cosmetics
D & C Blue No. 4	Externally applied cosmetics

Exempt from Certification

Titanium Dioxide	
Henna	Hair coloring
Pyrophyllite	Externally applied cosmetics
Potassium Sodium Copper Chlorophyllin	Dentifrices
Dihydroxyacetone	Suntan lotion
Disodium EDTA Copper	Shampoos
Azulene	
Iron oxides	
Manganese violet	External including eye area
Ultramarine blue	External including eye area
Ultramarine green	External including eye area
Ultramarine pink	External including eye area
Ultramarine red	External including eye area
Ultramarine violet	External including eye area

4. Color Additives Provisionally Listed for Use in Drugs

Subject to Certification

FD&C Green No. 3	External use only.
FD&C Yellow No. 5	External use only.
FD&C Yellow No. 6	External use only.
FD&C Red No. 3	External use only.
FD&C Blue No. 1	External use only.
FD&C Blue No. 2	Ingested drugs.
D&C Green No. 5	
D&C Green No. 6	
D&C Yellow No. 10	
D&C Red No. 6	
D&C Red No. 7	
D&C Red No. 8	
D&C Red No. 9	External use only.
D&C Red No. 10	External use only.
D&C Red No. 11	External use only.
D&C Red No. 12	
D&C Red No. 13	External use only.
D&C Red No. 19	
D&C Red No. 21	
D&C Red No. 22	
D&C Red No. 27	
D&C Red No. 28	
D&C Red No. 30	
D&C Red No. 33	
D&C Red No. 36	
D&C Red No. 37	
D&C Orange No. 4	External use only.
D&C Orange No. 5	
D&C Orange No. 10	
D&C Orange No. 11	
D&C Orange No. 17	External use only.
D&C Blue No. 6	
Ext. D&C Yellow No. 1	
Ext. D&C Green No. 1	

Exempt from Certification

Logwood

Surgical suture use only.

5. Color Additives Provisionally Listed for Use in Cosmetics

Subject to Certification

FD&C Green No. 3
 FD&C Yellow No. 5
 FD&C Yellow No. 6
 FD&C Red No. 3
 D&C Blue No. 1
 D&C Green No. 5
 D&C Green No. 6
 D&C Yellow No. 10
 D&C Red No. 6
 D&C Red No. 7
 D&C Red No. 8
 D&C Red No. 9
 D&C Red No. 10
 D&C Red No. 11
 D&C Red No. 12
 D&C Red No. 13
 D&C Red No. 19
 D&C Red No. 21
 D&C Red No. 22
 D&C Red No. 27
 D&C Red No. 28
 D&C Red No. 30
 D&C Red No. 33
 D&C Red No. 36
 D&C Red No. 37
 D&C Orange No. 4
 D&C Orange No. 5
 D&C Orange No. 10
 D&C Orange No. 11
 D&C Orange No. 17
 D&C Blue No. 6
 Ext. D&C Yellow No. 1
 Ext. D&C Green No. 1

External use only.
 External use only.

Exempt from Certification

Aluminum powder
 Annatto
 Bismuth citrate
 Bismuth oxychloride
 Bronze powder
 Caramel
 Carmine
 Carotene
 Chromium hydroxide green
 Chromium oxide greens
 Copper, metallic powder

For use as a color component in
 hair dye.

continued, color additives exempt from certification, for use
in cosmetics -

Ferric ferrocyanide (iron blue)

Graphite

Guanine (pearl essence)

Lead acetate

**For use as a color component in
hair dye.**

Mica

Zinc oxide

PARTIAL LIST OF PRIOR SANCTIONED SUBSTANCES

TAB F

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FOOD DRUG COSMETIC LAW JOURNAL—DECEMBER, 1958

LIST OF CHEMICALS APPROVED UNDER FEDERAL MEAT INSPECTION ACT BEFORE SEPTEMBER 6, 1958, WHICH ARE EXEMPTED FROM 1958 FOOD ADDITIVES AMENDMENT OF FEDERAL FOOD, DRUG, AND COSMETIC ACT

UNITED STATES DEPARTMENT OF AGRICULTURE

Agricultural Research Service
Meat Inspection Division
Washington 25, D. C.

ZYLY-
MID-57-27
November 8, 1957

TO: Inspectors in Charge of Meat Inspection
FROM: R. M. Mehurin, Chief, Chemical Control Section
SUBJECT: Permitted Chemical Additives

<i>Additive</i>	<i>Reference</i>	<i>Purpose</i>	<i>Products</i>	<i>Amount</i>
Acetic acid	Reg. 18.7(i)	Refining	Rendered fats	Sufficient for purpose
Amines, filming	Correspondence	Volatile boiler additive to retard corrosion in pipes	Steam	See chemical names
Anti-foam A (methyl polysilicone)	Correspondence	To retard foaming	Soup	10 ppm
Antioxidants (oxygen interceptors)	Reg. 16.13(e); 18.7(d); 17.9(d)	To retard rancidity development	Lard and shortening	0.01% singly; 0.02% combinations
Ascorbic acid	Reg. 18.7(s); 28.3(b)(7)	Accelerate color fixing in curing	Cured cuts; cured comminuted product	7½ ozs. to 100 gals. pickle; ¾ oz. to 100 lbs. meat
Bacterial starter; lactic acid starter culture	Reg. 18.7(p) Memo. 234	To develop flavor	Dry sausage; pork roll	0.5%
Benzoate, sodium; benzoic acid	Reg. 28.1(a)(3)(ii), (b)(1)(v)	To retard flavor reversion	Oleomargarine	0.1%
BHA (butylated hydroxyanisole)	Reg. 17.9(d) 18.7(d)(5); Reg. 18.7(t) 16.13(c)	Antioxidant: To retard rancidity development Antioxidant: To retard rancidity development	Lard and shortening Unsmoked dry sausage	0.01% 0.003%

<i>Additive</i>	<i>Reference</i>	<i>Purpose</i>	<i>Products</i>	<i>Amount</i>
BHT (butylated hydroxy-toluene)	Reg. 18.7(d) (6); 17.9(d)	Antioxidant: To retard rancidity development	Lard and shortening	0.01%
Bicarbonate of soda	Reg. 18.7(l); Correspondence	To neutralize excess acidity; cleaning vegts.	Rendered fats; tomato soup; beans, etc.	Sufficient for purpose
Borax	Reg. 17.9; 18.8	Preservative	Export	Sufficient for purpose
Bromelin	Correspondence	Same use as papain		
Carbon (purified charcoal)	Reg. 18.7(i)	Refining	Rendered fat	Sufficient for purpose
Carbon dioxide gas	Correspondence	Immobilizing	Hogs	Sufficient for purpose
Caseinate, sodium	Manual 18.28	Binder and extender	Imitation sausage; non-specific loaves; soups; stews; etc.	Sufficient for purpose
Caustic soda (sodium hydroxide)	Reg. 18.7(i) (j)	Refining; denuding	Fats; tripe	Sufficient for purpose
Cellulose gum (carboxymethyl cellulose)	Correspondence	Extender, stabilizer	Baked pies, etc.	Sufficient for purpose
Citric acid	Reg. 18.7(d) (9)	Synergist: To increase effectiveness of antioxidants	Lard and shortening	0.01%
	Reg. 18.6(t)	Synergist: To increase effectiveness of antioxidants	Unsmoked dry sausage	0.01%
	Reg. 28.1(a) (3) (viii), (b) (1) (viii) Correspondence	To protect flavor Flavoring	Oleomargarine Chili con carne	Sufficient for purpose Sufficient for purpose
Citric acid and sodium citrate	Reg. 18.7(o)	Prevent coagulation	Beef blood	0.2%
Coloring material, vegetable, and synthetic	Reg. 28.1(a) (3) (i), (b) (1) (iv); 18.7(m); (CFR Amendment 57-29); 17.9(a) (b); 16.7(a); 16.13(d)	To color casings, rendered fat, marking ink, etc.	Sausage casings; oleomargarine; shortening; branding ink	Sufficient for purpose

<i>Additive.</i>	<i>Reference</i>	<i>Purpose</i>	<i>Products</i>	<i>Amount</i>
Corn syrup	Reg. 18.7(b) (q); Memo. 243	Flavor; cure	Cured product; hamburger	2.5%
Corn syrup dried	Reg. 18.7(b) (q); Memo. 243	Flavor; cure	Cured product; hamburger	2.0%
Cyclamate, sodium	Memo. 249	Sweetener	Bacon	0.15%
Cyclohexylamine	Correspondence	To retard corrosion	Boiler water	10 ppm from approved feeder
Dextrose	Reg. 18.7(b); 28.2(a) (4); Memo. 215	Flavor; cure; seasoning	Sausage, ham, etc.	Sufficient for purpose
Diacyl	Reg. 28.1(a) (3) (iv), (b) (1) (vii)	Flavor	Oleomargarine	Sufficient for purpose
Diatomaceous earth	Reg. 18.7(i)	Refining	Rendered fats	Sufficient for purpose
Dry ice (carbon dioxide-solid)	Memo. 239	Cooling	Chopping of meat; packaging of product	Sufficient for purpose
Enzymes, proteolytic	See papain, bromelin, ficin			
Ficin	Correspondence	Same use as papain		
Fullers earth	Reg. 18.7(i)	Refining	Rendered fats	Sufficient for purpose
Glycerol (glycerine)	Reg. 18.6(a) (5) Correspondence	Inhibit drying; manufacture of mono- & diglycerides	Seasonings, curing mixes	Sufficient for purpose
Glycerol lactopalmitate, etc.	Correspondence	A type of mono- & diglycerides	Shortening	Sufficient for purpose
Gums, vegetable (tragacanth, karaya, etc.)	Correspondence	Emulsifying agent; binder	Spice emulsion; egg roll, breading mix	Sufficient for purpose
Hydrogen peroxide	Reg. 18.7(j)	Bleach	Tripe	Sufficient for purpose
Hydrolyzed plant protein	Reg. 28.2(b) (7) Manual 18.52 to 18.56	Flavor	Various	Sufficient for purpose
Isoscorbic acid	Reg. 18.7(s); 28.3(b) (7)	Accelerate color fixing in curing	Cured cuts; cured comminuted product	7½ ozs. to 100 gals. pickle; ¾ oz. to 100 lbs. meat
Isopropyl citrates	Reg. 28.1(a) (3) (ix), (b) (1) (ix)	To protect flavor	Oleomargarine	0.02%

<i>Additive</i>	<i>Reference</i>	<i>Purpose</i>	<i>Products</i>	<i>Amount</i>
ascithin	Reg. 18.7(d) (4); Correspondence	To retard rancidity development; emulsifier	Lard and shortening	Sufficient for purpose
Lignin	Reg. 28.1(a)(9) Correspondence	Emulsifier Loosen scale	Oleomargarine Steam boilers	0.5% Sufficient for purpose
Lime	Reg. 18.7(j)	Denude	Tripe	Sufficient for purpose
Malt syrup, Mono- and diglycerides	Correspondence Reg. 17.8(c) (43); 18.7(c); 28.1(a)(9)	Flavor; cure Emulsifiers	Cured products Lard, shortening; oleomargarine	2.5% Sufficient for purpose; 0.5% in oleomargarine
Monoisopropyl citrate	Reg. 18.7(d) (9); 28.1(a) (3) (ix), (b) (1) (ix)	To increase effectiveness of antioxidants	Lard, shortening; oleomargarine	0.01% in lard & shortening; 0.02% in oleomargarine
Monosodium glutamate	Reg. 28.2(b) (6); Manual 18.52 to 18.56	Flavor	Various	Sufficient for purpose
Morpholine	Correspondence	To retard corrosion	Boiler water	10 ppm from approved feeder
Nickel	Reg. 18.7(i)	Catalyst	Hydrogenated fats	Sufficient for purpose
Nitrate of soda or potassium	Reg. 18.7(k)	Source of nitrite	Cured products	See sodium or potassium nitrate
Nitrite of soda or potassium	Reg. 18.7(k)	Fix color	Cured products	See sodium or potassium nitrite
Nitrogen	Correspondence	Exclude oxygen	Sealed products	Sufficient for purpose
Nordihydroguaiaretic acid (NDGA)	Reg. 18.7(d)(2)	Antioxidant: To retard rancidity development	Lard and shortening	0.01%
Octadecylamine	Correspondence	Boiler additive to retard corrosion in steam pipes	Steam	25 ppm in condensed steam (analysis required)
Oxygen interceptor	Reg. 16.13(e); 17.9(d); 18.7(d)	See antioxidants		
Papain	Reg. 17.8(c) (56); Memo. 226	Soften tissue	Frozen cuts	Sufficient for purpose

<i>Additive</i>	<i>Reference</i>	<i>Purpose</i>	<i>Products</i>	<i>Amount</i>
Phosphates: disodium; sodium hexameta-; tripoly-; sodium pyro-; sodium acid pyrophosphate	Reg. 18.7(r); 18.7(t); 28.3 (b) (6)	Decrease amount of cooked-out juices	Ham, pork shoulder picnics; canned chopped ham	5.0% in pumping pickle; 0.5% in canned chopped ham
Phosphate, sodium hexameta-	Correspondence	Retard scale formation in pipes	Potable water supply	10 ppm from approved feeder
Phosphate, trisodium	Reg. 18.7(j)	Denuder	Tripe	Sufficient for purpose
Phosphoric acid	Reg. 18.7(d) (9)	Synergist: To increase effectiveness of antioxidants	Lard and shortening	0.01%
Propyl gallate	Reg. 18.7(d) (7)	Antioxidant: To retard rancidity development	Lard and shortening	0.01%
Resin guaiac	Reg. 18.7(d) (1)	Antioxidant: To retard rancidity development	Lard and shortening	0.1%
Silica gel	Correspondence	Anti-caking agent	Curing mixes, etc.	0.5%
Sodium ascorbate; sodium isoascorbate	Reg. 18.7(s); 28.3(b) (7); Memo. 217, Supl. 1 & 2	Accelerate color fixing in curing	Cured cuts; cured comminuted product	7½ ozs. to 100 gals. pickle; ¾ oz. to 100 lbs. meat; 10% soln. to surface of cured product prior to packaging
Sodium bicarbonate	See bicarbonate of soda			
Sodium carbonate	Reg. 18.7(i); 18.7(j)	Refining; denuding	Fats; tripe	Sufficient for purpose
Sodium caseinate	Manual 18.28	Binder and extender	Imitation sausage; non-specific soups; stews; etc.	Sufficient for purpose
Sodium hydroxide (caustic soda)	Reg. 18.7(j)	Denuder	Tripe	Sufficient for purpose

<i>Additive</i>	<i>Reference</i>	<i>Purpose</i>	<i>Products</i>	<i>Amount</i>
Sodium metasilicate	Reg. 18.7(j)	Denuder	Tripe	Sufficient for purpose
Sodium or potassium nitrate	Reg. 18.7(k)	Source of nitrite	Cured products	7 lbs. to 100 gals. pickle; 3½ ozs. to 100 lbs. meat (dry cure); 2¼ ozs. to 100 lbs. chopped meat
Sodium or potassium nitrite	Reg. 18.7(k)	Color fixing	Cured products	200 ppm (0.2%) in product; 2 lbs. to 100 gals. pickle; 1 oz. to 100 lbs. meat (dry cure); ¼ oz. to 100 lbs. chopped meat
Sodium sulfoacetate derivatives of mono- and diglycerides	Reg. 28.1(a) (9)	Emulsifier	Oleomargarine	0.5%
Sorbitol	Correspondence	Retard drying	Seasonings	Sufficient for purpose
Starter distillate	Reg. 28.1(a) (3) (iv), (b) (1) (vii)	Flavor	Oleomargarine	Sufficient for purpose
Stearyl citrate	Reg. 28.1(a) (3) (x), (b) (1) (x)	To protect flavor	Oleomargarine	0.15%
Sugars, approved (sucrose and dextrose)	Reg. 18.7(b); 28.2(a) (4); Memo. 215	Flavoring; curing; seasoning	Sausage, ham, miscellaneous	Sufficient for purpose
Sulphites with strong alkali	Correspondence	Retard corrosion	Boiler water	Sufficient for purpose
Tannic acid	Reg. 18.7(i); Correspondence	Refining; loosen scale	Rendered fats; boiler water	Sufficient for purpose
Tocopherols	Reg. 18.7(d) (3)	Antioxidant: To retard rancidity development	Lard and shortening	0.03%

<i>Additive</i>	<i>Reference</i>	<i>Purpose</i>	<i>Products</i>	<i>Amount</i>
Trisodium phosphate	Reg. 18.7(j)	Denuder	Tripe	Sufficient for purpose

This memorandum supersedes "Meat Inspection Division Memorandum No. 57-3, dated February 25, 1957.

R. M. Mehurin
[Signature]

ACCEPTABLE MATERIALS FOR PLASTICS, UNITED STATES DEPARTMENT OF AGRICULTURE, MEAT INSPECTION DIVISION, JANUARY, 1958

SYNTHETIC RESINS, PLASTICIZERS, STABILIZERS, DRIERS, DRYING OILS, COLORANTS AND RELEASE AGENTS APPROVED FOR USE IN RUBBER OR SYNTHETIC RESIN PLASTICS INTENDED FOR CONTACT WITH FEDERALLY INSPECTED MEAT FOOD PRODUCT

Resins

A. Can enamels

The classes of resins that have been met with most frequently are:

Bisphenol-epichlorhydrin
Bisphenol-epichlorhydrinesters
Epoxy ester
Bisphenol-epichlorhydrin-vinyl
Certain modified phenols
Certain modified vinyls
Oleoresins
Alkyl oleoresinous
Polyester
Alkyl ester

The basic types of resins may be listed as follows:

Fossil resins, specifically gilsonite, and natural East Indian and Congo resins
Bisphenol-formaldehyde
Certain substituted phenol formaldehydes
Phenol formaldehyde
Urea formaldehyde
Bisphenol-epichlorhydrin
Bisphenol-epichlorhydrin esters
Maleic anhydride resin ester

Esterified Congo resin
Esterified resin
Melamine-formaldehyde
Polyvinyl chloride and acetate
Cellulose acetate butyrate
Polystyrene
Polyvinyl butyral
Polyethylene
Petroleum hydrocarbon

B. Films for wrapping foods

The acceptable resins for wrapping foods are:

Condensate of dimethyl terephthalate and ethylene glycol
Resins from high and low viscosity polyvinyl alcohol for fatty foods only
Polyvinyl chloride
Polyvinyl acetate
Polyvinyl chloride-acetate
Vinylidene chloride
Polystyrene
Polyethylene of the high and low pressure type
Cellulose acetate
Regenerated cellulose
Butadiene-styrolonitrile synthetic rubber

Methyl and ethyl acrylate
 Ethyl cellulose
 Rubber hydrochloride
 Polyester resin—ethylene terephthalate and ethylene isophthalate
 Butadiene acrylonitrile-styrene copolymer
 Butadiene-styrene copolymer
 Terephthalic acid-ethylene glycol copolymer

C. Grease-proofing and wet-strength resins for paper wraps

Polymer of 2-chloro-butadiene
 Polymer of melamine-formaldehyde
 Polymer of urea-formaldehyde
 Polymer of dimethyl-polysiloxane of 350 centistokes viscosity
 Copolymer of styrene and isobutylene (for foods of high water content only)

Plasticizers

Acetyl tributyl citrate
 Acetyl triethyl citrate
 Butyl stearate
 Butyl phthalyl butyl glycolate
 p-Tertiary butyl phenyl salicylate
 Dibutyl sebacate
 Di-iso butyl adipate
 Di-2-ethylhexyl phthalate (for foods of high water content only)
 Di-iso-octyl phthalate (for food of high water content only)
 Diethyl phthalate
 2-Ethylhexyl diphenyl phosphate
 Ethyl phthalyl ethyl glycolate
 Glyceryl monooleate
 Glycerin triacetate
 Monisopropyl citrate
 Paraplex G-60
 Paraplex G-62
 Stearyl citrate
 Triethyl citrate
 3-(2-Xenoxyl)-1,2-epoxypropane

Stabilizers

Aluminium monostearate
 Ammonium citrate

Ammonium potassium phosphate
 Calcium acetate
 Calcium ethyl acetoacetate acetate
 Calcium carbonate
 Calcium glycerophosphate
 Mono-, di- and tricalcium phosphate
 Calcium oleate
 Calcium ricinoleate
 Calcium stearate
 Magnesium glycerophosphate
 Magnesium stearate
 Mono-, di- and trimagnesium phosphate
 Potassium citrate
 Disodium hydrogen phosphate
 Sodium citrate
 Sodium pyrophosphate
 Sodium tetrapyrophosphate
 Tin stearate

Inorganic salts of copper manganese, and zinc. zinc stearate and zinc resinates, are acceptable if the leaching of the metal contributes less than 50 parts per million to the food.

Driers

Cobalt caprylate, linoleate, naphthenate, and tallate
 Iron caprylate, linoleate, naphthenate, and tallate
 Manganese caprylate, linoleate, naphthenate, and tallate

Drying Oils

Chinawood oil
 Dehydrated castor oil
 Linseed oil
 Tall oil

Colorants (Pigments)

Carbon black
 Oxides of iron
 Titanium dioxide (National Formulary grade)
 Ultramarine blue

Release Agents

Acrowax C
 DC Fluid 200 of 50,000 centistokes viscosity
 Methyl polysiloxane of 350 centistokes viscosity

Microcrystalline wax
 Linoleic acid amide
 Oleic acid amide
 Palmitic acid amide
 Stearic acid amide

Polyethylene glycol 400
 Polyethylene glycol 1500
 Polyethylene glycol 4000
 Polytetrafluoroethylene

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
 FOOD AND DRUG ADMINISTRATION
 (21 CFR PART 121)

FOOD ADDITIVES

PROPOSED DEFINITIONS AND PROCEDURAL AND
 INTERPRETATIVE REGULATIONS

[*Federal Register*, December 9, 1958, pages 9511-9517]

The Commissioner of Food and Drugs, in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (secs 201, 402, 409, 701; 72 Stat. 1784, 1785 et seq.; 52 Stat. 1055, as amended, 70 Stat. 919, 72 Stat. 948; 21 U. S. C. 321, 342, 348, 371) and pursuant to authority delegated to him by the Secretary of Health, Education, and Welfare, proposes the promulgation of the following regulations with respect to food additives, and hereby offers an opportunity to all interested persons to present their views in writing to the Hearing Clerk, Department of Health, Education, and Welfare, 330 Independence Avenue S. W., Washington 25, D. C., within 30 days from the date of publication of this notice in the FEDERAL REGISTER. Comments may be accompanied by a memorandum or brief, and it is requested that all comments be submitted in quintuplicate.

PART 121—FOOD ADDITIVES

SUBPART A—DEFINITIONS AND PROCEDURAL AND
 INTERPRETATIVE REGULATIONS

Sec.

- 121.1 Definitions and interpretations.
- 121.2 Pesticide chemicals in processed foods.
- 121.3 Substances added to food which are not generally recognized as safe.
- 121.4 Tolerances for related food additives.
- 121.5 Generally recognized safety factors to be considered.
- 121.6 General principles for the evaluation of the safety of food additives.
- 121.7 Food additives for which new-drug applications are required.
- 121.8 Food additives proposed for use in foods for which definitions and standards of identity have been prescribed.
- 121.9-121.50 [Reserved]
- 121.51 Petitions proposing regulations for food additives.
- 121.52 Withdrawal of petitions without prejudice.
- 121.53 Substantive amendments to petitions.
- 121.54 Effective date.
- 121.55 Objections to regulations and requests for public hearings.
- 121.56 Public hearing; notice.
- 121.57 Presiding officer.

Poultry Additives

The following important list of food additives approved under the Poultry Products Inspection Act and exempted by the 1958 food-additives amendment

was received just in time for inclusion in this December JOURNAL. It is presented in full text:

UNITED STATES DEPARTMENT OF AGRICULTURE

Agricultural Marketing Service

Poultry Division

Washington 25, D. C.

November 20, 1958

TO: All Poultry Inspectors, Inspection Branch

FROM: G. S. McKee, Pathology Section Head, Inspection Branch

SUBJECT: PERMITTED FOOD ADDITIVES

<i>Additive</i>	<i>Purpose</i>	<i>Amount</i>
Acetic acid	Refining	Sufficient for purpose
Amines, filming	Volatile boiler additive to retard corrosion in pipes	See chemical names
Agar Agar	Stabilizer	Sufficient for purpose
Animal & Vegetable Oil	Shortening	Sufficient for purpose
Anti-foam A (methyl polysilicone)	To retard foaming	10 ppm
Antioxidants (oxygen interceptors)	To retard rancidity development	0.01% singly; 0.02% combinations
Ascorbic acid	Accelerate color fixing in curing	7½ ozs. to 100 gals. pickle; ½ oz. to 100 lbs. chicken meat
Benzoate, sodium; benzoic acid	To retard flavor reversion	0.1%
BHA (butylated hydroxyanisole)	Antioxidant: To retard rancidity development	0.01%
BHT (butylated hydroxytoluene)	Antioxidant: To retard rancidity development	0.01%
Bicarbonate of soda	To neutralize excess acidity; cleaning vegs.	Sufficient for purpose
Caramel	Flavor	Sufficient for purpose
Carbon (purified charcoal)	Refining	Sufficient for purpose
Caseinate, sodium	Binder and extender	Sufficient for purpose
Cellulose gum (carboxymethyl cellulose)	Extender, stabilizer	Sufficient for purpose
Chlortetracycline	To retard spoilage	7 parts per million
Citric acid	Synergist: To increase effectiveness of antioxidants	0.01%
Citric acid and sodium citrate	Prevents coagulation	0.2%
Condiments	To develop flavor	Sufficient for purpose
Sodium chloride (NaCl)	Flavoring and deboning process	Sufficient for purpose

<i>Additive</i>	<i>Purpose</i>	<i>Amount</i>
Coffee extract	To develop flavor	Sufficient for purpose
Coloring material, vegetable and synthetic	Casings and ink only	Sufficient for purpose
Corn syrup	Flavor, cure	2.5%
Corn syrup dried	Flavor, cure	2.0%
Cyclamate, sodium	Sweetener	0.15%
Cyclohexylamine	To retard corrosion	10 ppm from approved feeder
Dextrose	Flavor; cure seasoning	Sufficient for purpose
Diacetyl	Flavor	Sufficient for purpose
Diatomaceous earth	Refining	Sufficient for purpose
Dry ice (carbon dioxide solid)	Cooling	Sufficient for purpose
Enzymes, proteolytic	Soften tissue	Sufficient for purpose
Ficin	Soften tissue	Sufficient for purpose
Flavoring (chemical and synthetic)	Flavor	Sufficient for purpose
Flour (rice, potato, wheat)	Thickener	Sufficient for purpose
Fullers earth	Refining	Sufficient for purpose
Gelatin	Thickener	Sufficient for purpose
Glycerol (glycerine)	Inhibit drying; manufacture of mono & diglycerides	Sufficient for purpose
Glycerol lactopalmitate, etc.	A type of mono- & diglycerides	Sufficient for purpose
Gums, vegetable (Tragacanth, karaya, etc.)	Emulsifying agent; binder	Sufficient for purpose
Hydrolyzed plant protein	Flavor	Sufficient for purpose
Isoascorbic acid	Accelerate color fixing in curing	7½ ozs. to 100 gals. pickle; ¼ ozs. to 100 lbs. chicken meat
Lactic acid	To develop flavor	0.5%
Lecithin	To retard rancidity development; emulsifier	Sufficient for purpose
Lignin	Loosen scale	Sufficient for purpose
Malt syrup	Flavor; cure	2.5%
Mono-and diglycerides	Emulsifiers	Sufficient for purpose
Monoisopropyl citrate	To increase effectiveness of antioxidants	0.01% in lard & shortening
Monosodium glutamate	Flavor	Sufficient for purpose
Nitrate of soda or potassium	Source of nitrite	See Sodium or potassium nitrate
Nitrite of soda or potassium	Fix color	See sodium or potassium nitrite
Nitrogen	Exclude oxygen	Sufficient for purpose
Nordihydroguaiaric acid (NDGA)	Antioxidant: To retard rancidity development	0.01%
Oxygen interceptors	See antioxidants	
Papain	Soften tissue	Sufficient for purpose

<i>Additive</i>	<i>Purpose</i>	<i>Amount</i>
Phosphates: disodium; sodium hexameta-; tripoly-; sodium pyro-; sodium acid pyrophosphate	Decrease amount of cooked-out juices	5.0% in pumping pickle; 0.5% in canned chopped chicken
Phosphate, sodium hexameta- Phosphoric acid	Retard scale formation in pipes Synergist: To increase effectiveness of antioxidants	10 ppm from approved feeder 0.01%
Propyl gallate	Antioxidant: To retard rancidity development	0.01%
Resin guaiac	Antioxidant: To retard rancidity development	0.1%
Silica gel	Anti-caking agent	0.5%
Sodium ascorbate; sodium isoascorbate	Accelerate color fixing in curing	7½ ozs. to 100 gals. pickle; ¾ oz. to 100 lbs. chicken meat; 10% soln. to surface of cured product prior to packaging
Sodium bicarbonate	To neutralize excess acidity; cleaning vegts.	Sufficient for purpose
Sodium caseinate Sodium or potassium nitrate	Binder and extender Source of nitrite	7 lbs. to 100 gals. pickle; 3½ ozs. to 100 lbs. chicken meat (dry cure); 2¼ oz. to 100 lbs. chopped chicken meat
Sodium or potassium nitrite	Color fixing	200 ppm (0.2%) in product; 2 lbs. to 100 gal. pickle; 1 oz. to 100 lbs. chicken meat (dry cure); ¼ oz. to 100 lbs. chopped chicken meat
Sodium sulfoacetate derivatives of mono- and diglycerides	Emulsifier	0.5%
Sorbitol	Retard drying	Sufficient for purpose
Starch	Thickener	Sufficient for purpose
Stearyl citrate	To protect flavor	0.15%
Sugars, approved (sucrose and dextrose)	Flavoring; curing; seasoning	Sufficient for purpose
Tannic acid	Refining; loosen scale	Sufficient for purpose
Tapioca	Thickener	Sufficient for purpose
Tocopherols	Antioxidant: To retard rancidity development	0.03%
Yeast (dry and wet)	Flavor	Sufficient for purpose

The food additives herein listed are permitted for use in official poultry processing plants provided a letter of approval or an approved label and formula is in the official file. These approvals outline specific conditions for use.

Authority for use of food additives is found in Sections 81.52 and 81.130 of the Regulations, and AMS PY-Instruction No. 918-10 Supplement No. 2 Revised 2/13/57.

§ 121.2000—Prior-Sanctioned Food Ingredients

§ 121.2000—General

(a) An ingredient whose use in food or drug packaging is subject to a prior sanction of approval within the meaning of section 201(s)(4) of the act is exempt from identification as a food additive. The Commissioner will publish in this chapter all known prior sanctions. Any interested person may submit to the Commissioner a request for publication of a prior sanction, supported by evidence to show that it falls within section 201(s)(4) of the act.

(b) Based upon scientific data or information that shows that use of a prior-sanctioned food ingredient may be injurious to health, and thus in violation of section 402 of the act, the Commissioner will establish or amend an applicable prior sanction regulation to impose whatever limitations or conditions are necessary for the safe use of the ingredient, or to prohibit use of the ingredient. (secs. 201(s), 409, 701(a), 52 Stat. 1055 and 22 Stat. 1784-1788, as amended; 21 U.S.C. 321(s), 348, 371(a.) [38 FR 12738, May 15, 1973]

§§ 121.2001—121.2004 [Reserved]

§ 121.2005 Substances employed in the manufacture of food-packaging materials.

Prior to the enactment of the food additives amendment to the Federal Food, Drug, and Cosmetic Act, sanctions were granted for the usage of the following substances in the manufacture of packaging materials. So used, these substances are not considered "food additives" within the meaning of section 201(s) of the act, provided that they are of good commercial grade, are suitable for association with food, and are used in accordance with good manufacturing practice. For the purpose of this section, good manufacturing practice for food-packaging materials includes the restriction that the quantity of any of these substances which becomes a component of food as a result of use in food-packaging materials shall not be intended to accomplish any physical or technical effect in the food itself, shall be

reduced to the least amount reasonably possible, and shall not exceed any limit specified in this section:

(a) *Antioxidants* (Limit of addition to food, 0.005 percent).

Butylated hydroxyanisole.
Butylated hydroxytoluene.
Dilauryl thiodipropionate.
Distearyl thiodipropionate.
Gum guaiac.
Nordihydroguaiaretic acid.
Propyl gallate.
Thiodipropionic acid.
2,4,5-Trihydroxy butyrophenone.

(b) *Antimicrobials*.

Calcium propionate.
Methylparaben (methyl *p*-hydroxybenzoate).
Propylparaben (propyl *p*-hydroxybenzoate).
Sodium benzoate.
Sodium propionate.
Sorbic acid.

(c) *Driers*.

Cobalt caprylate.
Cobalt linoleate.
Cobalt naphthenate.
Cobalt tallate.
Iron caprylate.
Iron linoleate.
Iron naphthenate.
Iron tallate.

Manganese caprylate.
Manganese linoleate.
Manganese naphthenate.
Manganese tallate.

(d) *Drying oils* (as components of finished resins).

Chinawood oil (tung oil).
Dehydrated castor oil.
Linseed oil.
Tall oil.

(e) *Plasticizers*.

Acetyl tributyl citrate.
Acetyl triethyl citrate.
p-tert-Butylphenyl salicylate.
Butyl stearate.
Butylphthalyl butyl glycolate.
Dibutyl sebacate.
Di-(2-ethylhexyl) phthalate (for foods of high water content only).
Diethyl phthalate.
Diisobutyl adipate.
Diisooctyl phthalate (for foods of high water content only).
Diphenyl-2-ethylhexyl phosphate.
Epoxidized soybean oil (iodine number maximum 6; and oxirane oxygen, minimum, 6.0 percent).
Ethylphthalyl ethyl glycolate.
Glycerol monooleate.
Monoisopropyl citrate.
Mono, di-, and trisethyl citrate.
Triacetin (glycerol triacetate).
Triethyl citrate.
3-(2-Xenyl)-1,2-epoxypropane.

38 FR 12738, May 15, 1973.

§ 121.2500

Title 21—Food and Drugs

(f) Release agents.

Dimethylpolysiloxane (substantially free from hydrolyzable chloride and alkoxy groups, no more than 18 percent loss in weight after heating 4 hours at 200° C.; viscosity 300 centistokes, 600 centistokes at 25° C., specific gravity 0.96 to 0.97 at 25° C., refractive index 1.400 to 1.404 at 25° C.)
 Linoleamide (linoleic acid amide).
 Oleamide (oleic acid amide).
 Palmitamide (palmitic acid amide).
 Stearamide (stearic acid amide).

(g) Stabilizers.

Aluminum mono-, di-, and tristearate.
 Ammonium citrate.
 Ammonium potassium hydrogen phosphate.
 Calcium glycerophosphate.
 Calcium phosphate.
 Calcium hydrogen phosphate.
 Calcium oleate.
 Calcium acetate.
 Calcium carbonate.
 Calcium ricinoate.
 Calcium stearate.
 Disodium hydrogen phosphate.
 Magnesium glycerophosphate.
 Magnesium stearate.
 Magnesium phosphate.
 Magnesium hydrogen phosphate.
 Mono-, di-, and trisodium citrate.
 Mono-, di-, and tripotassium citrate.
 Potassium oleate.
 Potassium stearate.
 Sodium pyrophosphate.
 Sodium stearate.
 Sodium tetrapyrophosphate.
 Stannous stearate (not to exceed 50 parts per million tin as a migrant in finished food).
 Zinc orthophosphate (not to exceed 50 parts per million zinc as a migrant in finished food).
 Zinc resinates (not to exceed 50 parts per million zinc as a migrant in finished food).

(h) Substances used in the manufacture of paper and paperboard products used in food packaging.

Aliphatic polyoxyethylene ethers.*
 1-Alkyl (C₈-C₁₈)-amino-3-aminopropane monoacetate.*
 Borax or boric acid for use in adhesives, sizes, and coatings.*
 Butadiene-styrene copolymer.
 Chromium complex of perfluoro-octane sulfonyl glycine for use on paper and paperboard which is waxed.*
 Disodium cyanodithioimidocarbamate with ethylene diamine and potassium *N*-methyl dithiocarbamate and/or sodium 2-mercaptobenzothiazole (slimicides).*
 Ethyl acrylate and methyl methacrylate copolymers of itaconic acid or metha-

crylic acid for use only on paper and paperboard which is waxed.*
 Hexamethylene tetramine as a setting agent for protein, including casein.*
 1-(2-Hydroxyethyl)-1-(4-chlorobutyl)-2-alkyl (C₈-C₁₈) imidazolium chloride.*
 Itaconic acid (polymerized).
 Melamine formaldehyde polymer.
 Methyl acrylate (polymerized).
 Methyl ethers or mono-, di-, and tripropylene glycol.*
 Myristo chromic chloride complex.
 Nitrocellulose.
 Polyethylene glycol 400.
 Polyvinyl acetate.
 Potassium pentachlorophenate as a slime control agent.*
 Potassium trichlorophenate as a slime control agent.*
 Resins from high and low viscosity polyvinyl alcohol for fatty foods only.
 Rubber hydrochloride.
 Sodium pentachlorophenate as a slime control agent.*
 Sodium trichlorophenate as a slime control agent.*
 Stearate-chromic chloride complex.
 Titanium dioxide.*
 Urea formaldehyde polymer.
 Vinylidene chlorides (polymerized).

(Sec. 409, 72 Stat. 1786; 21 U.S.C. 348) [30 F.R. 15845, Dec. 23, 1965, as amended at 31 F.R. 2897, Feb. 18, 1966; 31 F.R. 11609, Sept. 2, 1966; 33 F.R. 15282, Oct. 15, 1968. Redesignated at 38 F.R. 12738, May 15, 1973]

Subpart F—Food Additives Resulting From Contact With Containers or Equipment and Food Additives Otherwise Affecting Food

AUTHORITY: The provisions of this Subpart F issued under sec. 409, 72 Stat. 1785; 21 U.S.C. 348.

§ 121.2500 General provisions applicable to Subpart F.

(a) Regulations prescribing conditions under which food additive substances may be safely used predicate usage under conditions of good manufacturing practice. For the purpose of this Subpart F, good manufacturing practice shall be defined to include the following restrictions:

(1) The quantity of any food additive substance that may be added to food as a result of use in articles that contact food shall not exceed, where no limits are

*Under the conditions of normal use these substances would not reasonably be expected to migrate to food, based on available scientific information and data.

**MEMORANDUM TO ASSOCIATE COMMISSIONER FOR SCIENCE FROM ACTING DIRECTOR
BUREAU OF FOODS: BUREAU OF FOODS COMMENTS ON BACKLOG LIST OF CHEMICALS
IN THE NCI CARCINOGEN BIOASSAY PROGRAM (STUDY COMPLETED BUT NOT
YET REPORTED) JANUARY 17, 1977**

TAB G

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Associate Commissioner for Science (HFS-1)

DATE: 1/17/77

FROM : Acting Director, Bureau of Foods (HFF-1)

SUBJECT: Bureau of Foods Comments on the Backlog List of Chemicals in the
NCI Carcinogen Bioassay Program (Study Completed but not yet
Reported)

The list of chemicals provided by you to Dr. Angelotti on 11/18/76 and identified as those chemicals in the NCI Carcinogen Bioassay Program for which studies have been completed but not yet reported (backlog list) has been reviewed within the Bureau of Foods.

This review was undertaken to provide you with information relative to current or potential uses of these compounds in foods, meat animals, cosmetics and packaging and to identify those chemicals permitted for use through FDA regulations. In addition, and as appropriate, we have provided you with an indication of impact upon FDA and the regulated industries should certain of these chemicals be deemed unsuitable for use in the future in foods, meat animals, cosmetics, and packaging.

Should you need additional input from the Bureau of Foods relative to the backlog list in your efforts as the primary FDA liaison person to NCI for their Carcinogen Bioassay Program on Environmental Chemicals please contact Dr. Angelotti. As you will note from the following review many chemicals of interest to FDA exist on the list and unilateral and uncoordinated public issuances by NCI about these chemicals may be expected to impact heavily on the FDA and the Bureau. For example, of the approximate 200 chemicals on the list about 116 of them are permitted for use by FDA as GRAS, direct or indirect additives, as color additives or chemicals permitted for use in the production of colors, and as chemicals for which an NADA has been approved or which are used as ingredients in approved animal drugs. If substantial numbers of these 116 chemicals are identified as carcinogens by NCI the FDA will be hard pressed in terms of Bureau resources to either generate the banning regulations that may follow or to provide the appropriate scientific justification for not banning them. In either case, the milestones in the approved cyclic review of food additives may be adversely affected.

The subject list of chemicals has been reviewed and divided into eight general categories: pesticides, dye chemicals, color additives, food additives, indirect food additives, cosmetics, chemicals used in food producing animals, and other chemicals.

Most of the chemicals within the dye chemical, cosmetic chemicals and other chemical list have no direct use in foods, and only have the potential to contaminate foods through accidental or environmental means.

Most of the pesticides are used directly on foods. In some cases, they may be ubiquitous food contaminants by way of the environment. DDT and its analogs are good examples of such chemicals. EPA, of course, is responsible for the registration and establishment of tolerances for pesticides. Should any be found to be carcinogenic, it would be EPA's responsibility to cancel the registration so as to remove the pesticide from commerce. Although replacement chemicals would be available in most cases, the economic consequences might be serious. FDA methodology is presently suitable for the detection and quantification of these materials, and so no serious problems should confront FDA in that regard.

In the case of the chemicals listed under dye chemicals and other chemicals, few have the potential to become serious food contaminants. These would include only those chemicals which are highly lipid soluble (and highly water insoluble). Aroclor 1254 is a good example. Since this environmental contaminant is ubiquitous, FDA can do little but to continue to monitor foods for violative samples. Should a "zero" tolerance be established, our food supply as we know it would in some instances have to undergo drastic changes---

Many of the dye chemicals have intermediate lipid solubilities, and should they be released to the environment through industrial discharge, they could bioaccumulate in fish to some extent. Our present pesticide methodology would probably be inadequate to determine many of them. New methodology, therefore, would have to be developed in order to monitor for them in foods.

In the area of the use of these chemicals in cosmetics, 24 are identified as potential cosmetic ingredients with varying levels of importance to the cosmetic industry. Several of these materials are covered by regulations restricting their use in cosmetics. For each of these materials listed the number of formulas voluntarily submitted under the cosmetic registration has been included. Our files at present reflect approximately 20,000 formulas.

Pesticide Chemicals

- A. Where available, the type of use, volume of use in a recent year, and whether they are included on EPA's Rebuttable Presumption Against Reregistration List (RPAR) are given. The latter is a working list of pesticides that may be too hazardous to man or the environment to allow continued use. EPA will analyze data submitted by manufacturers, users, and other interested parties either in support of or challenging the presumption. EPA will then decide whether to allow reregistration or begin cancellation proceedings.

1. Captan. Very important fungicide, 1969 production estimated to be 12-14 M* lbs. Removal from market would have serious consequences.
2. Chlordecone (Kepone). Formerly used as insecticide in ant and roach baits; no longer produced in U.S. On RPAR list.
3. Dichlorvos (Vapona, DDVP). Insecticide, about 2.7 M lbs used in 1974. On RPAR list.
4. Dimethoate. Insecticide, about 2.3 M lbs used in 1974. On RPAR list.
5. Malathion. Very important insecticide, about 16 M lbs used in 1974. Removal from market would have serious consequences.
6. Dieldrin. Production discontinued.
7. Aldrin. Production discontinued.
8. Lindane. Insecticide, about 0.6 M lbs used in 1974. On RPAR list.
9. Endrin. Insecticide; about 1.2 M lbs used in 1974. On RPAR list.
10. Chlordane. Insecticide, all uses suspended. About 21 M lbs used in 1974.
11. Thiodan (Endosulfan). Insecticide, 1.5 M lbs used in 1974.
12. DDT (includes DDD, DDE). Insecticide, registration cancelled for nearly all uses.
13. PCNB. Fungicide, on RPAR list.
14. Chlorobenzilate. Acaricide, on RPAR list.
15. Heptachlor. Insecticide, 2 M lbs used in 1974. All crop uses suspended.
16. Methoxychlor. Insecticide, 3.4 M lbs used in 1974.
17. Parathion. Insecticide, 10.4 M lbs used in 1974. Important insecticide, removal would have serious consequences.
18. Toxaphene. Insecticide, 45 M lbs used in 1974. On RPAR list. Very important, removal would have very serious consequences.

* M = one million

Associate Commissioner for Science

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19. Tetrachlorvinphos (Gardona). Insecticide.
20. Nitrofen (TOK). Herbicide.
21. Daconil. Fungicide for turf and ornamentals.
22. Phosphamidon. Insecticide.
23. Picloram. Herbicide.
24. Chloramben (Amiben). Herbicide. Estimated use in 1964 was 1.2 M* lbs.
25. Treflan (Trifluralin). Herbicide. Estimated domestic use 5M lbs. Holds largest share of cotton herbicide market and 20% of the soybean herbicide market. Removal of this product would be serious.
26. Zectran. Systemic insecticide.
27. Photo-Dieldrin. See Dieldrin. This material is formed on exposure of dieldrin to sunlight.
28. Azinphosmethyl (Guthfon). Insecticide, 3.1 M lbs used in 1974.
29. Dicofol (Kelthane). Acaricide, 2.6 M lbs used in 1974.
30. Delnav (Dioxathion). Acaricide, insecticide.
31. Triphenyltin Hydroxide. Fungicide.
32. Hexachlorophene. Fungicide, bactericide. Current production very limited.
33. Bayluscide. Molluscicide.
34. Perthane. Insecticide, 0.2 M lbs used in 1974.
35. Methyl Parathion. An extremely import insecticide, especially on cotton. Estimated use in 1974 was 53 M lbs total. Removal would have serious consequences.
36. Piperonyl Butoxide. Synergists in insecticide formulations. On RPAR list.

* M = one million

37. 1,2 Dibromoethane. Insecticide, nematocide. On RPAR list.
331 M* lbs used in 1973.

Dye Chemicals

- B. Many different dyestuffs are produced and used in the U.S., and the number of chemicals that are used to manufacture them are myriad. As a consequence, although the total dye production is high, most of the intermediates are produced in relatively low tonnages. The following list gives some uses, and where available, production volumes.
1. 5-Nitro-o-toluidine. Dye intermediate, 0.35 M lbs made in 1973. Used in about 10 dyes.
 2. Hydrazobenzene. Dye intermediate, stabilizer for explosives, laboratory reagent.
 3. 2-Amino anthraquinone. Intermediate for anthraquinone vat dyes.
 4. 2-Chloro-p-phenylenediamine sulfate. Intermediate. Possibly used in some hair dyes.
 5. 4-Nitroanthranilic acid. Not listed as dye intermediate; 5-nitro compound is.
 6. p-Anisidine. Azo dye intermediate, more than 100,000 lbs/yr used.
 7. 2,5-Toluenediamine sulfate. Intermediate for a number of dyes.
 8. N,N-Dimethyl-p-nitrosoaniline. Used in about 36 dyes.
 9. o-Anisidine HCl. Dye intermediate, about 2M lbs used in 1973.
 10. Vat Yellow 4 (Amanthrene). About 12,000 lbs used in 1973.
 11. 3,3'-Dimethoxybenzidine (Dianisidine). Important dye intermediate.
 12. The following are believed to be dye intermediates, but information on them was not readily available:

* M = one million

2-methyl-1-nitroanthraquinone, 3-chloro-p-toluidine, 1-nitro-naphthalene, p-aminodiphenylamine, 5-chloro-o-toluidine, 1,5-naphthalenediamine, 4-chloro-m-phenylenediamine, N-(1-naphthyl) ethylenediamine, azobenzene, o-toluidine.HCl, 4-chloro-o-toluidine.HCl, 5-nitro-o-anisidine, 2,4-diaminoanisole, 4-amino-2-nitrophenol, meta and p-cresidines.

Color Additives

- C. Aniline.HCl is an intermediate used in the production of D&C Red No. 31 and D&C Red No. 33. D&C Red No. 31 is limited to external application and D&C Red No. 33 is permitted with tolerance limits for use in drugs and cosmetics. It is anticipated that Aniline, in most cases, is a component of the certified color and is permitted up to 2000 ppm.

EDTA is used in the production of disodium EDTA - copper listed for use in cosmetic shampoos, and would be present at significant levels.

o-Anthranilic acid is an intermediate used in the production of D&C Red No. 39 which is listed for use at a level of 0.1% in quaternary ammonium type germicidal solutions intended for external application. This compound would possibly be a metabolite of D&C Blue No. 6. D&C Blue No. 6 is listed for use in drugs and cosmetics with no restrictions.

Phthalic Anhydride as Acid is an intermediate used in the production of most halogenated fluoresceins (FD&C Red No. 3; D&C Orange Nos. 5, 10, 11; D&C Red Nos. 21, 22, 27, and 28; D&C Yellow Nos. 7 and 8) and in the production of D&C Red Nos. 19 and 37. The amount of free phthalic acid permitted varies from 0.1% in FD&C Red No. 3 to 1.0% in D&C Yellow No. 8.

The following compounds are used in the production of practically all azo colors:

Sodium Nitrite is used in diazotization of amines prior to coupling with other compounds to form color additives. (Food colors include FD&C Red No. 40, FD&C Yellow Nos. 5 and 6; and Orange B).

Urea is used to destroy the excess nitrous acid (sodium nitrite plus HCl) used to diazotize amines.

If adequate urea is used, we would not expect to find any sodium nitrite in any color additives. We would not expect to find more than a trace of urea in any of the color additives because of its great solubility in water. We have not looked for either in color additives.

<u>Direct Additives</u>				
<u>D.</u>	<u>Compound</u>	<u>Regulation</u>	<u>Use</u>	<u>Amount in Food</u>
	L-Tryptophan	121.1002	Amino acid nutrient	Max. 4.3% of total protein weight
	Calcium disodium EDTA	121.1017	Preservative	25 - 800 ppm
	Disodium EDTA	121.1056	Preservative	36 - 500 ppm
	Tetrasodium EDTA	121.1088	Boiler water additive	Est. less than 5 ppm
	BHT	121.1034	Preservative	10 - 200 ppm
	NTA	121.1088	Boiler water additive	Est. 0.1 ppm
	Trisodium NTA	121.1088	Boiler water additive	Est. 0.1 ppm
	1,2-dichloroethene (ethylene dichloride)	121.1040	Spice Extraction solvent	Max. 30 ppm in spice oleoresin
	Sodium nitrite	121.1064 121.1230	Preservative	10 - 200 ppm

In addition to the chemicals directly added to food there may be residues from the use of other additives which are on the NCI list. Azodicarbonamide (121.1085) used in flour at 45 ppm reacts to form biurea which is the dimer of urea and urea is on the NCI list. Azodicarbonamide is also covered under .2514, .2550, .2562 in indirect additives.

The biocides regulated in 121.1155 for use in sugar mills and in 121.2506 as slimicides pass through various thiourea intermediates before they are degraded to innocuous products. The intermediates include: thiourea, ethylenethiourea and dimethylthiourea. The NCI list does not include these specific thioureas but does mention diethylthiourea, 2,5-dithiodiurea and N,N-dicyclohexylthiourea. The biocides were approved based on data in the petitions which demonstrated that the thioureas were not present at an analytical sensitivity of 2 ppm along with lab scale studies which indicated that the actual residues are probably less than 1 ppb.

Arabinosylcytosine is an adduct of a sugar, arabinose, and a naturally occurring pyrimidine, cytosine. Adducts of carbonyl-amine compounds are formed in the browning reactions used to produce food flavorings. It is possible for arabinosylcytosine to be formed in the production of meat flavorings, etc., since cytosine is found in RNA and DNA and arabinose is a commercially available sugar.

The NCI list includes numerous solvents which might be used in food processing without our knowledge because their residues are so low that the food processor considers them "generally recognized as safe" or non-food additive residues. For example, some enzyme preparations use unusual solvents to isolate the enzyme and at the rate of enzyme usage in food the projected solvent residues are in the low ppb range. The Division of Chemistry considers this situation a possibility because the development of enzymes for food uses has been progressing very rapidly in relation to FDA's ability to review the status of these enzymes.

Cosmetic Chemicals

<u>E. Material</u>	<u>No. Formulas Filed</u>	<u>Reported Use</u>	<u>Important to Cosmetic Industry</u>
1. Captan	33	Preservative	No
2. p-phenylenediamine	329 (not listed as HCl)	Oxidative hair dye	Yes*
3. p-anisidine	not reported but in CTFA Dictionary	Oxidative hair dye(?)	No
4. 1H-Benzotriazole	1	Preservative	No
5. 2-Chloro-p-Phenylene-diamine Sulfate	31	Oxidative hair dye	?
6. Chloroform	5		No
7. Methyl Chloroform	4		No
8. NTA, Trisodium Salt	not reported but in CTFA Dictionary		No
9. Tetrachloroethylene	1		No
10. EDTA	507		Yes*
11. 2-Nitro-p-Phenylene-diamine	276	Oxidative hair dye	Yes**
12. 4-Nitro-o-Phenylene-diamine	212	Oxidative hair dye	Yes**
13. 1,1,2,-Tetrachloro-ethylene	1		No
14. 2,5-Toluenediamine Sulfate	106	Oxidative hair dye	Yes**

Associate Commissioner for Science

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<u>Material</u>	<u>No. Formulas Filed</u>	<u>Reported Use</u>	<u>Important to Cos- metic Industry</u>
15. p-Aminodiphenyl-amine	40 (59 more as HCl)	Oxidative hair dye	?
16. 2,4-Diaminoanisole	70 (229 more as sulfate)	Oxidative hair dye	?
17. 4-Amino-2-Nitrophenol	77	Oxidative hair dye	?
18. Hexachlorophene	8	Preservative	No but regulated
19. Trichlorofluoro-methane	334	Aerosol Propellant	Yes but regulated
20. L-Tryptophane	2		No
21. Sodium Nitrite	3	Preservative	No
22. Urea	114		Yes*
23. 2,4-Toluene diamine	5		No
24. BHT	240	Antioxidant	Yes**

* A determination of carcinogenicity would have a significant impact on the cosmetic industry. No known substitutes.

** A determination of carcinogenicity may have significant impact. Substitution of compound is possible but difficult.

Indirect Additives

- F. 1. Dioxane. Is permitted for use in adhesives.
2. NTA. Nitritotriacetate trisodium permitted as a boiler water additive.
3. Tetrachloroethylene. Is permitted as a foaming agent for polystyrene.
4. 1,1,2-Trichloroethane. Is permitted for use in adhesives.
5. β -Nitrostyrene. Basic polymer-paper-dry food.
6. Iodoform. Permitted for use in adhesive.

7. Piperonyl butoxide. Used for insect control in paper bags for dry food.
8. Phthalic anhydride. Permitted for use as a modifier for rosins, reactant for polyurethane, reaction control reagent, retarder for rubber articles.
9. Toluene diamine. Permitted for use as an antioxidant in rubber articles.
10. BHT. Prior sanctioned. Widely used in polyolefin and saran coatings.
11. Ethyl Tuads. Tetraethylthiuramdisulfide. Permitted for use as a rubber accelerator.
12. Dibutyltin diacetate - Cleared for use as an optional adjuvant under § 121.2522 (Polyurethane resins) as a catalyst, which may be used as the food-contact surface of articles intended for contact with bulk quantities of dry food. No food uses.

Chemicals Used in Food-Producing Animals

- G. 1. 2-Amino-5-Nitrothiazole and its N-acetyl derivative
Approved NADA used for blackhead disease in turkeys
2. Daraprim - One approved NADA - antimalarial and antimetabolite
 3. Dichlorvos - Three approved NADA's - antihelminthic
 4. Dibutyltin Dilaurate - Three approved NADA's - antihelminthic
The diacetate salt in on the NCI list
 5. Prednisone - One NADA approved
 6. Niathiazide - Approved for the treatment of blackhead disease turkeys
 7. Chloroform)
 8. EDTA)
 9. Uidifirn) These items may be used in formulations but not
 10. Carbon Disulfide) as the principal items, or as sanitizers, or as
 11. Hexachlorophene) antihelminthics without approved use or as feed
 12. Urea) constituents.
 13. BHT)

Other Chemicals

- H. This general category includes chemicals of many different types. Uses and production volumes are included where available.
1. 2,4-Dinitrotoluene. Used in dyes, explosives, intermediate for 2,4-diaminotoluene. About 471 M* lbs produced in 1973.
 2. 2,4-Toluenediamine (2,4-diaminotoluene). Used as intermediate for polyurethane foams, about 193 M lbs produced in 1973.
 3. Chloroform. About 253 M lbs made in 1973. Solvent, on RPAR list.
 4. Methyl chloroform. Solvent, intermediate for vinylidene chloride. Nearly 600 M lbs used in 1974.
 5. Dapsone. Epoxy hardening agent, some drug and veterinary use.
 6. Aniline. Intermediate for isocyanates, rubber chemicals, dyes, etc. About 412 M lbs produced in 1975.
 7. Tris (2,3-dibromopropyl) phosphate. Flame retardant for textiles. About 10 M lbs produced in 1975, use expected to grow significantly.
 8. 1,2-Dichloroethane. Solvent, about 6000 M lbs produced in 1975.
 9. Cupferron. Laboratory reagent.
 10. 1,1-Dichloroethane. Solvent.
 11. Tetrachloroethylene (perchloroethylene). About 728 M lbs produced in 1974. Primarily used as solvent.
 12. p-Phenylenediamine.HCl. Intermediate for antioxidants; about 74 M lbs of substituted p-phenylenediamines were used in 1974.
 13. EDTA. About 64 M lbs made in 1974. Used as chelating agent, in soaps and detergents, dye baths, boiler water, chemical processing aid, etc.
 14. Dichlorodimethylhydantoin. Used as chlorinating agent, disinfectant, industrial deodorant, in laundry bleach, PVC stabilizer.
 15. NTA. Proposed detergent booster. Also used in metal plating, mineral separations; chelating agent.

* M = one million

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Associate Commissioner for Science

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16. p-Nitrosodiphenylamine. Rubber processing chemical.
17. p-Dioxane. Solvent, also used in some cosmetics.
18. Trimethylphosphate. No longer produced.
19. Aroclor 1254. No direct food uses. Production will be halted in the very near future. Formerly used as flame retardant plasticizer, electrical fluid, hydraulic fluid.
20. Hexachloroethane. Intermediate for Fluorocarbon 114, of which 80 M lbs were produced in 1975.
21. Trichlorofluoromethane (Fluorocarbon 11). About 334 M lbs produced in 1973. FDA is suggesting warning labels on regulated products, may eventually require its withdrawal.
22. Carbon Disulfide. Intermediate, solvent. About 479 M lbs produced in 1975.
23. Phthalic anhydride. Intermediate. About 709 M lbs produced in 1975 (has ranged to 1000 M lbs in recent years).

* M = one million

S/
Howard R. Roberts, Ph.D.

RAngelotti:vs 1/12/77

cc HFF-100(Kolbye)
 HFF-200(Forbes)
 HFF-300(Quinn)
 HFF-400(Schaffner) —
 HFF-2(Riester)
 HFF-330(Ronk)
 HFF-150(Blumenthal)
 HFF-150(Shibko)
 HFF-3(Angelotti)

NCI LIST OF KNOWN HUMAN CARCINOGENS

TAB H

NCI LIST OF KNOWN HUMAN CARCINOGENS

1. beta-naphthylamine
2. benzidine
3. 4-aminobiphenyl
4. 4-nitrobiphenyl
5. clornaphazine (bis-2-chloroethyl-2-naphthylamine)
6. mustard gas (bis chloroethyl sulphide)
7. nickel carbonyl
8. diethylstilbestrol
9. bis (chromethyl) ether
10. vinyl chloride
11. aflatoxin
12. asbestos
13. arsenicals
14. chromates
15. estrogenic compounds
16. tobacco
17. tobacco smoke
18. soots
19. tars
20. pitches
21. asphalts
22. cutting oils
23. shale oils
24. creosote oils
25. high boiling petroleum oils
26. coke over effluents
27. various combustion products
28. betel nut (chewing)
29. radium (note a)
30. thorotrast (note a)
31. uranium ores (radon and radon daughters)
32. other radioactive materials (note a)
33. auramine (note b)
34. magenta (note b)
35. isopropyl oil
36. wood dust (note b)

PACKET OF INFORMATION ON FD&C YELLOW NO. 5

TAB I



U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

P77-4
 FOR IMMEDIATE RELEASE
 February 3, 1977

(Food and Drug Administration)
 PINES--(301)--443-3285
 (Home)--(202)--363-4104

The Food and Drug Administration today proposed a series of actions to assure that people allergic to Yellow No. 5, the most widely-used color additive, will be able to avoid it in foods and drugs.

FDA estimates that 47,000 to 94,000 people in the United States may be allergic to Yellow No. 5, also known as tartrazine. Allergic reactions to Yellow No. 5 include asthmatic symptoms, such as wheezing and difficulty in breathing; hives; and stuffy or runny nose.

The Agency proposed that:

-- the labels of foods containing Yellow No. 5 identify the color by name in the list of ingredients. Previously, all colors used in food products only needed to be identified in the ingredient listing as "artificial coloring," as provided for by law. The principal uses of Yellow No. 5 in foods are in beverages, candy, desserts, cereals, bakery goods, ice cream and sherbet, dairy products and snack foods.

-- Yellow No. 5 be prohibited in drugs used frequently by allergic people. The ban would apply to five categories of non-prescription drugs (pain relievers, antihistamines, cough-cold remedies, anti-asthmatic drugs and nasal decongestants taken by mouth) and to seven categories of prescription drugs (the previous five plus steroidal and non-steroidal anti-inflammatory drugs).

-- Drugs that continue to be colored with Yellow No. 5 be required to carry on the front label the warning statement: "This product contains FD&C Yellow No. 5 which may cause allergic-type reactions in certain susceptible people." Coloring is used in drugs to help identify medicines by kind and dosage.

Sherwin Gardner, Acting FDA Commissioner, said: "Yellow No. 5, as currently used, poses no hazard to the general population. But these people who are allergic to Yellow No. 5 ought to be able to identify it in their foods and drugs and avoid products containing it. The proposals we are issuing are designed to accomplish these objectives."

Estimates of the number of people allergic to Yellow No. 5 are based on the number of people estimated to be allergic to aspirin. Allergic responses to Yellow No. 5 seem to occur primarily in people allergic to aspirin. About half of the people who have had allergic reactions to aspirin are also allergic to Yellow No. 5.

There is no evidence of allergic reactions to Yellow No. 5 from its use on the skin, so the proposed regulations do not apply to externally-applied drugs or cosmetics.

The Commissioner proposes to make the exemption effective upon publication of a final order in the Federal Register, and he advises that, pending issuance of a final regulation in this matter, he is delaying application of § 1.17 to declarations of fat content that comply with all requirements of this proposal.

The Commissioner has carefully considered the environmental effects of the proposed regulation and, because the proposed action will not significantly affect the quality of the human environment, has concluded that an environmental impact statement is not required. A copy of the environmental impact assessment is on file with the Hearing Clerk, Food and Drug Administration.

Therefore, under the Federal Food, Drug, and Cosmetic Act (secs. 201(n), 303(a), 701(a), 52 Stat. 1041 as amended, 047 as amended, 1035 (21 U.S.C. 321(n), 343(a), 371(a))) and under authority delegated to the Commissioner (21 CFR 1.1) (codification published in the Federal Register of June 15, 1976 (41 FR 32422)), it is proposed that § 1.17 be amended by adding new paragraph (h) 1) to read as follows:

§ 1.17 Food; nutrition labeling.

(h) (1) A percentage declaration of the fat (milkfat, butterfat) content appearing in the ingredient statement on the label of a food listed in § 1.1(c)(7) (1) does not constitute a "nutrition claim or information" within the meaning of paragraph (a) of this section provided, that:

(i) The declaration appears on the information panel (for requirements for information panels, see § 1.8d) with no greater prominence than any other printed matter appearing on the panel, and in a type size no larger than the minimum type size required by § 1.8b(1) or the declaration of net quantity of contents, and

(ii) The declaration is not required by other regulations in this chapter.

Interested persons may, on or before April 5, 1977, submit to the Hearing Clerk, Food and Drug Administration, Rm 4-65, 5600 Fishers Lane, Rockville, MD 20857, written comments (preferably in quintuplicate and identified with the hearing clerk docket number found in brackets in the heading of this document) regarding this proposal. Received comments may be seen in the above office between the hours of 9 a.m. and 4 p.m., Monday through Friday.

The Food and Drug Administration has determined that this document does not contain a major proposal requiring preparation of an inflation impact statement under Executive Order 11831 and OMB Circular A-107. A copy of the inflation impact assessment is on file with the Hearing Clerk, Food and Drug Administration.

Dated: January 27, 1977.

JOSEPH P. HILL,
Associate Commissioner for
Compliance.

[FR Doc. 77-3222 Filed 2-3-77; 8:48 am]

LEGAL NOTICE

[Docket No. 77N-0009]

FDC Yellow No. 5

Labeling in Food and Drugs for Human Use and Restriction on Use in Certain Human Drugs

The Food and Drug Administration (FDA) is proposing to require the label declaration of FDC Yellow No. 5 when used to color foods and ingested drugs and to prohibit its use in certain drugs for human use. These restrictions are considered necessary because of mounting evidence of allergic-type reactions to FDC Yellow No. 5. Interested persons have until April 5, 1977 to submit comments.

In the Federal Register of May 8, 1969 (34 FR 7447), the Commissioner of Food and Drugs issued an order listing FDC Yellow No. 5 (also known as tartrazine when not certified by FDA for use in food, drugs, and cosmetics) for use in food under § 2.275 (21 CFR 2.275) and for use in ingested drugs under § 4.175 (21 CFR 4.175). This action was supported by safety data in a color additive petition (CAF 23) and other relevant data. The petition was submitted by the Certified Color Industry Committee, c/o Hazleton Laboratories, Falls Church, VA (now the Certified Color Manufacturers Association, 900 17th St. NW, Washington, D.C. 20008); notice of filing was published in the Federal Register of May 27, 1969 (34 FR 4083).

No specific restrictions were placed on the use of FDC Yellow No. 5 other than the requirements of batch certification by FDA. The color is provisionally listed for use in externally applied drugs and in cosmetics under § 8.501(a). The closing date for this provisional listing is January 31, 1977. A proposal was published in the Federal Register of September 23, 1976 (41 FR 41850) to postpone this closing date to December 31, 1980, conditioned upon the timely submission of reports from new chronic toxicity studies. Regulations finalizing this proposal are expected to be published in the near future.

An order listing FDC Yellow No. 5 for use in externally applied cosmetics under § 8.7255 (21 CFR 8.7255) was published in the Federal Register of January 21, 1974 (39 FR 2358). However, the effective date of that order was stayed by the submission of objections to, among other things, certain restrictions that were to be placed on use of the color. Published elsewhere in this issue of the Federal Register is a notice announcing the stay of the effective date of that order.

DISCUSSION OF PROBLEM

Since FDC Yellow No. 5 was listed for use in food and ingested drugs, evidence of allergic-type responses caused by ingestion of substances containing the color has accumulated. These responses to FDC Yellow No. 5 occur primarily in patients who also have aspirin intolerance, although an absolute association has not been established. The phenomenon of aspirin intolerance in certain persons with underlying allergic

nasal polyposis, vasomotor rhinitis, and skin allergies to various substances, has been known for over 50 years. The aspirin reaction is manifested by asthmatic symptoms, urticaria, angioedema, or nasal symptoms. The overall incidence of aspirin intolerance in the United States is unknown. Samter and Beers (Ref. 1) cited a report by Pearson on a large asthmatic population in which 2.3 percent were said to be aspirin intolerant. Chafee and Settipane (Ref. 2), on the other hand, reported an incidence of 4.3 percent in their large population of asthmatics. These figures were obtained solely on the basis of clinical history. Chafee and Settipane found that among their patients with rhinitis, 0.7 percent were aspirin intolerant.

It has also long been known that some persons are sensitive to organic chemicals. However, the first person to report an association between FDC Yellow No. 5 and allergic-type reactions was Lockey (Ref. 3). In 1919, Lockey reported generalized urticaria in three patients after ingestion of one or more tablets of a corticosteroid containing FDC Yellow No. 5. The patients were an asthmatic, a patient known to be very sensitive to drugs of coal tar origin who was taking a steroid for a skin rash due to a topical mercurial, and a patient with a collagen disease who was known to be aspirin intolerant.

Since the 1890's, there has been increasing numbers of reports establishing that there is a strong association between aspirin intolerance and FDC Yellow No. 5 intolerance. Chafee and Settipane (Ref. 4) described an asthmatic patient with aspirin sensitivity (angioedema) whose chronic asthma and acute attacks were exacerbated after taking certain antiasthmatic drugs, vitamins, promarin, and certain foods. In double-blind studies, she was reported to be allergic to FDC Yellow No. 5 and, mildly, to FDC Red No. 4. Drugs containing FDC Yellow No. 5 could provoke symptoms with a single dose. On the basis of these findings, the authors recommended that FDC dyes be required to be listed on food and drug packages.

The precise incidence of intolerance of FDC Yellow No. 5 in the total population or even in aspirin-intolerant patients is not known. Over an 11-year period, Samter and Beers (Ref. 1) followed over 1,000 aspirin-intolerant patients diagnosed on the basis of history. They hospitalized for study 182 of these aspirin-intolerant patients. All were asthmatic, but they had had vasomotor rhinitis and nasal polyps for years before developing asthma. Of the 182 aspirin-intolerant patients, nine (5 percent) were intolerant of tartrazine, FDC Yellow No. 5. In a double-blind study using some of these patients, 3 of 40 aspirin-intolerant patients (7.5 percent) receiving 25 milligrams of tartrazine developed symptoms.

Juhlin et al. (Ref. 5) found that seven of seven aspirin-intolerant patients developed urticaria or urticaria to only 1 to 2 milligrams of tartrazine. One of the seven reacted only slightly to 1 milli-

gran but reacted strongly to 5 milligrams of tartrazine. Thus, these authors found a 100-percent incidence of tartrazine intolerance in their limited studies. Although the test was single blind, there were no reactors to a placebo. One of the patients had been taking an antihistamine containing only 30 micrograms of tartrazine per tablet for a month in an attempt to relieve urticaria which began after taking an aspirin tablet. There was no improvement in the urticaria until 3 days after the patient stopped taking the antihistamine.

Michaels and Jullin (Ref. 8), in a study involving provocation tests with aspirin, azo dyes, and two commonly used food preservatives in patients with recurrent urticaria or angioedema, found that 39 of 52 patients developed a reaction to something—e.g., 35 of these had urticaria to aspirin; 19 to tartrazine (12 cases after 1 to 3 milligrams, the rest after 8 to 18 milligrams); and 22 to sodium benzoate (42 percent). There were also 10 cases of urticaria due to Sunset Yellow (FD&C Yellow No. 8) and some of these were not sensitive to tartrazine. It is not possible from this paper to ascertain the precise percentage of aspirin-intolerant patients who were also intolerant of FD&C Yellow No. 8, but it would appear to be about 50 percent.

Seltipane and Pudupakkam (Ref. 7) recently performed a tartrazine-placebo-controlled double-blind crossover study in 40 patients who had a history of aspirin intolerance and in 40 normal controls. Most of the aspirin-intolerant patients had asthma, the remainder had rhinitis and rhinorrhea. Many of these also had urticaria. The patients were challenged with 44 milligram of tartrazine or placebo (except for two who received 0.22 milligram). Six (15 percent) of the 40 aspirin-intolerant patients given tartrazine developed urticaria or bronchospasm, together with at least a 29-percent reduction in three pulmonary function tests. There were no reactions to the placebo, and none of the normal controls developed any reactions.

It is not possible to state precisely the incidence of intolerance to FD&C Yellow No. 8 in the United States. Further, there is a broad range of degree of intolerance, some patients reacting to a fraction of a milligram and others requiring 6 milligrams or more (the dosages found in foods). Using the incidence of 4.3 percent aspirin intolerance in a population of asthmatics and 0.7 percent in a population with rhinitis, as reported by Chafce and Seltipane (Ref. 4) in their large practice involving over 3,531 patients with these diseases, calculations of the incidence can be estimated. In Chafce and Seltipane's report, about half the patients had allergic rhinitis only. The other patients had asthma alone or asthma plus rhinitis. The incidence of asthma in the United States is estimated to be 1 to 2 percent. Thus, there are 2 to 4 million asthmatics in the United States and about a 4-percent incidence of aspirin intolerance in asthmatics, there could be 80,000 to 160,000 cases of aspirin intolerance among asthmatics. If Chafce and Seltipane's prac-

tice is indicative of the relative incidence of asthma vs. allergic rhinitis, there are 2 to 4 million patients with allergic rhinitis, of whom 14,000 to 28,000 could be aspirin intolerant. Thus, a total of 94,000 to 198,000 know aspirin-intolerant patients could be assumed. If it is further assumed that 50 percent of aspirin-intolerant patients are intolerant to FD&C Yellow No. 8, using Michaels and Jullin's urticaria and angioedema population (Ref. 8), then there would be 47,000 to 94,000 FD&C Yellow No. 8 intolerant patients.

The amount of FD&C Yellow No. 8 ingested is undoubtedly important in the potential provocation of a reaction. In many cases, however, it is not possible to ascertain the amount of FD&C Yellow No. 8 ingested by the people who reported the allergic-type symptoms to their physicians and, accordingly, the threshold value for provocation of a reaction to FD&C Yellow No. 8 has not been defined in the literature. The determination of the threshold amount may be particularly difficult in those persons who show allergic-type reactions to FD&C Yellow No. 8 only after having ingested the color additive in foods or drugs over a prolonged period. Santer and Deers (Ref. 11) tested one dose of 25 milligrams of FD&C Yellow No. 8 in 49 aspirin-intolerant patients, three of whom reacted positively. Jullin et al. (Ref. 8), on the other hand, reported that some patients promptly showed allergic-type reactions after a single ingestion of as little as 1 milligram of tartrazine (FD&C Yellow No. 8).

The Food and Drug Administration has long been concerned about allergies involving food, drugs, and cosmetics. The Commissioner recognizes that many substances to which man is exposed, including those occurring in nature, may elicit allergic-type reactions in some unusually susceptible or idiosyncratic individuals. A great variety of materials has been implicated in allergic-type reactions, e.g., dusts of various kinds, pollens, feathers, insect fragments, bee stings, seeds, dandruff, and a number of foods. In addition, hypersensitive persons may react by exhibiting a number of responses, which may include angioedema, urticaria, bronchial asthma, pruritus, and vascular purpura.

In evaluating the reports described above, the Commissioner concludes that there is no evidence in the available information on FD&C Yellow No. 8 that demonstrates a significant hazard to the general population when the color is used at current levels and in the manner now practiced. However, because of the evidence of a causal relationship between FD&C Yellow No. 8 and serious allergic-type responses in certain susceptible individuals, the Commissioner concludes that action must be taken to limit the potential for exposure of such persons to the color through ingestion of food or drugs.

There are no reports of reactions to FD&C Yellow No. 8 from external application and, accordingly, the use of the color additive in externally applied drugs

and cosmetics is not considered to present a likelihood of allergic-type responses. Cosmetic articles such as mouthwashes, dentifrices, and liniments are also unlikely to induce allergic-type responses because of the very small amount of the cosmetic that may actually be ingested. Furthermore, as of May 31, 1976, all newly ordered labels for cosmetics have been required to declare the specific colors present. Under these circumstances, persons who are hypersensitive to FD&C Yellow No. 8 will, by careful review of the product labeling, be able to avoid cosmetic products containing the color. The Commissioner concludes, therefore, that no further action is required as to cosmetics in general or for externally applied drugs.

FOODS FOR FOODS

Persons who know they are intolerant of FD&C Yellow No. 8 are likely to be selective in the types of foods that they use and, with appropriate label declaration, would be able to avoid the potential hazard from allergic-type reactions to the color in food by reading the label. Accordingly, a label declaration of the presence of FD&C Yellow No. 8 in food for humans, whether added as the natural color, a mixture, or a lake, would enable persons intolerant to FD&C Yellow No. 8 to minimize exposure to the color.

The basis for the proposed action is the provision of section 108(b) (3) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 378(b) (3)), which provides that regulations for the listing of a color additive shall "prescribe the conditions under which such additive may be safely employed for such use or uses (including, but not limited to * * * and directions or other labeling or packaging requirements for such additive)." FD&C Yellow No. 8 has clearly been shown to produce allergic-type responses in humans and thus a requirement for label declaration of the color is justified. The evidence that other color additives may elicit similar responses is limited and, accordingly, the Commissioner concludes that similar labeling requirements should not be extended to other color additives at this time. Under the proposed amendment, foods containing colors other than FD&C Yellow No. 8 can continue to be labeled in accordance with the requirements concerning the label declaration of color additives prescribed by section 402 (1) and (k) of the act (21 U.S.C. 343 (1) and (k)), which permits declaration collectively as artificial color.

There is no evidence that any color, including FD&C Yellow No. 8, elicits allergic-type reactions in animals. Accordingly, label declaration of FD&C Yellow No. 8 in animal feeds and pet food would not be required.

The Commissioner concludes that labeling for food products should be revised as soon as possible to include the declaration of FD&C Yellow No. 8 among the list of ingredients. Therefore, he proposes that the effective date for this portion of the final regulation be 1 year after the date of publication in the Federal Register. The Commissioner believes this

OTC drugs having the presence of FD&C Yellow No. 5 declared on the label would enable a physician to identify more readily persons intolerant of FD&C Yellow No. 5.

PRESCRIPTION DRUG PROPOSAL II

The second proposal applicable to prescription drugs would include the labeling requirements of the first prescription drug proposal and a prohibition against the use of FD&C Yellow No. 5 in seven classes of drugs. The following classes of untested prescription drugs, as well as those that may be administered orally or vaginally, would not be permitted to contain FD&C Yellow No. 5: analgesic drugs, antihistaminic drugs, cough and cold drugs, oral nasal decongestant drugs, antialthmatic drugs, non-steroidal anti-inflammatory drugs, and lucoecorticoid drugs.

The reasons for this proposal are the same as those set forth under OTC Drug proposal II.

PROPOSED DRUG REGULATIONS

In the proposed drug regulations set forth below, the Commissioner has decided to propose only the second approach for both OTC and prescription drugs for human use because it provides in optimal degree of safe conditions of use for the color. The second approach, while including the provisions of the first, would be more restrictive. Therefore, the Commissioner believes that the proposed changes to Parts 5 and 201 that could be made if the first proposed approach (i.e., a labeling requirement for all drugs containing FD&C Yellow No. 5) were finalized are readily apparent and do not require presentation. Even though only the second approaches are set forth in the proposed regulations, the Commissioner requests comments on both the OTC and prescription drug proposals. The Commissioner is also interested in receiving comments on the availability of drugs that do not contain FD&C Yellow No. 5 within the five OTC drug classes and the seven prescription drug classes included in the proposal set forth below.

EFFECTIVE DATES

As with the food labeling proposal, the Commissioner believes that the effective date of the final regulations as it pertains to labeling drugs for human use should also be 1 year after the date of their publication in the Federal Register. He believes this will provide sufficient time for manufacturers to obtain new labels. Each drug for human use containing FD&C Yellow No. 5 labeled after 1 year after the date of publication of the final regulations in the Federal Register, should bear a label indicating the presence of FD&C Yellow No. 5.

If the second proposal were adopted, the effective date of the labeling portion of the final regulation would be 1 year as stated above. With respect to the classes of drugs that would have to be reformulated to remove FD&C Yellow No. 5, the Commissioner proposes to make this portion of the final regulations

publication in the Federal Register. After the effective date of this portion of the final regulations, the use of FD&C Yellow No. 5 in the manufacture of any drug among the classes of drugs prohibited from containing FD&C Yellow No. 5 would render the drug adulterated within the meaning of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 et seq.) and subject to regulatory action. Further, the Commissioner proposes that the distribution by a manufacturer of any drug prohibited from containing FD&C Yellow No. 5 eighteen months after the date of publication of the final regulations will cause the product to be adulterated and subject to regulatory action. The prohibition of FD&C Yellow No. 5 would apply to its use as a straight color, a lake, or mixtures of straight colors. The Commissioner is not proposing to recall from the market any drugs containing FD&C Yellow No. 5 if they were manufactured or in process within 6 months of the date of publication of the final regulations or were distributed for sale within 18 months of the date of publication of the final regulations.

Manufacturers of new drugs containing FD&C Yellow No. 5 may revise their labeling to conform to this proposal at the earliest possible time after the effective date of the final regulations and, should not wait until their supplemental application submitted under § 314.8 (21 CFR 314.8) has been approved. If the second proposal were adopted, a manufacturer of a new drug containing FD&C Yellow No. 5 in one of the classes of drugs that would be prohibited from containing the color additive would be allowed to either delete the use of any color additive or substitute other color additives in accordance with § 314.8(d)(3) and (e) pertaining to supplemental new drug applications.

To be in compliance with § 314.8, the holder of a new drug application would be required to submit data providing the new composition and showing that the change in composition does not interfere with any assay or control procedure used in manufacturing the drug, or that the assay and any other control procedure have been revised to make them adequate. The supplement would be required to include data available to establish the stability of the revised formulation. If the data are limited to support a conclusion that the drug will retain its declared potency for a reasonable marketing period, a commitment to test the stability of marketed batches at reasonable intervals and to submit the data as they become available is required. Additionally, there must be a commitment to recall from the market any batch found to fall outside the approved specifications for the drugs.

The articles and publications cited in this preamble are listed below. In addition, other articles and publications used in support of this proposal are listed. Copies of the journal articles and other information forming the basis for the proposed actions are on public display in the office of the Hearing Clerk, Food and Drug Administration, Rm. 4-85,

between 9 a.m. and 4 p.m., Monday through Friday.

REFERENCES

- (1) Samter, M. and R. F. Beers, "Concerning the Nature of Intolerance to Aspirin," *Journal of Allergy*, 40:291-293, 1967.
- (2) Chafetz, F. H. and G. A. Settipane, "Aspirin Intolerance as a Precipitant in an Allergic Population," *Journal of Allergy and Clinical Immunology*, 53:193-199, 1974.
- (3) Lockey, S. D., "Allergic Reactions due to FD&C Yellow No. 5, Tartrazine, an Azo Dye Used as a Coloring and Identifying Agent in Various Steroids," *Annals of Allergy*, 17:718, 1959.
- (4) Chafetz, F. H. and G. A. Settipane, "Asthma Caused by FD&C Approved Dyes," *Journal of Allergy*, 40:48, 1967.
- (5) Juhlin, L., G. Michaelsson, and O. Zetterstrom, "Urticaria and Asthma Induced by Food- and Drug Additives in Patients with Aspirin Hypersensitivity," *Journal of Allergy and Clinical Immunology*, 59:192, 1972.
- (6) Michaelsson, G. and L. Juhlin, "Urticaria Induced by Preservatives and Dye Additives in Foods and Drugs," *British Journal of Dermatology*, 88:529, 1973.
- (7) Settipane, G. A. and R. K. Pudupakam, "Aspirin Intolerance. III. Subtypes, Familial Occurrence of Cross Reactivity with Tartrazine," *Journal of Allergy and Clinical Immunology*, 52:315, 1974.

OTHER ARTICLES AND PUBLICATIONS

- (1) Speer, S., Management of Childhood Asthma, Springfield: Charles C. Thomas, 1958.
- (2) Griep, L. H., "Allergic Vascular Purpura," *Journal of Allergy and Clinical Immunology*, 48:7, 1971.
- (3) Yurchak et al., "Immunologic Studies with Aspirin, Clinical Studies with Acetylsalicylic Acid," *Journal of Allergy*, 48:245, 1970.
- (4) Johnson, H. M. et al., "Tartrazine: Solid-phase Radioimmunoassay Studies of an Azo Dye Implicated in Allergic Reactions (Azo dye and Allergy)," (unpublished paper).
- (5) Samter, M. and R. F. Beers, "Intolerance to Aspirin, Clinical Studies and Consideration of its Pathogenesis," *Annals of Internal Medicine*, 68:973-983, 1969.
- (6) Cohen, H. S., "Tartrazine Revisited," *Drug Intelligence and Clinical Pharmacy*, 9:199, 1975.
- (7) Smith, L. V. and R. J. Slavia, "Drugs Containing Tartrazine Dye," *Journal of Allergy and Clinical Immunology*, 56:456, 1976.

The Commissioner has carefully considered the environmental effects of the proposed regulation, and because the proposed action will not significantly affect the quality of the human environment, has concluded that an environmental impact statement is not required. A copy of the environmental impact assessment is on file with the Hearing Clerk, Food and Drug Administration.

Therefore, under the Federal Food, Drug, and Cosmetic Act (secs. 501, 502, 701, 706 (b), (c), and (d)), 52 Stat. 1049-1051 as amended, 1053-1058 as amended, 74 Stat. 399-403 (21 U.S.C. 351, 352, 371, 376 (b), (c), and (d))) and under authority delegated to the Commissioner (21 CFR 5.1) (recodification published in the Federal Register of June 15, 1976 (41 FR 24262)), it is proposed that Chapter I of Title 21 of the Code of Federal Regulations be amended as follows:

PART 1—REGULATIONS FOR THE ENFORCEMENT OF THE FEDERAL FOOD, DRUG AND COSMETIC ACT AND THE FAIR PACKAGING AND LABELING ACT

1. In § 1.113 by revising paragraph (c) to read as follows:

§ 1.12 Food labeling; spices, flavorings, colorings, and chemical preservatives.

(c) A statement of artificial flavoring, artificial coloring, or chemical preservative shall be placed on the food, or on its container or wrapper, or on any two or all three of these, as may be necessary to render such statement likely to be read by the ordinary person under customary conditions of purchase and use of such food. The specific artificial color used in food shall be identified on the labeling when so required by its listing in Part 8 to assure safe conditions of use for the color additive.

PART 8—COLOR ADDITIVES

2. In § 8.275(d) by redesignating the text that follows the italicized heading as paragraph (d) (1) and by adding new paragraph (d) (2) to read as follows:

§ 8.275 FD&C Yellow No. 5.

(d) Labeling requirements. (1) . . .

(2) Foods for human use that contain FD&C Yellow No. 5, including butter, cheese, and ice cream, shall specifically declare its presence by listing the color additive in the list of ingredients.

3. In § 8.4175 by revising paragraphs (b) and (c) to read as follows:

§ 8.4175 FD&C Yellow No. 5.

(b) Uses and restrictions. (1) Except for the categories of drugs for human use in paragraph (b) (2) of this section, FD&C Yellow No. 5 may be used for coloring ingested drugs in amounts consistent with good manufacturing practice.

(1) FD&C Yellow No. 5 may not be used in the following categories of ingested prescription drugs for human use as well as those that may be administered rectally or vaginally:

Anesthetic drugs
Antibiotic drugs
Cough and cold preparations
Oral nasal decongestants
Antihistamines
Nonsteroidal anti-inflammatory drugs
Otitic/ear-drip drugs

(2) FD&C Yellow No. 5 may not be used in the following categories of ingested OTC drugs for human use as well as those that may be administered rectally or vaginally:

Anesthetic drugs
Antibiotic drugs
Cough and cold preparations
Oral nasal decongestants
Antihistamines

(c) Labeling requirements. (1) The label of the color additive and any mixtures prepared therefrom intended solely

or in part for coloring purposes shall conform to the requirements of § 8.22.

(2) Ingested drugs for human use (as well as those that may be administered rectally or vaginally) containing FD&C Yellow No. 5 shall bear the statement "This product contains FD&C Yellow No. 5 which may cause allergic-type reactions in certain susceptible individuals" on their label and in the labeling on or within the package, if any. For prescription drugs containing FD&C Yellow No. 5, the labeling required by § 201.100(d) of this chapter shall bear the statement "This product contains FD&C Yellow No. 5 which may cause allergic-type reactions in certain susceptible individuals." This statement shall be set forth in the "How Supplied" section of the labeling.

PART 200—GENERAL

4. In Subpart B by adding new § 200.55 to read as follows:

§ 200.55 Drugs for human use not permitted to contain FD&C Yellow No. 5.

Although § 8.4175 of this chapter provides for the use of FD&C Yellow No. 5 in most drugs, it prohibits FD&C Yellow No. 5 from being used in certain categories of systemically administered drugs for human use. If a drug within one of the categories of drugs for human use listed in § 8.4175 of this chapter contains any quantity of FD&C Yellow No. 5, the drug is deemed adulterated and subject to regulatory action.

PART 201—LABELING

5. In subpart C by adding new § 201.64 to read as follows:

§ 201.64 Declaration of presence of FD&C Yellow No. 5.

The labeling for each ingested over-the-counter drug for human use containing FD&C Yellow No. 5 (as well as those that may be administered rectally or vaginally) shall, as required by § 8.4175 of this chapter, bear the statement "This product contains FD&C Yellow No. 5 which may cause allergic-type reactions in certain susceptible individuals." The labeling statement shall appear on the principal display panel of the OTC drug product. A statement indicating the presence of FD&C Yellow No. 5 shall also appear on any labeling on or within the package.

6. In § 201.100 by revising paragraph (b) (6) and by adding new paragraph (b) (7) and (8) to read as follows:

§ 201.100 Prescription drugs for human use.

(b) . . .

(6) An identifying lot or control number from which it is possible to determine the complete manufacturing history of the package of the drug.

(7) For all ingested drugs containing FD&C Yellow No. 5, the statement "This product contains FD&C Yellow No. 5

which may cause allergic-type reactions in certain susceptible individuals" as required by § 8.4175 of this chapter.

(8) If a container is too small or otherwise unable to accommodate a label of sufficient space to bear all the required information but is packaged within a outer container from which it is removed for dispensing or use, the information required by paragraph (b) (2), (3), (4), (5), (6), (7), (8) and (9) of this section may be contained on other labeling on or within the pack from which it is to be dispensed, the information referred to in paragraph (1) and (7) of this section may be placed on such outer container only, and the information required by paragraph (b) (8) of this section may be the crimp of dispensing tube.

Interested persons may, on or before April 5, 1977, submit to the Hear: Clerk, Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville MD 20857, written comments (preferably in duplicate and identified with Hearing Clerk docket number found brackets in the heading of this document) regarding this proposal. Receipts may be seen in the above office between the hours of 9 a.m. and 4 p.m. Monday through Friday.

Note.—The Food and Drug Administration has determined that this document does contain a major proposal requiring preparation of an inflation impact statement under Executive Order 11821 and OMB Circular A-107. A copy of the inflation impact assessment is on file with the Hearing Clerk Food and Drug Administration.

Dated: January 28, 1977.

SEAWIN GARDNER,
Acting Commissioner of
Food and Drug

[FR Doc. 77-3358 Filed 2-3-77; 8:45 am]

[21 CFR Part 1284]

[Docket No. 78N-0298]

PROCESSING AND BOTTLING OF BOTTLED DRINKING WATER

Proposed Amendments to Current Good Manufacturing Practice Regulations

Correction

In FR Doc. 77-121 appearing at 1:507 in the issue for Tuesday, January 1977, in the fifth line of the third paragraph, "radium-225", should read "dium-226".

DEPARTMENT OF TRANSPORTATION

Federal Highway Administration

[23 CFR Part 922]

[FHWA Docket No. 78-52]

SAFER OFF-SYSTEM ROADS PROGRAM

Notice of Proposed Rulemaking

• Purpose. The purpose of this document is to publish proposed rules for administration of the safer off-system roads program. •

Notice of Proposed Rulemaking

Concerning limitations on the use of FD&C Yellow No. 5 in Food and Drugs (Docket No.) Supporting Material.

CITED REFERENCES

1. Santer, M. and R. F. Beers, "Concerning the Nature of Intolerance to Aspirin," Journal of Allergy. 40:281-293, 1967.
2. Chafee, F. H. and G. A. Setticone, "Aspirin Intolerance, I. Frequency in an Allergic Population," Journal of Allergy and Clinical Immunology. 53:193-199, 1974.
3. Lockey, S. D., "Allergic Reactions Due to FD&C Yellow No. 5 Tartrazine, an Aniline Dye Used as a Coloring and Identifying Agent in Various Steroids," Annals of Allergy 17, 719, 1959.
4. Chafee, F. H. and G. A. Setticone, "Asthma Caused by FD&C Approved Dyes," Journal of Allergy 40:65, 1967.
5. Juhlin, L., G. Michaelsson, and O. Zetterstrom, "Urticaria and Asthma Induced by Food-and-Drug Additives in Patients with Aspirin Hypersensitivity," Journal of Allergy and Clinical Immunology 50:92, 1972.
6. Michaelsson, G. and L. Juhlin, "Urticaria Induced by Preservatives and Dye Additives in Foods and Drugs," British Journal of Dermatology 88:525, 1973.

is amended in paragraph (c)(2), by changing the reference to "§ 1.6(c)" to read "§ 2.707."

§ 1.100 (Amended)

5. Section 4.100 *Applicability: cross-reference to other regulations* is amended in paragraph (c)(1) by changing the reference to "§ 1.6(c)" to read "§ 2.707."

Effective date. This regulation shall become effective March 7, 1977.

(Secs. 305, 701(a), 70 Stat. 1045, 1056 (21 U.S.C. 356, 371(a).))

Dated: January 28, 1977.

SHERWIN GARDNER,
Acting Commissioner of
Food and Drugs.

[FR Doc. 77-3339 Filed 2-3-77; 8:46 am]

[Docket No. 77N-0008]

PART 8—COLOR ADDITIVES

Listing of FD&C Yellow No. 5 for Cosmetic Use Subject to Certification; Stay of Effectiveness

The Food and Drug Administration (FDA) is announcing a stay of the effectiveness of the order listing FD&C Yellow No. 5 for use in externally applied cosmetics.

In the FEDERAL REGISTER of January 21, 1974 (39 FR 2358), the Commissioner of Food and Drugs issued an order listing FD&C Yellow No. 5 for use in externally applied cosmetics other than hair straighteners, permanent wave preparations, and depilatories by adding new § 8.7255 (21 CFR 8.7255). The continued use of these three types of products has been permitted under the provisional listing of FD&C Yellow No. 5.

Timely objections to the order were received from a manufacturer of colors, a manufacturer of cosmetics, and a trade association. Two of the letters objected to the order's exclusion of the use of FD&C Yellow No. 5 in ingested cosmetics. Both letters claimed that such use should be included in the order and cited findings from teratological and multireproduction studies as supporting evidence for their safe use. It was also cited that the color was already listed for use in food and ingested drugs. Two of the letters objected to the exclusion of the use of the color in hair straighteners, permanent wave preparations, and depilatories. One letter objected to the omission of a final listing of lakes made from FD&C Yellow No. 5. One letter objected to the omission of the use of FD&C Yellow No. 5 in externally applied drugs. The filing of these objections automatically served to stay the effectiveness of the order because they involved its primary aspects.

A proposal was published in the FEDERAL REGISTER of September 23, 1976 (41 FR 41860) to postpone the closing dates for the provisional listing of certain color additives beyond December 31, 1976. One of the requirements that the proposal would impose is the submission of new data from chronic studies with certain color additives, including FD&C

Yellow No. 5. The Commissioner, in evaluating the listing of FD&C Yellow No. 5 for external cosmetic use, concludes that such action is inappropriate pending receipt of the new data from chronic studies with FD&C Yellow No. 5.

Accordingly, the Commissioner is announcing in accordance with section 701 (e) (2) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 371 (e) (2)), that the effectiveness has been stayed for the order listing FD&C Yellow No. 5 for use in externally applied cosmetics.

Therefore, under the Federal Food, Drug, and Cosmetic Act (sec. 701(e), 708 (b), (c), and (d), 70 Stat. 910, 74 Stat. 399-403 (21 U.S.C. 371(e), 376 (b), (c), and (d))) and under authority delegated to the Commissioner (21 CFR 8.1) (recodification published in the FEDERAL REGISTER of June 15, 1976 (41 FR 24202)), notice is given that the effective date of March 22, 1974 for the order amending Part 8 by adding new Subpart O consisting of § 8.7255 is stayed by the filing of timely and valid objections.

Until further notice, FD&C Yellow No. 5 will continue to be provisionally listed for use in cosmetics, generally, and in externally applied drugs.

Dated: January 28, 1977.

SHERWIN GARDNER,
Acting Commissioner of
Food and Drugs.

[FR Doc. 77-3337 Filed 2-3-77; 8:46 am]

provide sufficient time to permit use of current stocks of labeling and revision of labeling to include a declaration of the presence of FD&C Yellow No. 5. Manufacturers could, of course, revise their labeling before the effective date of the regulation, and the Commissioner encourages them to do so.

PROPOSAL FOR DRUGS FOR HUMAN USE

The use of color additives in ingested drugs for human use is an old, accepted practice in the pharmaceutical industry. The use of color additives in drugs serves necessary public health function because it permits drugs of identical size and shape to be distinguished. The distinction provided by the use of colors provides an important quality control tool in the dispensing of drugs to prevent mixups between otherwise similarly appearing drugs. The ability to distinguish among different products is also very important to persons taking many drugs, especially to the patient who may think in terms of taking a drug of a particular color rather than by the name of the drug. Color additives in drugs also assist in the identification of a drug in cases of accidental overdose.

Because yellow is a primary color, it is widely used as a color additive in drugs. Of the three yellow color additives available for use in ingested drugs, FD&C Yellow No. 5 is the most widely used. It is used to produce not only typically yellow shades but also variations of green, brown, orange, and other related colors. It is estimated that approximately 60 percent of all colored drug tablets for human use sold in the United States contain FD&C Yellow No. 5.

Thus, in view of the extent of use of FD&C Yellow No. 5, a substantial number of drugs would have to be reformulated if the color additive were prohibited in drugs for human use. Further, while reformulating their products to eliminate FD&C Yellow No. 5, some firms might decide to eliminate all color additives. The considerable time and effort necessary to reformulate drug products and the loss of product identification would be unimportant if considered necessary for the protection of public health and if there were no suitable alternative course of action. However, on the basis of the current information available concerning the nature and extent of the problem of tolerance of FD&C Yellow No. 5, the Commissioner believes that prohibiting all drug uses of FD&C Yellow No. 5 is not necessary for the protection of patients who are intolerant of FD&C Yellow No. 5, and that a labeling requirement similar to that for foods will be satisfactory.

The Commissioner concludes, however, that for drugs a simple listing of the color as FD&C Yellow No. 5 among the list of ingredients would not provide a sufficient safeguard for the person intolerant of FD&C Yellow No. 5. Generally, there is no uniform procedure for the declaration of ingredients on drug labeling; therefore, susceptible individuals might overlook such a listing. The listing of ingredients for ingested drug products has traditionally been used to designate active ingredients; conse-

quently, listing of FD&C Yellow No. 5 may give an incorrect impression that it is an active ingredient. Finally, there may be physicians who are unaware that FD&C Yellow No. 5 may elicit allergic-type responses in certain susceptible individuals and for whom a simple listing would be inadequate.

For these reasons, the Commissioner concludes that the use of FD&C Yellow No. 5 in drugs should be declared in the form of a precautionary statement, i.e., "This product contains FD&C Yellow No. 5 which may cause allergic-type reactions in certain susceptible individuals."

This above decision would, of course, be subject to modification if new information becomes available indicating that the only way to protect sensitive persons would be to prohibit the use of FD&C Yellow No. 5.

Although a total prohibition against the use of FD&C Yellow No. 5 is not warranted, the Commissioner concludes that some action must be taken to limit the potential for exposure of these sensitive individuals to drugs containing FD&C Yellow No. 5. To achieve this objective, the Commissioner is proposing two alternative approaches for both over-the-counter (OTC) and prescription human drugs. In addition to comments on the proposals themselves, the Commissioner requests views concerning the advantages and disadvantages of the two alternative approaches.

OTC DRUG PROPOSAL I

The first proposal applicable to OTC drugs would amend the color additive regulations (21 CFR Part 8) to require that the presence of FD&C Yellow No. 5 be declared on the labels of all OTC drugs that are ingested as well as those that may be administered rectally or vaginally. A declaration of the presence of FD&C Yellow No. 5 on the label of these OTC drugs would enable persons who know they are intolerant of FD&C Yellow No. 5 to avoid drugs containing this color additive. Further, by having the presence declared on the label, physicians would more easily be able to identify persons intolerant of FD&C Yellow No. 5.

Under this proposal, the principal display panel of OTC drugs containing FD&C Yellow No. 5 that are ingested, as well as those that may be administered rectally or vaginally, would be required to contain the statement "This product contains FD&C Yellow No. 5 which is capable of producing allergic-type reactions in certain susceptible persons." The quantity of FD&C Yellow No. 5 would not have to be given.

OTC DRUG PROPOSAL II

Persons intolerant of FD&C Yellow No. 5, like many other persons, may take a variety of OTC drugs at one time or another, to relieve or treat conditions or symptoms of a disease. Some of the drugs that may be taken are used to treat allergic or allergic-type conditions, including those allergic-type conditions that may arise as a result of ingestion of FD&C Yellow No. 5. As previously discussed, most persons reacting to FD&C

Yellow No. 5 have other basic allergic problems including, in many cases, a sensitivity to aspirin. Thus, drugs used to treat allergic problems may be used widely by persons intolerant of FD&C Yellow No. 5. However, if a person intolerant of FD&C Yellow No. 5 is administered a drug containing FD&C Yellow No. 5 to treat an existing allergic problem, severe aggravation of the basic allergic condition may result. Further, in the haste of treating a serious allergic problem, a drug containing FD&C Yellow No. 5 could be taken by a person who knows he is intolerant even though the drug is labeled as containing the color additive. Likewise, a drug containing FD&C Yellow No. 5 could also be taken by a sensitive person to treat a serious allergic problem before the person's intolerance of FD&C Yellow No. 5 had been ascertained.

Another possibility which would not be resolved by the OTC Drug Proposal I is that all available drugs of a particular class that are used to treat a sensitive person's allergic condition might contain FD&C Yellow No. 5. Alternatively, the only drugs in a class which are effective for a person might all contain FD&C Yellow No. 5; thus, it could be impossible to select a drug free of FD&C Yellow No. 5.

In view of these considerations, the Commissioner is offering, as an alternative to OTC Drug Proposal I, a second proposal applicable to OTC drug products. This second proposal would include the labeling requirements of the first proposal plus a requirement that would prohibit the use of FD&C Yellow No. 5 in certain classes of drugs that are ingested, as well as those that may be administered rectally or vaginally. The classes of OTC drugs that would not be permitted under this proposal to contain FD&C Yellow No. 5 are analgesic, antihistaminic, cough and cold, oral nasal decongestant, and antispasmodic drugs. These are the classes of OTC drugs that are most likely to be taken by persons intolerant of FD&C Yellow No. 5 to treat an allergic problem or as a substitute for aspirin.

PRESCRIPTION DRUG PROPOSAL I

The first proposal applicable to prescription drugs is a labeling requirement similar to that proposed for OTC drugs. In addition to a declaration of the presence of FD&C Yellow No. 5 on the label of all ingested prescription drugs (as well as those that may be administered rectally or vaginally) containing this color additive, the labeling required by § 201.100(d) (21 CFR 201.100(d)) would be required by proposed § 8.4175 (21 CFR 8.4175) to contain the statement "This product contains FD&C Yellow No. 5 which may cause allergic-type reactions in certain susceptible persons." This statement would be required to appear on the label and in the "How Supplied" section of the package insert, if present.

Although persons intolerant of FD&C Yellow No. 5 may not see the labeling on prescription drugs, they could remind their physicians of their intolerance. The physician could then avoid prescribing a drug containing FD&C Yellow No. 5 for

The proposed labeling requirements would take effect one year after the issuance of a final regulation. The ban on Yellow No. 5 in certain drugs would take effect six months after a final regulation is published. No product recalls would be required.

The proposals appear in the February 4, 1977 FEDERAL REGISTER. Comments may be submitted within 60 days to Hearing Clerk, Food and Drug Administration, Room 4-65, 5600 Fishers Lane, Rockville, Maryland 20857.

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7. Settupane, G. A. and R. K. Pudupakkam, "Aspirin Intolerance, III. Subtypes, Familial Occurrence of Cross Reactivity with Tartrazine," Journal of Allergy and Clinical Immunology 56:215, 1975.

OTHER REFERENCES

- A. Crip, L. H., "Allergic Vascular Purpura," Journal of Allergy and Clinical Immunology 48:7, 1971.
- B. Yurchak, et al., "Immunologic Studies with Aspirin, Clinical Studies with Aspiryl-protein Conjugates," Journal of Allergy 46:245, 1970.
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- E. Cohon, M. S., "Tartrazine Revisited," Drug Intelligence and Clinical Pharmacy, 9:198, 1975.
- F. Smith, L. V. and R. J. Slavia, "Drugs Containing Tartrazine Dye" Journal of Allergy and Clinical Immunology 58:456, 1976.

- 3 -

- G. Memorandum of Conference of April 24, 1975 between Dr. Max Samter, Professor of Medicine, Grant Hospital of Chicago and representatives of the Bureau of Foods.
- H. Memorandum of Conference of June 12, 1975, between Dr. Samter and representatives of the Bureau of Foods.

Concerning the nature of intolerance to aspirin

Luc Sauter, M.D.,* and Ray F. Beers, Jr., M.D.,** Chicago, Ill.

(1) Intolerance to aspirin is not uncommon in individuals near middle age who, as a rule, do not have a history of atopy. It is characterized by changes in the skin and respiratory mucous membranes—angioedema, perennial rhinitis, formation of nasal polyps, and bronchial asthma—which precede the development of intolerance to aspirin. (2) While reactions induced by aspirin simulate an immunological disease for an allergy to acetylsalicylic acid per se or acetylsalicylic acid as an antigenic determinant is unconvincing. Attempts to demonstrate antibodies to acetylsalicylic acid have been generally unsuccessful. (3) If an immunological etiology can be ruled out, aspirin must induce its effects by a direct action on effector organs. (4) Acetylsalicylic acid can inhibit at least one mediator-induced reflex, i.e., the histamine-induced vasodilatation in the skin of man and the histamine-induced bronchoconstriction of the guinea pig. (5) Patients who have an intolerance to aspirin have a comparable intolerance to other minor analgesics, e.g., pyrazolones and indomethacin. It appears reasonable to assume that they exert their effect on the same receptors. (6) It is proposed that aspirin produces characteristic symptoms in aspirin-sensitive patients by activating peripheral chemoreceptors which have been altered by a pre-existing disease. (7) A possible mechanism by which peripheral chemoreceptors might participate in the pathogenesis of bronchial asthma has been outlined and reviewed in the light of what is known about reflexes which control the vascular bed of the skin and the functional state of the respiratory mucous membranes.

reports that the ingestion of aspirin can produce severe allergic reactions which appeared shortly after the discovery of the drug.¹ Since then, a substantial number of related observations has been published, and their similarity is impressive.² In a recent tabulation, Bruce Pearson³ states that of asthmatic patients of all ages on his services, 2.3 per cent were aspirin-

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** In part by a grant from Asthmatic Children's Aid, Chicago, Ill., in part Health Service Research Grant No. FR 45 from the General Clinical Research Branch, National Institutes of Health.

† For publication June 22, 1967.

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sensitive, but the incidence increases in middle age; consequently, published statistics vary according to the type of patients who form the basis of the statistics.

By and large, the literature suggests that an immunological mechanism accounts for the peculiar features of aspirin sensitivity, i.e., the acute episodes of acute angioedema or acute and severe bronchial asthma which simulate anaphylaxis. Bruce Pearson states, "It is generally assumed that aspirin acts as a complement and forms an antigen in combination with serum protein," but raises some question about this "mysterious clinical entity." Friedlander and Feinberg⁴—in a review of 45 aspirin-sensitive patients—comment on the lack of specific skin reactions (in spite of the severity of symptoms), record a sensitivity to drugs other than aspirin in 13 patients, and conclude that "repeated exposure to other substances of a chemical nature, also incapable of giving positive skin tests, may be responsible for the chronic asthma encountered in these individuals. Allergy to aspirin may be an indicator that the individual acquired the mechanism to react to simple chemical compounds." Feinberg concluded that in the absence of positive skin reactions the antibody with which aspirin reacts must have been induced by an extraneous antigen which has not been identified⁵ but might include infectious agents, bacterial toxins, and products of tissue breakdown; indeed, aspirin sensitivity has been classified as a form of infectious asthma.

It has been known for some time that aspirin-sensitive patients are able to take sodium salicylate with impunity, but, even in the absence of reactions to sodium salicylate, it is conceivable that a specific immunological sensitivity to acetylsalicylic acid exists.⁶ In spite of numerous attempts, however, it has not been possible to demonstrate the existence of unequivocal antibodies to acetylsalicylic acid: most of the studies so far either report antibodies to salicylates or permit alternate interpretations.⁷⁻¹²

BRIEF COMMENTS ON THE NATURAL HISTORY OF INTOLERANCE TO ASPIRIN

Intolerance to aspirin is not uncommon. From 1954 until 1965, we have followed more than 1,000 aspirin-sensitive patients who suffered from angioedema, rhinitis, and/or bronchial asthma; of these, 182 were hospitalized for intensive study.

The initial manifestation of the disease which is eventually associated with intolerance to aspirin is a vasomotor rhinitis, distinguished by profuse watery secretion, which develops in the majority of patients during the second or third decade, is at first intermittent, later perennial, and commonly followed by the formation of nasal polyps.

Bronchial asthma in aspirin-sensitive patients tends to occur in middle age. Precipitating factors are rarely defined, but nasal polypectomy seems to bring on the first attack of bronchial asthma in a suggestive number of patients. In the beginning, the bronchial obstruction is readily reversible with isoproterenol; and even later, bronchial asthma of aspirin-sensitive patients often can be controlled with surprisingly low maintenance doses of corticosteroids.

Many authors believe that bronchial asthma of aspirin-sensitive patients is invariably progressive and has a much higher mortality rate than bronchial asthma in general; statistical evaluation of our own group, however, makes us believe that the over-all prognostic picture is better than the literature suggests.

Intolerance to aspirin is not the cause of the disease. In the majority of our patients, respiratory symptoms precede the development of intolerance to aspirin, often by years; and religious avoidance of aspirin and aspirin-containing compounds by patients with established intolerance to aspirin does not, as a rule, alter the natural history of the disease, i.e., recurrence of symptoms and progression.

In contrast to surveys published by others, the incidence of atopy in our group of patients is low; only about 3 per cent of our patients had a personal history of atopy—compared with an expected incidence of atopy of at least 10 per cent, but probably significantly higher,¹² in any unselected group of the population—but the incidence of atopy in their offspring is substantial and reasonably close to the expected figure. We do not wish to imply that the relative absence of atopy in our group argues against a possible immunological mechanism, but it seems curious that the incidence of atopy in our patients is so much lower than one would expect.

THE SPECIFICITY OF INTOLERANCE TO ASPIRIN

In order to clarify the biochemical spectrum of intolerance to acetyl-salicylic acid, each of the patients under our care received (a) sodium salicylate and (b) one or several of the compounds which are listed in Figs. 1 and 2. The compounds include (1) salicylic acid esters with lengthening side chains, (2) choline-salicylate, (3) thio-aspirin, (4) acetyl-p-aminophenol, (5) diacetylfluorescein, and (6) Trasentine-azo-salicylate. Propionylsalicylic acid behaves *in vitro* like aspirin: it is hydrolyzed by the cholinesterase of washed red blood cells and of serum. Diacetylfluorescein is a white, nonfluorescent powder, but deacetylation produces brilliant fluorescence in minimal doses; consequently, the compound lends itself well to studies of gastrointestinal absorption, since onset and degree of deacetylation can be readily established by fluorometric analysis. Thio-aspirin and acetyl-p-aminophenol are not deacetylated either *in vivo* or *in vitro*. None of these compounds induced any symptoms in aspirin-sensitive patients.¹⁴

Extensive *in vitro* studies which we have carried out in recent years have failed to uncover any fundamental differences in the handling of acetylsalicylic acid by various test systems, e.g., plasma and red blood cells obtained from aspirin-sensitive patients and normal controls. In essence, the studies confirm the information which has been summarized in several excellent monographs in recent years.¹⁵⁻¹⁷ *In vitro* binding of sodium salicylate and acetylsalicylic acid by serum proteins is the same in aspirin-sensitive and non-aspirin-sensitive patients. The rate of deacetylation is the same in serum from aspirin-sensitive patients and from normal controls; and red blood cells obtained from aspirin-sensitive patients and normal controls deacetylate acetylcholine

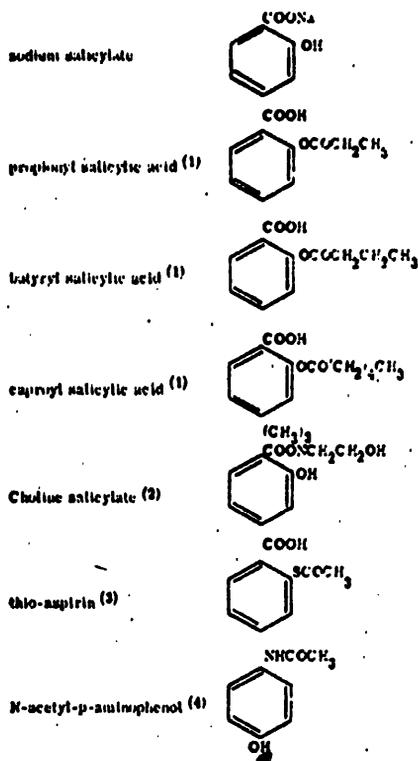


Fig. 1

Compounds tolerated by patients with established intolerance to acetylsalicylic acid. (1. Prepared for us by Dr. C. S. Marvel, The William Albert Noyes Laboratory, Department of Chemistry, and Dr. E. C. Taylor, Jr., Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Ill. 2. Supplied as Arthropan by Dr. Harry S. Tirsch, The Purdue Frederick Company, New York, N. Y. 3. Synthesized by Drs. M. L. Tainter and G. M. Suter, Sterling-Winthrop Research Institute, Kenilworth, N. Y. 4. Supplied as Apanale by Dr. Max Gillbert, The Ames Company, Elkhart, Ind.)

at a comparable rate (methods used: Soltzman,¹⁷ Trinder,¹⁸ Hofstee,²⁰ and Michel²¹).

In vivo absorption of sodium salicylate and acetylsalicylic acid varies greatly from one individual to another but is reasonably constant within the same individual. Separation of bound and unbound salicylate by equilibrium dialysis showed no difference in the handling of sodium salicylate by aspirin-sensitive and non-aspirin-sensitive patients. It is not possible, of course, to administer acetylsalicylic acid routinely to aspirin-sensitive patients for experimental studies, but it is of interest that the rate of deacetylation of diacetylfluorescein is the same in aspirin-sensitive and non-aspirin-sensitive patients.

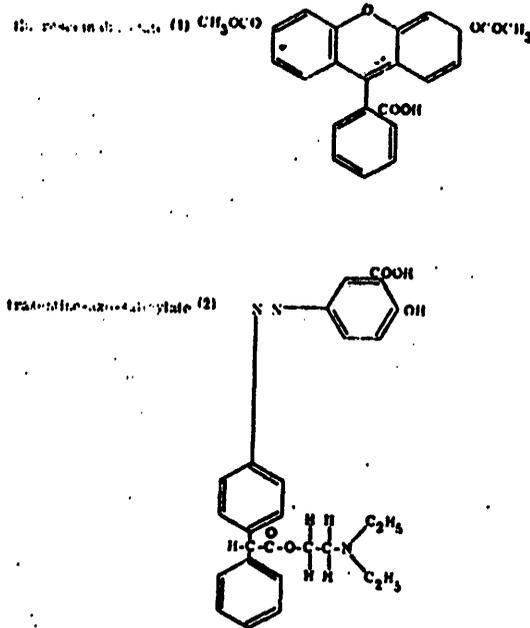


Fig. 2

Fluorescein diacetate and Tartrazine-azo-salicylate. (1, Prepared by Dr. J. O. Hauck, Knoll Pharmaceutical Company, Orange, N. J. 3, Synthesized by Patricia G. Sudar, the Graduate College, Chicago Professional Colleges, University of Illinois, 1953.)

REACTIONS TO CHEMICALS OTHER THAN ASPIRIN IN ASPIRIN-SENSITIVE PATIENTS

While intolerance to acetylsalicylic acid is not intolerance to salicylates, a number of chemicals cause rhinitis and bronchial asthma in aspirin-sensitive patients; they are identified in Fig. 3. Aminopyrine (and antipyrine) have been responsible for several severe reactions, but their number is limited because physicians are reluctant to prescribe these drugs. Tartrazine, FD&C yellow No. 5, produced nasal and bronchial symptoms in 9 of our 182 hospitalized patients. During the summer of 1965, we administered 25 mg. of tartrazine in aqueous solution to 80 ambulant and hospitalized patients under rigidly controlled, double-blind conditions. One half of the participating patients were aspirin-sensitive. Only 3 patients reacted to the administration of tartrazine with rhinorrhea and bronchoconstriction, but the 3 reactors were aspirin-sensitive; even so, the low incidence of reactions surprised us.

After we observed, by chance, that indomethacin precipitated bronchial asthma in one of our aspirin-sensitive asthmatics, we have given indomethacin (25 mg.) to 18 hospitalized aspirin-sensitive patients; each of these patients

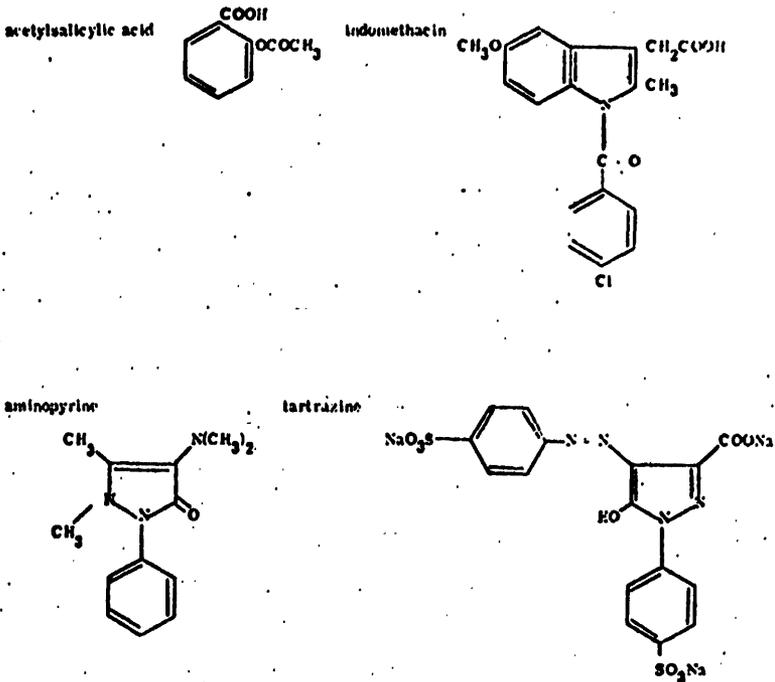


Fig. 3

Compounds which induce reactions in patients with established intolerance to acetylsalicylic acid. (Indomethacin supplied as Indocin by Dr. Gilbert M. Rayne, Merck, Sharp and Dohme Laboratories, West Point, Pa. Tartrazine supplied by Mr. W. H. Kretlow, the Wm. J. Stange Company, Chicago, Ill.)

developed severe bronchial obstruction which required extensive symptomatic therapy.

Since an intolerance to acetylsalicylic acid seems to be associated with an intolerance to pyrazolones and indomethacin, a correlation might exist between the manner in which aspirin, and other minor analgesics, relieve pain and the manner in which aspirin induces nasal and bronchial reactions in aspirin-sensitive patients.

In recent years, it has been shown that the relief of pain by acetylsalicylic acid and by some other minor analgesics involves peripheral as well as central action. Lim and associates²² have offered convincing evidence that aspirin acts by competitive inhibition of chemoreceptors of pain. It appears that bradykinin and related polypeptides stimulate pain receptors; and that certain, but not all, effects of bradykinin can be prevented by pretreatment with acetylsalicylic acid. In man, aspirin abolishes the bradykinin-induced axon reflex, i.e., the vasodilatation considered part of the defenses against various forms

of injury; in guinea pigs, aspirin inhibits bradykinin-induced bronchoconstriction.²⁵ The assumption that aspirin causes severe reactions in either respiratory tract or skin by direct activation of sensory receptors implies (1) that chemoreceptors of skin and mucous membranes are capable of inducing increased blood flow and increased vascular permeability in skin and mucous membranes, as well as bronchoconstriction and secretion of mucous glands, and (2) that aspirin in an abnormal receptor system might, in aspirin-sensitive patients, activate rather than inhibit peripheral chemoreceptors. It is the objective of this report to examine this postulate.

THE NATURE OF AXON REFLEX AND ANTIDROMIC VASODILATATION

Like histamine, as bradykinin acts on chemoreceptors of the skin, it initiates an axon reflex. Studies of the effects of physical, thermal, or chemical irritation on the vascular bed have been carried out on the skin, and it is generally agreed that a variety of stimuli are followed by antidromic vasodilatation.^{24, 25} Crossland²⁴ describes the pathway of antidromic vasodilatation.

Mild mechanical stimulation of the skin causes vasodilatation in the stimulated area, it is widely believed, to the operation of an axon reflex. Cutaneous sensory nerves arising from some skin receptors also receive branches from arterioles in the same areas of the skin. Impulses from the receptors travel along the sensory fibers into the cord in the usual way, but in addition as they pass the point where the vascular branches join, they initiate impulses which pass centrifugally in these branches—in the opposite direction, that is, to that normally taken by impulses in sensory nerves—to produce a vasodilator response.⁴

The nature of the chemical mediator, or mediators, which produce vasodilatation is uncertain. Some investigators believe that the mediator which initiates the sequence of the receptor, e.g., histamine, also elicits the vascular response, but Parrot and Lefebvre²⁷ have shown that antihistamines which inhibit histamine-induced vasodilatation will not abolish vasodilatation elicited by stimulation of the peripheral end of posterior root fibers.

Paton,²⁸ in a thoughtful review of the pharmacology of antidromic vasodilatation, acknowledges that the proper mediators for rapid vascular responses remain to be identified:

But now we know that sensory nerves contain very little histamine or acetylcholine, and since atropine or potent antihistamines do not reduce antidromic vasodilatation, nor (as I have observed myself) the flare response evoked by histamine, one has to seek for other mediators of the vasodilatation. I have speculated whether the vasodilatation seen in blushing may not be partly mediated by the same means. Blushing is usually attributed to a relief of sympathetic tone. Yet the patchiness of it is of a kind that I have not seen in subjects undergoing ganglion block; and the latency is rather short. The other day I asked a laboratory assistant whether he blushed; within a period of about 10 seconds he was scarlet. In contrast, the rise in blood-flow of the forearm in response to heating the feet, which is certainly due to release of vasoconstrictor tone in the arm, appears to take several minutes to develop.¹

Of the substances which induce an axon reflex after local application to, or release from within, the skin, histamine has been studied most extensively.

⁴Crossland, J.: Some Possible Mediators of Non-cholinergic Central Transmission, in Elliot, K. A. C., Page, Irvine H., and Quastel, J. H., editors: *Neurochemistry, the Chemistry of Brain and Nerve*, Springfield, Ill., 1962, Charles C. Thomas, Publisher, p. 657.

¹Paton, W. D. M.: Pharmacology of Vasodilator Drugs With Special Reference to the Skin, in Book, A., editor: *Progress in the Biological Sciences in Relation to Dermatology*, London, 1960, Cambridge University Press, p. 120.

It has not been possible, however, to demonstrate the existence of "histaminergic" nerves; and it has not been shown that histamine can be released from nerve fibers by electrical stimulation.

The same general statement holds true for most, if not all, of the chemicals which, like serotonin, ATP, and polypeptides, e.g., bradykinin or substance P, act on chemoreceptors. It is fair to state that the hypotheses which assign the activation of the chemoreceptor and the subsequent antidromic vasodilatation to the same substance have become less and less attractive.

SIMILARITIES BETWEEN REFLEXES IN THE SKIN AND IN THE RESPIRATORY MUCOUS MEMBRANE

In the skin, histamine has been the most widely examined mediator and Ungar, Grossiord, and Brincourt²⁰ have suggested as early as 1936 that histaminergic fibers produce antidromic vasodilatation in the lungs of dogs. In recent years, Aviado^{20, 21} has suggested (1) that histamine induces parasympathetic bronchoconstriction and (2) that parasympathetic stimulation initiates a feedback mechanism which in turn inhibits the release of additional histamine. Aviado suggests that bradykinin might act in a similar fashion, but this suggestion, while attractive, remains to be substantiated by experimental evidence.

It is likely that stimuli which are generated at chemoreceptors of the respiratory mucous membrane not only induce an axon reflex but are carried to the dorsal root ganglion and beyond.²² In other words, bronchoconstriction might represent not only the result of abnormal stimulation of peripheral receptors, but the combined result of exaggerated peripheral responses transmitted to an unduly responsive system of autonomic controls within the bronchial tree.

THE EFFECT OF ASPIRIN ON PERIPHERAL CHEMORECEPTORS

Aspirin acts as a competitive inhibitor for many, but not all, bradykinin receptors. The axon reflex which is induced by topical application of tetrahydrofurfuryl nicotinate (Fig. 4)—thought to release vasoactive kinins—can be effectively inhibited by pretreatment with comparatively small doses of acetylsalicylic acid in normal subjects.²³ Aspirin does not inhibit axon reflexes which are induced by other irritants, including reactions induced by application of histamine and methacholine.²¹ It is interesting to note that

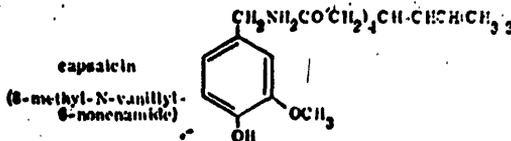


Fig. 4

Tetrahydrofurfuryl nicotinate. (Supplied as Trafuril by Dr. J. H. Fyfe, CIBA Limited, Basel, Switzerland.)

aspirin does not abolish the direct effect (e.g., the formation of a wheal) of injected bradykinin, but that aspirin prevents secondary effects, i.e., the axon reflex and the antidiuretic vasodilatation.

Normally, the chemoreceptors of the nasal and bronchial mucosa are not activated by aspirin; in fact, it has been said that acetylsalicylic acid is a helpful adjunct in the treatment of bronchial asthma.²⁵

In aspirin-sensitive patients, aspirin produces—either immediately or after a latent period which might extend to up to 3 hours—angioedema, rhinitis, and bronchial asthma. We believe that aspirin causes these effects by direct stimulation of chemoreceptors in either skin or nasal and bronchial mucous membrane; and we shall present, briefly, the rationale for this interpretation.

The question arises, of course, why acetylsalicylic acid, an antagonist to kinins, should act as an agonist in aspirin-sensitive patients and initiate rather than prevent stimuli at the site of the chemoreceptor. In recent years, several attempts have been made to clarify the biochemical difference between agonists and antagonists. Paton,^{26, 27} for instance, has proposed a kinetic theory of drug-receptor interaction, applied to acetylcholine and acetylcholine antagonists and, more recently, to adrenergic mediators and receptors. Paton suggests that only the agonist disassociates rapidly; the antagonist forms a comparatively stable complex with receptor protein which prevents short-acting agonists from reaching and stimulating receptor sites. If a pre-existing disease, therefore, would decrease or destroy the capacity of the receptor to bind antagonists, antagonists might be expected to stimulate, rather than to block, receptor sites.

In spite of extensive studies, the relative significance of sympathetic and parasympathetic control in the maintenance of normal bronchomotor tone has not been clearly established; specifically, it appears that results obtained in normal men²⁸⁻³⁰ do not necessarily apply to patients with pre-existing bronchoconstriction. Even so, Widdicombe,³¹ after a comprehensive review of the existing evidence, concludes, "It is probably wise to accept the conventional view that vagal nerves are constrictor, and sympathetic dilator, to the tracheo-bronchial tree; while remembering that many experiments which indicate that this is an oversimplification have yet to be explained."

Atropine fails to control the aspirin-induced bronchoconstriction of aspirin-sensitive patients; their response to isoproterenol remains intact. While this does not necessarily preclude a possible role of sympathetic and parasympathetic abnormalities in the pathogenesis of the syndrome, it is tempting and, we believe, logical to trace the bronchoconstriction of aspirin-sensitive patients to receptors which have not been previously identified as a cause of nasal and bronchial congestion; and which, like the peripheral chemoreceptors, are equipped for rapid control of the air-conditioning structures of the respiratory tract.

The natural history of what we have called "aspirin disease" suggests that aspirin-sensitive patients develop changes in the chemoreceptors, in the nasal and bronchial mucous membranes, or of the skin, which, at first, result in exaggerated responses to physiological stimulation. After a variable interval, often years after

onset, patients record an intolerance to acetylsalicylic acid. If the responsible receptors are kinin receptors, they should, and would normally, be inhibited by aspirin, but aspirin-sensitive receptors are not normal. If the receptor distortion at this particular moment has progressed to a point where aspirin can no longer form a stable complex with the receptor, it would be no longer an antagonist, but an agonist, and initiate the series of reflexes which we have described.

If intolerance to aspirin is caused by changes in peripheral chemoreceptors, other peripheral analgesics, i.e., chemicals reacting on the same or similar receptors should induce comparable reactions. We have demonstrated that this assumption is correct for (1) aminopyrine (and antipyrine), (2) indomethacin, and (3) tartrazine (FD&C yellow No. 5), which is a pyrazole derivative.^{12, 13} Indomethacin will induce severe reactions in most, if not all, aspirin-sensitive patients, but FD&C yellow No. 5—which is capable of inducing reactions—will induce these reactions only in a certain, comparatively small percentage of aspirin-sensitive patients. It is our impression that tartrazine requires biotransformation before it becomes an effective metabolite, and that this biotransformation occurs only in a limited number of patients under conditions which are currently under study.⁹

Anti-inflammatory drugs, on the other hand, might not act on the same chemoreceptors: neither phenylbutazone nor 1-N-methyl-piperidyl-4'-3-phenyl-4-benzyl-pyrazolone-5 induce untoward reactions, and they can be taken with impunity by aspirin-sensitive patients. Consequently, it appears that not only anti-inflammatory drugs and peripheral analgesics differ in their mode of action, but that minor analgesics which induce antidiromic responses in aspirin-sensitive patients might differ in their mode of action from minor analgesics which, like N-acetyl-p-aminophenol or sodium salicylate, are tolerated without untoward reactions.

DESENSITIZATION OF CHEMORECEPTORS

Attempts to desensitize hypersensitive peripheral receptors have been made, but have not been very successful. On the basis of Janesko's¹⁴ studies, we have used the active principle of red pepper, capsaicin, a highly irritating substituted benzamide of a long-chain unsaturated fatty acid (Fig. 5). Capsaicin sensitizes peripheral receptors,¹⁵ but has the peculiar ability to desensitize the same receptors on continued application, either topically or by injection. Janesko demonstrated that capsaicin does not only induce nonresponsiveness of chemoreceptors to capsaicin, but also to nicotine and acetylcholine, while the response to physical stimulation is unimpaired.

Desensitization, of course, is not new. Repeated application of nicotine desensitizes to nicotine. Repeated application of acetylcholine desensitizes to acetylcholine, but none of these desensitizes to capsaicin, formalin, and similar

⁹In a recent study of a patient sensitive to acetylsalicylic acid as well as to FD&C approved dyes, Chaffer, F. H., and Settipane, G. A. (*J. Allergy* 40: 65, 1967) call attention to the possible importance of free carboxyl groups attached to aromatic rings for the development of symptoms.

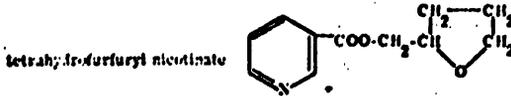


Fig. 5

Capsaicin, the active principle of various species of *Capiscum anatum*.

irritants. Desensitization to capsaicin, on the other hand, “. . . blocks the axon reflexes throughout the body and blocks them permanently.”¹⁰ We are still uncertain, however, whether capsaicin will desensitize not only normal receptors but also the abnormal receptors of aspirin-sensitive patients.

CONCLUSIONS

In the absence of convincing evidence that acetylsalicylic acid is an antigen or an antigenic determinant (except under in vitro conditions which have no relation to the clinical syndrome) it seemed desirable to examine alternate explanations for the severe reactions which it produces in selected patients. Our assumption that aspirin acts directly and paradoxically on peripheral chemoreceptors has been strengthened by the recognition that other, chemically unrelated, peripheral analgesics produce identical symptoms on first exposure. The possible role of altered components of the autonomic nervous system in the pathogenesis of bronchial asthma¹¹—with particular emphasis on the vagus¹²⁻²⁰—has been debated for many years, but none of the theories which have been offered has wholly explained the natural history of bronchial asthma. Investigation of the normal and abnormal behavior of peripheral chemoreceptors might provide a missing link and a better understanding of the nonspecific factors which determine the course of a specific disease.

Intolerance to aspirin has some features which we cannot understand at present, e.g., the etiology of nasal polyps or the meaning of the eosinophilia which tends to be associated with the syndrome. Even so, we feel justified in proposing that aspirin-induced reactions are the result of a receptor disease which requires further study and clarification.

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Aspirin intolerance

I. Frequency in an allergic population

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The frequency of intolerance to aspirin in a total population of asthma and/or rhinitis was 89 out of 3,781 or 2.3 per cent. In the asthmatic group, the frequency was 4.3 per cent. Those asthmatics with negative allergy skin tests had significantly more aspirin intolerance (6.8 per cent) than did those with positive skin tests (3.5 per cent) ($p < 0.01$). The frequency of intolerance to aspirin increased with advancing years (1.4 per cent for those under 40 years of age compared to 4.3 per cent for those 50 years of age or over). In those patients with rhinitis alone it was 0.7 per cent, a significantly lower value than found in those who had both asthma and rhinitis, 4.5 per cent ($p < 0.001$). Of the 89 patients, 59 or 66 per cent reported bronchospasm, 39 or 43 per cent reported urticaria, and 9 or 10 per cent reported rhinitis after ingestion of aspirin. Bronchospasm was the primary manifestation of aspirin intolerance in patients with known asthma, while the manifestations of urticaria were the predominant symptom of patients with known rhinitis (alone). It is important to compare the characteristics of an aspirin-intolerant group to that of the population from which it is selected.

Adverse reactions to acetylsalicylic acid, aspirin, have been known for over 70 years.¹ A review of the literature reveals a definite need for population studies to determine the frequency and nature of intolerance to this drug. In this report, we have attempted to evaluate the frequency and characteristics found in a selected group of patients intolerant to aspirin with the characteristics found in a given population of allergic subjects from which the former group was derived.

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TABLE 1. Frequency of aspirin intolerance in various age groups of asthmatic patients

Age when first seen (years)	No. with asthma	No. with ASA intolerance	% *	No. with broncho-spasm	% *	No. with urticaria/ angioedema	% †
10-19	353	5	1.4	3	0.8	2	0.6
20-29	351	13	3.7	6	1.7	6	1.7
30-39	343	13	3.5	11	3.2	3	0.9
40-49	357	24	6.7	18	5.0	7	2.0
50 and over	367	22	6.0	17	4.6	2	0.5
Total	1,775	76	4.3	55	3.1	20	1.1

*The trend of progressive differences by decades is statistically significant ($p < 0.01$).

†The trend of differences is not statistically significant. Included are 3 patients who manifested both bronchospasm and urticaria. Four patients with aspirin intolerance manifested solely by rhinorrhea are not included in this table.

MATERIAL AND METHODS

The records of private patients of an allergist practicing in Providence, Rhode Island, were reviewed. This was facilitated by a program of coding pertinent details of each record onto McBee Keysort cards, which had been in use for many years to provide quick and accurate retrieval of information. The few records of children first seen under the age of 10 years were excluded. A diagnosis of asthma or rhinitis was found in 3,731 records, and these cases formed the population of this study. All patients had been tested by the scratch technique to a battery of inhalant allergens.* When the scratch test was negative for a particular allergen that was suspected by history, the intracutaneous test was also done. Allergens included tree, grass, and ragweed pollens, animal danders, and selected molds. A positive reaction had to be definitely larger than the control, and all questionable reactions were considered as negative tests. The cases were sorted into those with a diagnosis of asthma alone (1,133); those with only rhinitis, seasonal or nonseasonal (2,006); and those with both of these conditions (642). The records for each diagnosis were subdivided into those patients who had positive allergy skin tests to one or more pollen, dander, or mold and those with negative reactions to those antigens. Cases of infectious rhinitis were excluded from this study. Vasomotor rhinitis was classified with those cases of rhinitis with negative skin tests. A diagnosis of asthma was accepted if symptoms consisted of clinically reversible signs of wheezing, shortness of breath, and cough on a recurrent basis, not due to any other organic disease. A diagnosis of rhinitis was accepted if symptoms consisted of repeated nasal stuffiness, rhinorrhea, and frequent sneezing on a seasonal or nonseasonal basis. The records were then divided according to sex, and further arranged by decades according to the age when first seen. Those patients 50 years of age and over were classified into one group.

All patients had been routinely questioned for any symptom of possible intolerance to aspirin, and their answers had been specifically included on the Keysort cards. Since aspirin is in such common use, it is assumed that all patients had at one time ingested it. Acute wheezing, rhinorrhea, sneezing, urticaria, angioedema, or symptoms of shock occurring within 2 hours after ingestion were our criteria for intolerance to aspirin. There were 39 records in which one or more of these symptoms occurred. There was no instance of shock. Angioedema was included in the category of urticaria. These 39 cases were carefully scrutinized as to the family history of allergy, the presence of nasal polyps, and the type of response to aspirin ingestion.

RESULTS

Out of 3,731 patients, 1,133 or 30 per cent had asthma alone, 642 or 17 per cent had both asthma and allergic rhinitis, and 2,006 or 53 per cent had allergic

*The standard scratch tests as purchased by Hollister-Stier Laboratories.

TABLE II. Frequency of aspirin intolerance in rhinitis alone compared to rhinitis with asthma

Sex	Rhinitis alone			Rhinitis and asthma		
	Number	Aspirin intolerance	%	Number	Intolerance	%
Male	668	6	0.7	280	13	4.6
Female	1,138	7	0.6	362	16	4.3
Total	2,006	13	0.7*	642	29	4.5*

*This difference is statistically significant ($p < 0.001$).

rhinitis alone. Of the total population 44 per cent were males and 56 per cent were females, and this sex ratio was approximately similar in each of the preceding categories. These 3,781 patients were further classified into age when first seen. The age range was from 10 to over 50 years of age with a slight predominance in the younger age groups. There were 21.5 per cent in the 10 to 19 year age group, 23.3 per cent in the 20 to 29 year age group, 20.6 per cent in the 30 to 39 year age group, 17.1 per cent in the 40 to 49 year age group, and 15 per cent were 50 years of age or older.

The frequency of aspirin intolerance in our total population was 89 out of 3,781 or 2.4 per cent. In the asthmatic group of 1,775 cases the frequency of aspirin sensitivity was 4.3 per cent. Those asthmatics with negative skin tests to inhalant allergens, intrinsic asthma, had significantly ($p < 0.01$) more aspirin intolerance (29 out of 427 or 6.8 per cent) than asthmatics with positive skin tests, extrinsic asthma (47 out of 1,348 or 3.5 per cent). A greater frequency of intolerance to aspirin in intrinsic asthma was noted in all age groups, and this frequency of intolerance appeared to increase with advancing years. For example, those individuals under 20 years of age had a frequency of 1.4 per cent, while those 50 years of age or older had a frequency of 6.0 per cent (Table I).

The frequency of aspirin sensitivity in patients with rhinitis (alone) was 13 out of 2,006 or 0.7 per cent. There was essentially no difference in the rhinitis with positive allergy skin tests compared to the rhinitis with negative allergy skin tests. However, when the frequency of aspirin intolerance in patients with rhinitis alone (0.7 per cent) was compared to the frequency found in patients with rhinitis and asthma (29 out of 642 or 4.5 per cent), this difference became highly significant ($p < 0.001$) (Table II). The two manifestations of aspirin intolerance, bronchospasm and urticaria, were found in different frequencies in aspirin-intolerant patients with known asthma compared to those with known rhinitis alone (Table III). The manifestation of urticaria was found to be in greater proportion in those aspirin-intolerant patients with known rhinitis alone. The manifestation of bronchospasm in aspirin-intolerant patients with known asthma was statistically greater ($p < 0.01$) than the manifestation of urticaria in these same patients. In addition, the frequency of bronchospasm in aspirin-intolerant patients with intrinsic asthma was greater than in those with extrinsic asthma ($p < 0.01$). However, the frequency of urticaria was not significantly different in the extrinsic compared to the intrinsic asthmatic. The frequency of one or more positive skin tests was greater in those intolerant

TABLE III. Frequency of bronchospasm and urticaria as manifestations of aspirin intolerance in asthmatic patients

	Total No.	No. with bronchospasm	%	p value	No. with urticaria/angioedema	%	p value
Extrinsic asthma (positive skin tests)	1,848	30	2.2	< 0.01	17	1.9	N.S.
Intrinsic asthma (negative skin tests)	427	25	5.9		8	0.7	
Total asthma	1,775	55	3.1*	< 0.01	20	1.1*	< 0.01
Rhinitis alone	2,006	4	0.2†		8	0.4†	

N. S. = not significant.

Three cases who reacted with both bronchospasm and urticaria are included. Four cases of aspirin intolerance manifested solely by rhinorrhea are not included in this table.

*The difference between the number of asthmatics whose symptoms of aspirin intolerance were manifested by bronchospasm (3.1%) as compared to those whose symptoms were manifested by urticaria (1.1%) is statistically significant ($p < 0.01$).

†Not significantly different.

patients who on ingestion of aspirin manifested only urticaria (22 of 25 or 88 per cent) than in those who manifested only acute bronchospasm (31 of 55 or 56.4 per cent) ($p < 0.01$).

In known asthmatics, the increased frequency of intolerance to aspirin with advancing age appeared to be significantly influenced by the symptom of bronchospasm ($p < 0.01$) rather than by that of urticaria (Table I). There was no significant sex difference found in any of our categories. Of the 89 patients with aspirin sensitivity, the frequency of symptoms that occurred after aspirin ingestion was as follows: bronchospasm, 66 per cent; urticaria or angioedema, 33 per cent; and severe rhinitis, 10 per cent. There was a history of multiple drug allergy in 18 per cent and also a tartrazine sensitivity in 2 per cent. Other characteristics found in these patients with aspirin sensitivity are listed in Table IV. Nasal polyps were noted either at the physical examination or by ENT consultation.

DISCUSSION

Epidemiological surveys should include two types of detailed examinations. The first examination should be the characteristics of the selected or afflicted group and the second examination must include a search for these same characteristics in the population from which the group was selected. In this manner a particular characteristic found in the selected group is evaluated for its uniqueness in the population.

In our population of 3,781 allergic patients, 89 had aspirin intolerance and presented the following characteristics: a predominance of females; a relatively older age group; a positive family history of asthma or allergic rhinitis in 51 per cent; and one or more positive skin tests to pollens, animal danders, or molds in 65 per cent. Most studies showed similar findings in those with aspirin

TABLE IV. Eighty-nine patients with aspirin intolerance

Clinical data	Number	%
Males	35	39
Females	54	61
Positive allergy skin tests	58	65
Nasal polyps	32	36
Positive family history of atopy	45	51
Symptoms of aspirin intolerance*		
Bronchospasm	59	66
Urticaria/angioedema	29	33
Rhinitis	9	10

*Includes patients whose symptoms of aspirin intolerance involve more than one of these categories.

intolerance but their results were not evaluated and compared with the total population from which the aspirin-intolerant group was chosen.

In our total allergic population of 3,781, a predominance of females exists (56 per cent). The 61 per cent of females found in our intolerant group may actually be reflecting this disproportionate sex ratio of the total allergic population. There was no statistically significant sex difference found in any of our categories. Most past studies³⁻⁶ showing a predominance of females in individuals with aspirin intolerance failed to show the sex ratio of the total population from which the intolerant group was selected.

The frequency of aspirin intolerance in a population is also dependent on the characteristics of that population. Factors that may affect this frequency are as follows: The number and type of asthma and allergic rhinitis, the age group of the population, and method of study. Our data indicates that a population consisting largely of patients with allergic rhinitis or of a younger age group will have a lower frequency of aspirin intolerance.

Our frequency of intolerance in our asthmatic population was 4.3 per cent. This frequency is similar to other studies done on asthmatic individuals. Walton and Randle⁴ reported a frequency of intolerance to be 3.2 per cent in 2,580 asthmatic patients. Although they stated that their intolerant group was equally divided between extrinsic and intrinsic asthma, they did not report what percentage of their total asthmatic population had extrinsic or intrinsic asthma. Pearson¹⁰ reported a frequency of aspirin intolerance to be 2.3 per cent of 1,205 asthmatics. Although he demonstrated that this intolerance occurred more frequently in an older age group, as was noted in our study, he did not report how many of his asthmatics had intrinsic or extrinsic asthma. In their survey Gardner and Blanton¹¹ asked 95 allergists, "How many cases of aspirin sensitivity have you encountered and what per cent does this represent of all the allergic patients you have seen?" Their estimate revealed a frequency of 0.2 per cent. McDonald, Mathison, and Stevenson⁹ reported a frequency of 5 per cent in 282 asthmatic patients. However, after challenging certain selected asthmatics, their frequency of aspirin intolerance increased to 8 per cent.

Past reports of a positive family history of atopy in those individuals with aspirin intolerance range from 50 per cent, reported by Samter and Beers,^{7,8}

to 65 per cent by Walton and Randle.⁴ If the total population from which the aspirin-intolerant group was selected is essentially an atopic population, a positive family history of over 50 per cent is to be expected.⁷ Therefore, the increased frequency of a positive family history of allergy may not be a unique property of intolerance to aspirin.

A positive allergy skin test was found in 65 per cent of our aspirin-intolerant group, and this increased frequency probably reflects the general characteristics of the total population⁸ from which our group was chosen. Other investigations^{4, 5, 9, 10} on aspirin-intolerant patients who were selected from an allergic population also have showed high frequencies of positive allergy skin tests. Samter and Beers³ selected 183 patients with aspirin intolerance not from an allergic population but from the medical wards of a hospital. Their data proved to be dramatically different in that only 10 per cent of their 182 patients with intolerance had positive allergy skin tests to seasonal and environmental inhalant allergens. This demonstrates that positive allergy skin tests may not be a characteristic of aspirin intolerance. Our data also supports this finding by showing that significantly more intrinsic than extrinsic asthmatics have an intolerance to aspirin.

Similar to other reports,⁴⁻⁶ the most common manifestations of aspirin intolerance in our patients were bronchospasm (66 per cent), urticaria and angioedema (33 per cent), and rhinitis (10 per cent). Our data also agrees with other studies^{3, 4, 9, 10} in that a significant number of our aspirin-intolerant patients had nasal polyposis (36 per cent). We were not able to judge the severity of asthmatic symptoms in our aspirin-intolerant patients in relation to the severity of asthma in our total population.

The fact that bronchospasm is the predominant symptom of aspirin intolerance in patients with known asthma compared to those aspirin-intolerant patients with known rhinitis alone may demonstrate that aspirin may somehow enhance or interact with a defect already present in selected asthmatic patients. This enhancement of an existing defect may account for the greater frequency of aspirin intolerance in the asthmatic compared to the patient with rhinitis alone. Patients with rhinitis alone do not have an increased frequency of bronchospasm, but when the primary diagnosis of rhinitis and asthma coexists in the same patient, the symptom of bronchospasm as a manifestation of intolerance again predominates.

The frequency of positive skin tests in those patients whose aspirin intolerance is manifested by urticaria/angioedema is greater (88 per cent) than in those whose manifestation is bronchospasm (56 per cent) ($p < 0.01$). This difference together with our other data presented may imply that a reaction of urticaria/angioedema may be related to atopy while one of acute bronchospasm is not.

The frequency of multiple drug allergies in our aspirin-intolerant group (18 per cent) is similar to that reported by Samter and Beers (22 per cent).^{3, 4} Our frequency of intolerance to tartrazine was only 2 per cent of the group that was intolerant to aspirin. However, the association of intolerance to aspirin and

to tartrazine has been reported in the literature,^{12, 13} and if all our aspirin-intolerant patients were challenged with tartrazine, the frequency of associated tartrazine intolerance might be increased.

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ALLERGIC REACTIONS DUE TO F D & C YELLOW No. 5
TARTRAZINE, AN ANILINE DYE USED AS A COLORING
AND IDENTIFYING AGENT IN VARIOUS STEROIDS

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AGENTS of coal tar origin are used to flavor and identify corticosteroids which today are used widely in the treatment of allergic reactions of all types.¹ It has been established that sensitization to drugs derived from coal tar is not infrequent.

This is a report on three patients who developed allergic-type reactions after ingesting: Case 1—dexamethasone (Decadron®) 0.5 mg tablets; Case 2—prednisolone (Paracortol) 5 mg tablets; Case 3—dexamethasone (Deronil) 0.75 mg tablets. Doses administered orally:

Case 1.—A sixty-six-year-old man suffering from severe bronchial asthma of an infectious type was advised to take 0.5 mg tablets of dexamethasone (Decadron). About forty minutes after he used his first prescribed 0.5 mg tablet of dexamethasone (Decadron) he developed severe generalized pruritus, itching of his tongue and uvula followed by generalized urticaria. His reaction progressed until many of his urticarial lesions were confluent.

His reaction was brought under control with the use of sympathomimetic drugs administered parenterally and in addition, Epinephrine sulfate gr $\frac{3}{16}$ administered orally.

When tested, this patient reacted sublingually to 1 cc of a 1:1000 dilution of Tartrazine dissolved in triple distilled water.

His therapeutic response to undyed 0.5 mg tablets of dexamethasone (Decadron), graciously supplied by Merck Sharp and Dohme has been excellent.

Case 2.—A forty-nine-year-old orthopedic surgeon, known to be violently sensitive to drugs of mercurial and coal tar origin, developed a severe type of generalized reaction after using several 5 mg prednisolone (Paracortol) tablets to aid in controlling a generalized pruritus and a macular papular rash from which he was suffering due to exposure to Tincture of Merthiolate. Shortly after using several 5 mg prednisolone (Paracortol) tablets he developed severe generalized urticaria and in addition a localized edema of his lips, tongue and uvula. Exacerbations of all his previous symptoms occurred.

The prednisolone (Paracort) 5 mg tablets do not contain Tartrazine. The patient is able to take prednisolone (Paracort) 5 mg tablets without experiencing any untoward effects.

Case 3.—Dexamethasone (Deronil) 0.75 mg tablets were prescribed for a thirty-eight-year-old white woman collagen disease sufferer, also known to be sensitive to acetylsalicylic acid.

Shortly after this patient took her first tablet of Deronil, she experienced numbness and tingling of the mouth and tongue. She then developed generalized urticaria with vomiting associated with a severe headache. Her urticaria persisted for several days despite treatment with 1:500 Epinephrine in gelatine sol-

ALLERGIC REACTIONS—LADNEY

... injected intramuscularly and in addition, Ephedrine sulfate gr ʒʒ and Nicotinic acid ʒʒ 50 mg orally.

When tested, this patient reacted to a 1 cc dose of a 1:1000 dilution of Tartrazine administered sublingually. The patient developed tingling of the tongue and lips with a sensation of burning. Later she developed mild edema of her upper lip and uvula with itching of her palate.

Tartrazine, also known as F D & C Yellow #5, is an aniline dye. It is a permitted primary certified food color used for coloring food, drugs and cosmetics; also as a dye for wool and silk. This dye, a potential sensitizing agent, is added to the following corticosteroids as a coloring and dosage identifying agent:

1. Dexamethasone (Decadron)[®] 0.75 mg tablets contain Guinea Green F D & C (Green F D & C #1), dexamethasone (Decadron) 0.5 mg tablets contain F D & C Yellow #5 Tartrazine. Dexamethasone (Decadron) 0.75 mg and 0.5 mg tablets are manufactured by Merck Sharp & Dohme, division of Merck and Co., Inc., Rahway, New Jersey.

2. Prednisolone (Paracortol)[®] 5 mg tablets contain F D & C Yellow #5 Tartrazine. Prednisone (Paracort) 5 mg tablets contain no dye. Prednisolone (Paracortol) tablets 5 mg and prednisone (Paracort) 5 mg tablets are manufactured by Parke, Davis and Co., Detroit, Michigan.

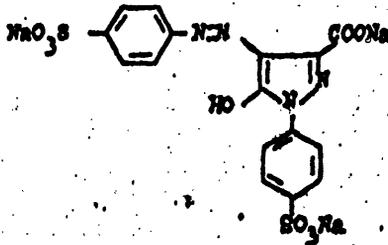
3. Dexamethasone (Deronil)[®] 0.75 mg tablets contain F D & C Yellow #5 Tartrazine and F D & C Yellow #6 Sunset Yellow F C F. Deronil (Dexamethasone) 0.75 mg tablets are manufactured by Schering Corporation, Bloomfield, New Jersey.

Three of the above mentioned corticosteroid hormone preparations contain one dye in common, F D & C Yellow #5 Tartrazine,² a coal tar derivative manufactured by the Aniline Division of the Allied Chemical Corporation.

The Merck Index³:

"Hydrazine yellow; F D & C Yellow #5 (when certified); trisodium salt of 3-carboxy-5-hydroxy-1-sulfophenylazopyrazole.

$C_{16}H_{11}N_3Na_3O_5S$; mol. wt. 534.39. C 35.9%, H 1.70%, Na 12.91%, S 12.00%.



ALLIED CHEMICAL COMPANY

Bright orange-yellow powder. Freely soluble in water. The aqueous solution is not changed by HCl but becomes redish with sodium hydroxide.

Use: It is a permitted coal tar color for coloring foods, drugs and cosmetics; also as a dye for wool and silk; commercially available."

SUMMARY

1. Tartrazine, also known as F D & C Yellow #5, is an aniline dye. It is a permitted primary certified food color used for coloring food, drugs and cosmetics; also as a dye for wool and silk. It is commercially available. The pure dye contains not less than 85.0 per cent.

2. Tartrazine F D & C Yellow #5, an aniline dye, is used to color and identify: dexamethasone (Decadron) 0.5 mg tablets; prednisolone (Paracortol) 5 mg tablets and dexamethasone (Derunil) 0.75 mg tablets.

3. Tartrazine F D & C Yellow #5 is an aniline dye and known sensitizing agent.

4. Three cases of sensitivity to Tartrazine are reported.

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BASIC RESEARCH

Basic research is the qualitative and quantitative investigation of the laws and phenomena of nature; it requires the highest degree of individualism, knowledge and idea productivity, and it provides the spark that sets the complicated machinery of applied research in motion. However, as Vannevar Bush has stated, "The scientist doing basic research may not be at all interested in the practical application of his work."—PAUL DE HAEN, A report to the President, 1945—quoted in "Today and Tomorrow," *Med. Science*, 5:483-504 (April 10) 1959.

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Asthma caused by FD&C approved dyes

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A case of severe intractable asthma due to sensitivity to certain of the FD&C approved dyes, notably Tartrazine (yellow No. 5), is reported. These dyes are commonly used to color drug tablets and capsules, as well as many foods. Required listing of these dyes on drug and food packages might be life-saving.

Artificial food colors, derived from coal tar and approved by the Food and Drug Administration, have long been suspected to be a cause of urticaria in children who have eaten colored candies. In 1958 Speer¹ reported that artificial colors were the cause of asthma in six children, but gave no further details. A year later Lockey² found that Tartrazine (yellow No. 5), the coloring agent in Decedron, Paracortol, and Deronil tablets, was the cause of urticaria in three adults.

We present in this paper an adult whose severe asthma was caused by certain of these artificial colors.

Case history

Mrs. D. T., age forty-two, was initially seen in 1958. At that time the family history and her past history of allergy were negative. In 1956 she noted the gradual development of chronic nasal blockage and the loss of her sense of taste and smell. She began to wheeze in early 1958, and a diagnosis of infectious asthma was made. Audible wheezing in the chest and evidence of nasal polyposis and sinusitis were the only positive findings of the physical examination. Submucous resection, ethmoidectomy, antrostomy, and excision of nasal polyps were performed, and treatment with a vaccine made from bacterial cultures at the time of operation was instituted. The asthma gradually disappeared under this program.

In January, 1960, her asthma suddenly returned and by the end of the month was only controlled by prednisone. She has had to receive steroid therapy since that time except for

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brief periods. The maintenance dose of prednisone has been 5 to 7.5 mg., or its equivalent, daily.

She had to be hospitalized three times during 1962 to control severe attacks. In 1963 she complained of menopausal symptoms which were relieved by a daily dose of Premarin, 1.25 mg. She was hospitalized three times for severe asthma, and at home her symptoms were never truly controlled. It was during this same year that she first was able to describe a tight unproductive cough and a very severe wheezing which was quite distinct from her "usual" asthma.

1964 brought no improvement. In July she took two aspirin tablets for headache, a drug which she often took for this complaint, and within a few moments developed a severe attack of angioedema. She was unable to accept a diagnosis of aspirin sensitivity and, on her own volition, took this drug on two separate occasions. Each time a severe attack of angioedema promptly resulted. She has had no further intake of aspirin, and no further attacks of angioedema. Acetaminophen (Tylenol) was substituted, and one tablet immediately produced severe asthma and the tight, unproductive cough described above. She discontinued all salicylates and antipyretics, but the tight cough and severe wheezing continued. The patient and her physician gradually became aware that this syndrome seemed to be enhanced whenever she took drugs for the relief of asthma, such as, Inuprel-Francl, Elixophyllin, Quilron capsules, or Teofat-25. Antibiotics, such as, Achromycin and Mysterlin-F, seemed to control her infections, but they also definitely enhanced the severe asthma and cough. There was no beneficial response to treatment, and her asthma became increasingly more severe.

In late November, 1964, she was referred to the Pratt Diagnostic Hospital where all medications were stopped. She was discharged a week later, essentially symptom free. Within two weeks the severe asthma returned, and she had to be hospitalized again. The wheezing never truly disappeared during this hospital stay, in spite of bronchoscopy. She was discharged after 3 weeks, only to be readmitted 6 days later, severely ill. She was treated with amloxyllin, hydrocortisone, and fluids intravenously. No oral medications were given. By the sixth hospital day her chest was completely clear, and the tight cough had gone. Hot flashes were her only complaint, so Premarin, 1.25 mg., which had been omitted at the time of admission, was reordered. The tight cough and severe wheeze returned within 3 hours, and continued for 24 hours. Ethisteral was substituted, the menopausal symptoms were relieved, and there was no return of wheezing. She has had only one attack of severe asthma since her discharge from the hospital in mid-January, 1965, and that attack was on a warm day in May. She had been sitting outdoors near her husband who was applying a lawn fertilizer which contained a fungicide. The severe asthma and tight cough appeared within 2 hours, and, "like the opening of a door," disappeared suddenly 45 hours later. The label showed that the product contained "dimethyl ester of tetrachloroterephthalic acid."

The essential clue that this patient was reacting to something in her medications was revealed by the attack after taking Premarin, and a review of her chart at the preceding hospitalization showed that she had been receiving this drug regularly. When it was learned that Tartrazine (yellow No. 5) was one of its ingredients, attention was directed to coloring agents as a possible cause of her asthma.

She continued to do well after discharge. There was some return of the cough after a few days, which was relieved when she stopped the routine use of a vitamin tablet, coated and colored with Tartrazine. From time to time there would be a similar flaring of symptoms, which would stimulate a search for a dye-containing product. Its removal always brought further improvement. The patient wanted to take a vitamin, so Poly-Vi-Sol liquid, a color-free product, was prescribed. Another flare of wheezing promptly recurred, and it was found that the product contained sodium benzoate as a preservative. Other foods containing this chemical were then seen to precipitate attacks, so a search was now directed toward chemical additives. By careful reading of labels, by correspondence with food and drug manufacturers, and by trial and error, a list of food and drugs was finally obtained which was free of FDA²¹ approved coal-tar dyes, sodium benzoate, and drugs derived from coal tar, such as aspirin and acetaminophen. A lipstick was obtained which contained only vegetable dyes. These measures have resulted in complete relief from the syndrome.

At this writing (October, 1956), the patient still has a little morning wheeze which is relieved by a whiff of Isuprel, and she occasionally has some symptoms of sinusitis. The picture is consistent with a diagnosis of infectious asthma. She takes Aristocort, 1 mg. daily, along with a capsule of Promarin, 1.25 mg. This has been especially prepared by the manufacturer and is free of color. She has had no severe attack since May, 1955, and is leading a normal full-life.

LABORATORY STUDIES

Extensive laboratory examinations, including bone marrow and electrophoretic studies, were performed during the patient's numerous hospitalizations. The findings were entirely within normal limits, except for a blood eosinophilia of 30 per cent which was found on admission at her last hospitalization. A repeat count the next day was within normal limits. Cultures of the sputum, nose, and throat, repeatedly revealed nonpathogenic flora. Scratch and intracutaneous skin tests to inhalants and foods were also repeatedly negative.

Ouchterlony plate analysis⁴ confirmed the presence of immunoglobulins A, G, and M. Large precipitin bands were observed between the center well, which contained the patient's serum, and the peripheral wells, which contained the separate goat antihuman immunoglobulins, A, G, and M.⁶

The patient's serum was also analyzed by Ouchterlony plate technique for precipitin antibodies against the dyes. The center well was filled with serum, and each of the peripheral wells contained a 2 per cent solution of one of the FD&C approved dyes. No precipitin bands were seen during a 7 day period of observation.

A double-blind procedure was used to challenge the patient with these dyes. Uniform capsules, colored with vegetable dye, were each filled by a pharmacist with a 2 per cent solution of one of the FD&C approved dyes. Three capsules containing a dye were sealed in each envelope, which the pharmacist then coded. These envelopes were handed to the physician, who, having no knowledge of their content, passed them on to the patient with instructions that she take the contents of one envelope before breakfast every third morning. A record of the reactions was kept, and the code was not broken until after all capsules had been taken. The result appears in Table I.

Patch tests with a 2 per cent solution of each of the dyes were applied to the skin of the patient's upper arm. No local dermatitis nor increase in wheezing was noted within the next 72 hours.

The patient was tested by the skin-window technique.⁵ After cleansing, sites on the volar surface of the forearm were prepared by scraping away the superficial layers of the skin with a sterile scalpel until a sheen of tissue fluid was obtained. A drop of 2 per cent solution of each dye was placed on a separate site which was then covered with a sterile glass cover slip, backed by a cardboard, and bound down with adhesive tape. A drop of normal saline for a control was placed on an additional site and similarly covered. Twenty-four hours later the cover slips were removed, coded, and then handed to a hematology technician. His report is given in Table II.

⁴Hyalab Laboratories, Los Angeles, Calif.

Table I. Result of oral ingestion of dyes

Envelope	Capulet	Symptoms
A	Red No. 3	No symptoms
B	Red No. 3	No symptoms
C	Yellow No. 5	Tickling of throat; tight cough and wheezing for 24 hours
D	Blue No. 1	No symptoms
E	Placebo	No symptoms
F	Red No. 4	Little heaviness of chest
G	Yellow No. 6	No symptoms
H	Placebo	No symptoms

Table II. Eosinophilotactic response to FD&C dyes

Slide	Dye	Total cells counted	% eosinophils
A	Blue No. 1	100	9
B	Red No. 2	100	2
C	Yellow No. 5	100	5
D	Yellow No. 6	100	6
E	Saline	4	0

Table III. Eosinophilotactic response to FD&C dyes by passive transfer

Slide	Dye	Primed site		Unprimed site	
		Total cells counted	% eosinophilia	Total cells counted	% eosinophilia
P	Blue No. 1	100	8	100	3
Q	Red No. 2	100	1	0	
R	Yellow No. 5	100	44	0	
S	Yellow No. 6	100	25	100	5
T	Saline	100	15		6

Passive-transfer testing on a nonallergic male by the same technique was then done. Sites on the volar aspect of the forearm were each primed with 0.10 ml. of the patient's serum 24 hours in advance of the skin scraping. An equal number of sites were also scraped on an area of skin which had not been primed. A 2 per cent solution of each dye was then placed on a primed and also an unprimed site, covered with a sterile glass cover slip, and bound in the usual manner. They were removed 24 hours later, coded, and handed to the hematology technician. Table III contains findings.

Skin-window tests with aspirin, acetaminophen, and sodium benzoate were not done, for it was felt that sensitivity to these substances had been adequately proven clinically.

DISCUSSION

Until mid-1962 the patient's asthmatic symptoms behaved according to the usual conception implied in the diagnosis of infectious asthma. She had chronic wheezing which was not related to environment or season, and which was defi-

nity made worse by respiratory infection. Patients with this disease often develop an aspirin sensitivity, and, when this happened to our patient, we at first considered it to be another manifestation of the disease.

After June, 1962, a new syndrome appeared which was superimposed upon her usual asthma. These symptoms consisted of a markedly tight cough, which was often sudden in its onset, and which was always associated with an unproductive cough and with a severe asthma. She frequently commented that "this asthma is different"—a remark with which her physician concurred, but did not know how to interpret. The dramatic reappearance of this syndrome following Premarin at her last hospitalization provided the means for its understanding.

Some drug manufacturers employ vegetable dyes to color their products. Most companies use one or more of the FD&C dyes which are derived from coal tar or petroleum, for they are quite stable and will not be changed by a long shelf life. The actual dye used in any individual product is a trade secret and will not readily be revealed. We have had cooperation from the medical departments of the companies concerned, when we have asked for the dyes in a specific product and have given the reasons for our request. Even so, it has been a time-consuming procedure. It is difficult for the average physician, and nigh impossible for the patient, to obtain this information. It would be far easier for the physician, let alone the patient sensitive to these chemicals, if the Food and Drug Administration were to require listing of the dyes on the package.

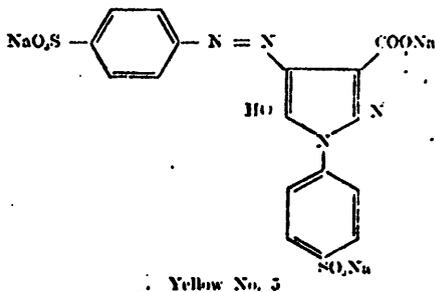
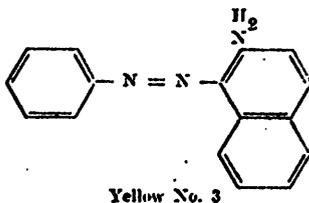
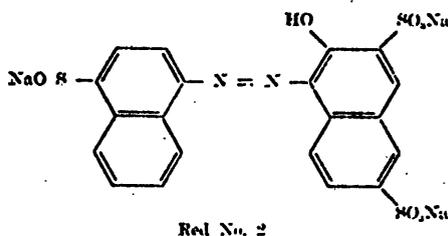
The same comment is applicable to food. These dyes, such as Tartrazine (yellow No. 5), are at times added without being listed on the label and some food manufacturers are loathe to admit that chemicals, other than those required to be listed, are added to their products. Other companies, in contrast, have freely answered our requests for information.

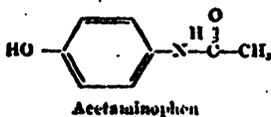
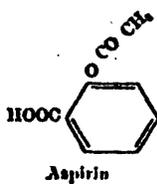
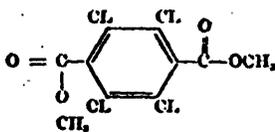
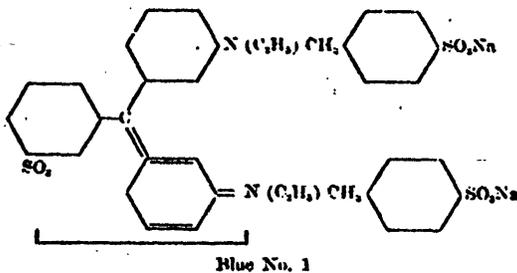
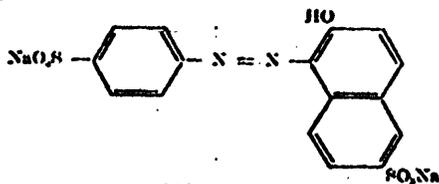
Another possible contact with these dyes occurs in the hospital operating room where solutions—notably of cocaine—are often colored for identification by the hospital pharmacy. Irrigating solutions employed by the ear, nose, and throat surgeon must also be screened. Neither of these is labeled as to dye content.

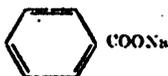
There are 11 coal-tar dyes which are certified by the Food and Drug Administration,⁴ and 5 of these (red No. 2, red No. 4, yellow No. 5, yellow No. 6, and blue No. 1) are most commonly used. Blue No. 1 was the only dye in one drug which caused symptoms in our patient, and yellow No. 6 was the sole one in another. Both of these dyes gave no response in the double-blind oral test, which might imply that these dyes were poor stimulators of antibody and must be given over a period of time to bring results. Positive results were obtained to each of them in the skin-window procedure on the patient's skin, so antibodies were present, confirming our impression of clinical sensitivity. Hidinger and his co-workers⁵ noted that a less-marked eosinophilic response was usually obtained in the recipient's skin on passive transfer, and Fowler and Lowell⁶ confirmed this finding in their need to prime the sites with larger amounts of serum than usual. This finding might explain the lack of response to blue No. 1 in the skin of our nonallergic recipient. The positive response to yellow No. 6, though, does show that some anti-

bodies were transmitted in quantity and that it is not always necessary to prime the passive-transfer site more heavily. In the clinical investigation we believed that yellow No. 5 was the most severe offender, and this impression was amply confirmed not only by the positive double-blind oral test, but also by the unequivocal results obtained in the skin-window procedures.

It is, therefore, interesting to speculate upon the possible relationship between her sensitivity and the chemical structure of yellow No. 5, yellow No. 6, aspirin, acetaminophen, sodium benzoate, and the compound of terephthalic acid.¹⁶







Sodium benzoate

All of these compounds are aromatic, and some include other heterocyclic rings, such as are found in Tartrazine. They all contain acidic carboxyl or sulfonic acid groups. Our patient apparently had no clinical reaction to yellow No. 3, and, although this compound is aromatic, it is free of acidic groups. The sensitivity to aspirin, sodium benzoate, and acetaminophen may, therefore, be ascribed to the presence of a carboxyl group on an aromatic ring. Her sensitivity to dimethyl tetrachloroterephthalic acid inhalation is explained on the basis of a ready hydrolysis of the methyl ester groups in vivo, thereby giving rise to free carboxyl groups—again on an aromatic ring. It has to be assumed that these reactive acidic groups are somehow involved in a haptene formation,¹¹ and it is not clear why other non acidic reactive groups, such as amino groups, play no part. At any rate, those dyes which contain only sulfonic acid groups appear to be less effective in producing a sensitivity reaction in our case, and no clinical reaction has occurred when our patient has ingested those chemicals which have basic, rather than acidic, groups.

Finally, we should like to give credit for the solution of this case to the patient herself. It can honestly be said that, if it were not for her objectivity and intelligence in observing, interpreting, and reporting of her symptoms, she probably would not be alive today.

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Urticaria and asthma induced by food-and-drug additives in patients with aspirin hypersensitivity

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Seven of 8 recently investigated aspirin-sensitive patients reacted with asthma, urticaria, or both after 1 to 3 mg. of the azobenzene dye, tartrazine. Tartrazine is commonly used as a food and drug color and a daily intake of several milligrams is possible. The patients also reacted to some benzoic acid derivatives. All of these food-and-drug additives may be difficult to avoid. It is important, therefore, that they are properly identified since they are dangerous for certain patients with asthma and urticaria. Administration of these drugs to patients with a history of reactivity is a procedure of considerable danger that should be done only with extreme caution and informed consent.

It is well known that many patients with bronchial asthma and chronic urticaria show a pronounced hypersensitivity to aspirin, sometimes leading to fatal reactions. There is evidence that the effect of aspirin becomes apparent only when several factors are present at the same time.^{1,2} Various hypotheses have been presented for the mechanism of the reactions to aspirin. In some patients the clinical symptoms are of an anaphylactic type and suggest an allergic pathogenesis. Acetylated proteins³ or aspirin anhydride impurities⁴ have been proposed as possible antigens. Yurchak and associates¹ have found no conclusive evidence of specific immunological reactions. The fact that not only aspirin but also other chemically different compounds, such as indomethacin, antipyrine, and tartrazine can precipitate attacks might suggest a non-allergic mechanism. Alternative explanations along this line include the theory of an alteration of kinin receptors in the lungs and the capillaries as proposed by Samter and Beers,^{5,6} Direct activation of the complement system or lack of an inhibitor to an enzymatic reaction are 2 other possibilities discussed by Yurchak and associates.¹

We have studied patients with aspirin hypersensitivity for some time, but only recently have we become aware of the importance of the associated sensitivity to the yellow azobenzene dye, tartrazine. This dye is commonly used, and it is an approved food-and-drug additive. Asthma and urticaria caused by tartrazine

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was first described by Lockey.⁷ Since then 18 such patients and one case of tartrazine-induced purpura have been described.^{8, 9, 11} Santer and Beers⁸ found a reaction to tartrazine in 3 out of 40 aspirin-sensitive patients. During the last few months we have been able to demonstrate a pronounced tartrazine hypersensitivity in 7 out of 8 of our aspirin-sensitive patients. We have also found an associated reaction to benzoates in some patients. This, and the surprisingly high number of foods and drugs which contain tartrazine as well as other additives, have prompted the following preliminary report. The administration of these drugs to patients with a history of reactivity is a potentially dangerous procedure that is not recommended for general use.

MATERIAL AND METHODS

Compounds used

The dye tartrazine, (FDC Yellow No. 5), was dissolved in water and given orally with 10 to 20 mL of water. The initial test dose was 1 mg. except in one case in which 0.75 mg. was given.

The following drugs were administered in colorless gelatin capsules with lactose to complete to filling of the capsule. No other additives besides lactose were used: (1) acetylsalicylic acid* (aspirin), capsules with 1, 2.5, 5, 10, 50, and 500 mg. were available; (2) purified acetylsalicylic acid† (free from the anhydrides) in amounts of 1, 2.5, 5, 10, 50, 100, 250, and 500 mg. per capsule; (3) acetylsalicylic acid anhydride‡ in capsules containing doses of 5, 10, 25, and 100 mg.; (4) 2-hydroxybenzyl alcohol (Saligenin),‡ 50 mg. per capsule; (5) 4-hydroxybenzoic acid; 50 mg. per capsule; (6) benzoic acid sodium salt; 250 mg. per capsule; (7) Lactose in 100 mg. dose was used as a placebo.

Testing procedure

The provocation tests were done when the patients were as free as possible from their symptoms. As a rule, other drugs were not used during the 3 days preceding the provocation test which was made in the morning after a light breakfast. The patients, all of whom were known from previous tests or history to react to aspirin, were informed that various products which might be present in food and drugs were to be tested. The amount or type of drug was unknown to them. We usually started with benzoate and its derivatives. The minimal dose of purified acetylsalicylic acid which gave symptoms was determined in the last provocation tests. The day of the placebo varied. The initial dose of acetylsalicylic acid was usually 50 mg. in patients with urticaria as the dominating symptom and 1 mg. in those with mainly or only asthma. If no response or only an uncertain one occurred within one hour, the dose was increased. Sodium benzoate was given in an initial dose of 250 mg. After 2 hours another 250 mg. was given if no reaction occurred. A single dose of 50 mg. of hydroxybenzoic acid and hydroxybenzyl alcohol was used except in 2 patients who were given another 50 mg. of hydroxybenzoic acid. Only one type of drug was tested per day and a new test with another drug was not made until the previous reaction had disappeared.

CASE REPORTS

Case 1

A 24-year-old man had urticaria which began after he had taken 0.5 Gm. of aspirin for a hangover. He discontinued the use of aspirin and used an antihistamine, clemastine, 3 times daily for one month without any effect. He was then admitted to the hospital where a medical

*Bofors AB, Molndal, Sweden.

†Kindly supplied by Prof. H. L. Vogel, Chemische Fabrik, Auling, Munich, Germany.

‡Sigma Chemical Company, St. Louis, Mo., U.S.A.

TABLE I. Reactions in 7 aspirin-sensitive patients to tartrazine, salicylates, and benzoates: Testing hour

Substance	Patient 1	Patient 2	Patient 3
Aspirin	500 mg., moderate angioedema and urticaria	100 mg., severe angioedema and urticaria	Not done
Acetylsalicylic acid with-out its anhydride	Not done	50 mg., severe angioedema and urticaria	35 mg., severe asthma and urticaria
Acetylsalicylic acid anhydride	125 mg., no reaction	Not done	15 mg., rhinitis and asthma
2-Hydroxybenzyl alcohol	50 mg., moderate urticaria	50 mg., moderate angioedema and urticaria	50 mg., severe asthma
4-Hydroxybenzoic acid	50 mg., slight urticaria	50 mg., moderate angioedema and urticaria	100 mg., slight asthma
Sodium benzoate	500 mg., no reaction	500 mg., no reaction	500 mg., slight asthma
Tartrazine	1 mg., severe urticaria	2 mg., facial erythema and urticaria	1.5 mg., severe asthma lasting four days
Placebo	No reaction	No reaction	No reaction

*Sodium salicylate 500 mg. (free from anhydride) provoked rhinitis, asthma, and urticaria. †A repeated test with 10 mg. showed more pronounced symptoms and general malaise.

examination revealed nothing abnormal except urticaria. His urticaria ceased 3 days after he stopped taking the antihistamine.

In the provocation tests (Table I) tartrazine produced a much more intense urticaria within one hour than any of the other chemicals. It was also revealed that the antihistamine with which he had been treated contained 30 μ g of tartrazine per tablet.

The patient was told to avoid drugs and, as far as possible, food additives. Since then he has not had any symptoms of urticaria.

Case 2

A 54-year-old woman has had a disabling urticaria and angioneurotic edema since 1969 and bronchial asthma since 1970. In 1970 she developed a severe attack of urticaria and angioedema after taking aspirin. She has been hospitalized for several months mainly for her urticaria and has been unable to work for long periods. In the summer of 1970 she had a period with only minor symptoms of urticaria and asthma. In the following autumn she started to take a new β -receptor-stimulating drug for her asthma. It had no effect on her asthmatic symptoms and after 1 to 2 weeks of treatment she had a relapse of her urticaria. A connection with the drug was suspected and its administration was discontinued. A number of drugs were then tried for treatment of her urticaria without any success. Finally she obtained some relief from an antihistamine which we now know is free from additives.

Provocation tests with aspirin, hydroxybenzyl alcohol, hydroxybenzoic acid, and tartrazine induced urticaria and facial erythema within one hour (Table I). She also complained of a feeling of tightness in her chest for one hour but had no rhonchi. The reaction to tartrazine might explain much of her persistent urticaria since several of the drugs given earlier to this patient contained up to 2 mg. of tartrazine per tablet. The relapse in the autumn of 1970 could certainly be caused by the new asthma drug as the ordinary daily dose of that drug contained 1.2 mg. of tartrazine. During the last few months the patient has been kept on a diet in which we have tried to avoid all food, drinks, and drugs containing dyes, salicylates, and benzoates. She has had only minor asthmatic symptoms and is practically free from urticaria.

was begun with a small dose and increased 2 to 10 times if no reaction developed within one

Patient 4	Patient 5	Patient 6	Patient 7
Not done	500 mg., angio- edema and urticaria	500 mg., angio- edema	Not done*
4.5 mg., severe asthma and rhinitis	Not done	Not done	Not done*
10 mg., no reaction	Not done	25 mg., no reaction	Not done
50 mg., no reaction	50 mg., itching and urticaria	50 mg., itching and urticaria	Not done
50 mg., moderate asthma, rhinitis and itching	50 mg., angio- edema and urticaria	100 mg., itching, urticaria and malaise	50 mg., asthma and severe urticaria
500 mg., no reaction	500 mg., pharyngeal edema and urticaria	300 mg., no reaction	250 mg., asthma and urticaria
1 mg., slight asthma; 5 mg., severe asthma	1 mg., facial flushing, itching and urticaria†	1.5 mg., swelling of pharynx, urticaria and malaise†	1 mg., rhinitis, dyspnea; 5 mg., also severe urticaria
No reaction	No reaction	No reaction	No reaction

Case 3

A 17-year-old girl had a history of nasal polyps and bronchial asthma which began at the age of 6. Her asthma has been of chronic character with many exacerbations which have required hospitalization. She has also suffered from itching, increased dermatographism, and urticaria. She had had a pronounced hypersensitivity to aspirin for 3 years and had also noticed that apple juice, broccoli, dill pickles and bananas may possibly increase her symptoms. Allergologic investigation, as well as a radical allergosorbent test (RAST), have not revealed any atopic allergy.

Provocation tests caused hypersensitivity reactions of rhinitis and asthma within 10 to 60 minutes to all drugs except the placebo. In addition, urticaria and a further increase of her dermatographism occurred at the same time following the administration of purified acetylsalicylic acid (Table I). The reactions were most pronounced after the administration of purified acetylsalicylic acid, 2-hydroxybenzyl alcohol, and tartrazine. Administration of epinephrine was needed to stop the attacks.

Case 4

A 20-year-old woman had nasal polyps and "bronchitis" which began at the age of 8. The first severe asthma attack occurred at the age of 12 when she took an aspirin tablet for the first time. Since then she has always had nasal swelling and moderate to severe asthma of a non atopic character. Frequent respiratory infections often increased her asthmatic symptoms. The patient had negative skin tests to common allergens.

In November, 1971, she came to the hospital as an emergency case with an acute asthma attack precipitated by an ampicillin tablet. A RAST test to penicilloyl and an intracutaneous test to benzyl-penicillin were negative as well as an oral provocation test with 0.6 Gm. of tartrazine-free ampicillin. Since the ampicillin tablet contained 0.2 mg. of tartrazine and provocation tests with tartrazine produced asthma (Table I), it seems probable that her attack was caused by tartrazine. The patient also remembers having an attack of asthma after taking a yellow C-vitamin drink (which contains tartrazine) and eating mayonnaise. She also used a contraceptive pill which contained 0.1 mg. of tartrazine.

Tests with purified acetyl-salicylic acid and tartrazine produced severe asthma. Within a half hour after 4-hydroxybenzoic acid she developed moderate asthma, rhinitis, and itching. Epinephrine was given to interrupt the reaction.

Case 5

A 47-year-old woman had migraine since childhood and recurrent urticaria for 40 years. She has detected that she vomits and has severe urticaria after taking aspirin. Beets, canned peas, and coffee also produce symptoms. Provocation tests were positive to benzoates and tartrazine. Flushing and itching were evident after approximately 30 minutes, whereas the urticaria was most evident after 6 to 12 hours. Since she has maintained a diet which is free from benzoates, she has scarcely any symptoms of urticaria.

Case 6

A 57-year-old woman with periods of asthma since the age of 17 had recurrent urticaria for the past 7 years. For many years she has suffered constantly from swollen nasal mucosa. She has a feeling of heaviness, tightness of the head, and general malaise before the onset of urticaria and asthma. She suspected an intolerance to lingonberry jam, "soft drinks," and some yellowish-colored buns. She rarely used aspirin and was not aware of any hypersensitivity to that drug.

At the time of testing she had been free from asthma for about one month and from urticaria for one week. One hour after the provocation test with aspirin anglo-eburn, increased nasal swelling, headache, and dyspnea developed, but no real asthma was noticeable. The patient complained of headache, malaise, sore and swollen throat, and increased dyspnea about one hour after provocation tests with benzoates and tartrazine. After 2 to 3 hours she had developed pronounced urticaria. Repeated tests were performed with tartrazine and they always induced the same reaction.

Case 7

A 40-year-old woman had pulmonary tuberculosis from 1945 to 1954 and was treated with para-amino salicylic acid. Perhaps it is coincidental, but from approximately that time she has also suffered from nasal congestion and complained about an asthma-like bronchitis. There has been a pronounced increase of her asthma during the last 5 years and she also developed symptoms of severe urticaria. She has not been aware of any precipitating factors but has consumed fresh and preserved fruits and berries daily since 1966 at which time she had moved to a house with a large fruit garden.

The provocation tests with 500 mg. of sodium salicylate induced nasal congestion, headache, sweating, rhinitis, and asthma within one hour and urticaria within 2 to 3 hours. The urticaria was most pronounced after 6 to 12 hours. She also reacted in the same way with asthma and urticaria after the administration of sodium benzoate and 4-hydroxybenzoic acid. These tests were repeated one month later with the same results. A 1 mg. dose of tartrazine induced rhinitis and dyspnea. When an additional dose of 5 mg. was given, the patient also developed urticaria. The patient has improved after being kept on a diet in which dyes and preservatives are avoided.

DISCUSSION

Santer and Beers² followed 182 aspirin-sensitive patients with asthma, and sometimes also urticaria, and found associated sensitivities to foods and drugs in 42. Fourteen patients (< 8 per cent) were sensitive to tartrazine. However, it is not obvious from that study if provocation tests had been made in all patients and, if so, whether the doses required were sufficient to induce symptoms. Santer and Beers described in another study³ 40 aspirin-sensitive patients of whom 3 reacted to 25 mg. of tartrazine. Thus, one has the impression that the frequency

of tartrazine reactions is fairly low among patients sensitive to aspirin. Our finding that 7 out of 8 of the aspirin-sensitive patients in our latest study are highly sensitive to even 1 mg. of tartrazine has made us re-evaluate the importance of this approved food additive. Why they also react to tartrazine is unknown. Tartrazine taken orally undergoes an azo-reductive cleavage in the gut. In man it is completely excreted as sulfanilic acid within 48 hours.¹²

The level of sensitivity to aspirin and tartrazine may vary from time to time. If Santer and Beers^{5, 6} tested the patients when they were in a phase of decreased sensitivity, it could possibly explain the lower incidence of reactions to tartrazine. We therefore think that, although repeated provocation tests involve a considerable risk for severe reactions, they should be undertaken because patients may obtain considerable relief when they are put on a suitable diet and drug regimen.

Cross-sensitivity to benzoates seems to be uncommon.¹³ The only case we have found described is that of Chafce and Settignano⁹ in which benzoates are shown to probably cause a reaction. The doses used in the provocation tests may seem to be high but this amount can easily be obtained merely by unknowingly consuming certain types of preserved foods or beverages which contain up to 1 to 2 Gm. of benzoates per 1,000 Gm. A hypersensitivity reaction to 4-hydroxybenzoic acid was seen in all of our patients, whereas only 3 patients reacted to sodium benzoate when given it in a 5 to 10 times larger dose. Why the hydroxy compounds tested are more reactive needs further investigation.

The presence of the immunogenic substance, acetylsalicylic acid anhydride, in commercially available aspirin has been shown by de Week.³ He proposed that the anhydride might be responsible for some of the untoward reactions to aspirin. In one of our patients it produced rhinitis and asthma within 30 minutes. However, this might be due to the formation of acetylsalicylic acid to which the patient is highly sensitive, reacting severely to only 3.5 mg. Acetylation of proteins has been regarded as another possible mechanism.³ It does not, however, seem to be involved in our patients who reacted to several nonacetylated substances.

When looking at the clinical symptoms of our patients we find that 4 of the 6 patients with urticaria also have asthma. Two of them reacted in the provocation tests with both asthma and urticaria, whereas 2 showed angio-edema and urticaria but no real asthma, although one complained of tightness of the chest. The reason might be that in the period before the testing, urticaria had been the prevailing symptom. One of our patients (Case 4) who had no history of urticaria reacted with asthma but also complained of itching with hydroxybenzoic acid. It therefore seems obvious that the dominating symptom is reproduced in the provocation tests. The doses of acetylsalicylic acid needed to produce a reaction in the 2 patients with mainly asthma and nasal polyps (Cases 3 and 4) were much lower than in the others. It is uncertain whether or not they represent a different type of aspirin hypersensitivity with a mechanism for reactions separate from those present in the other patients.

We have also been alarmed to find how often considerable amounts of tartrazine, as well as several other additives such as benzoates, are used without any information being available to the prescriber and consumer. Feingold¹⁴ discusses

the use of over 2,700 food additives which have been compiled and classified by the National Research Council, and Lockey¹¹ has recently exemplified the great number of various additives present in some common drugs. The problems associated with food and drug dyes in an asthma patient sensitive to aspirin, sodium benzoate, and tartrazine have been analyzed by Chafee and Settignano.⁷ They also discussed cross-reactions between chemically related red, yellow, and blue colors. Detailed declarations of additives in foods and drugs are therefore highly desirable.

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Clinical and Laboratory Investigations

Urticaria induced by preservatives and dye additives in food and drugs

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SUMMARY

Fifty-two patients with recurrent urticaria or angio-oedema and thirty-three controls have been provoked with five different food dyes and the preservatives sodium benzoate and 4-hydroxy-benzoic acid, as well as aspirin, sulphanilic acid and a placebo. The reaction was judged as positive in thirty-nine patients who developed urticaria within 14 h. Of these, thirty-five reacted to aspirin, twenty-seven to benzoic acid compounds and twenty-seven to azo dyes. The four patients who did not have urticaria after aspirin, reacted with urticaria to benzoic acid compounds, and three of them to azo dyes. No definite pattern for the reaction to the different azo dyes was seen. None had an urticarial reaction from sulphanilic acid, Patent Blue (a non-azo dye) or placebo. The doses of additives used in the provocation tests are easily exceeded in daily life by the consumption of foods and drugs. Recurrences of urticaria could be prevented through the avoidance of food and drugs containing azo dyes and preservatives.

Aspirin-sensitive patients sometimes react adversely to other analgesics, like the indol and pyrazolon derivatives, and to azo dyes such as tartrazine. Reactions to tartrazine were first described by Lockey (1959). Since then about twenty-five such cases have been described (Juhlin, Michaëlsson & Zetterström, 1972). Samter & Beers (1968) found that about 8% of their aspirin-sensitive patients also reacted to tartrazine. In a study of reactions in aspirin-sensitive patients, we found that seven out of ten patients with recurrent urticaria and/or asthma reacted to tartrazine. Furthermore, seven of them also reacted to benzoic acid and some of its derivatives (Juhlin *et al.*, 1972). The high frequency of reactions to these common food and drug additives has prompted us to extend with several dyes the provocation tests in patients with recurrent urticaria and angio-oedema. This paper reports the results obtained in a group of fifty-two patients with recurrent urticaria. Examples of food with and without azo dyes, benzoates and salicylates are also included.

Patients

Fifty-two patients with symptoms of recurrent urticaria and angio-oedema were tested. No patients with urticaria factitia, cold or heat urticaria were included in the group. All except five cases were investigated on the wards. The medical histories indicated the presence of an aspirin hypersensitivity in only seventeen patients. Five of the patients also had asthma. Several patients had had urticaria periodically or continually for years. In some of the cases, the symptoms had been so intense that they required long periods of sick leave and hospital care. All of the patients had been treated with

different antihistamines, mostly without any improvement. Sometimes an unexplainable worsening had been noted after certain antihistamine tablets which were coloured.

The sampling of patients is, to some extent, selective. Twelve of the patients had visited the dermatology clinic earlier and had been found to be sensitive to aspirin but had, despite avoidance of this medication, continued to have urticaria. The other patients had visited the clinic for the first time for recurrent urticaria of unknown aetiology during the period December 1971–November 1972.

Controls

Thirty-three healthy persons served as controls. Two of them have a moderate allergic rhinitis and one sometimes has an itching of unknown cause.

TABLE 1. Foods which often contain azo dyes and benzoic acids

(a) Azo dyes

Penny candies, caramels and chews, life savers and fruit drops, filled chocolates, but not pure chocolate.

Soft drinks, fruit drinks and ades.

Jellies, jams, marmalades, stewed fruit sauces, fruit gellatines, fruit yoghurts, ice cream, pie fillings, vanilla, butterscotch and chocolate puddings, caramel custard, whips, dessert sauces, such as vanilla, and cream in powdered form. Bakery goods except plain rolls, crackers, cheese puffs, etc., chips, cake and cookie mixes, waffle/pancake mixes, macaroni and spaghetti (certain brands).

Mayonnaise, salad dressings, catsup (certain brands), mustard, ready made salads with dressings, remoulade, bearnaise and hollandaise sauces, as well as sauces such as curry, fish, onion, tomato and white cream.

Mashed rutabagas, purées, packaged soups and some canned soups.

Canned anchovies, herring, sardines, fish balls, caviar, cleaned shellfish.

Colored toothpastes.

(b) Preservatives containing benzoic acid compounds

Soft drinks, ciders, fruit drinks and ades.

Jellies, jams, marmalades, fruit gellatines, stewed fruit sauces.

Cheese, especially cream cheeses, low-calorie margarines, salad dressings, remoulade, hollandaise, bearnaise and mustard sauces, and readymade salads with dressings.

Refrigerated preserves of herring, sardines, anchovies, shellfish and fish.

Surface-treated fish (can be rinsed away).

Diet and drugs during the testing period

For 3 days before admission to hospital, the patients were requested not to use antihistamines or any other drugs, if possible. The patients with frequent recurrences of urticaria had been on a diet free from dyes and preservatives before being admitted. All patients were on such a diet while in the hospital. Examples of food without additives which we have found that patients with aspirin hypersensitivity usually can tolerate are: bread, cereals, rice, sugar, potatoes, butter, salad oils, eggs, milk, cream, meat, including chicken and turkey, fish, lettuce, parsley and mushrooms. They are recommended to drink only plain water. The types of food products containing additives which should be avoided are shown in Table 1. Small amounts of naturally occurring benzoates and salicylates have been found, especially in blue berries, lingon berries, bananas, green peas and licorice (Juhlin & Michaëlsson, 1973). Many aspirin-sensitive patients do not tolerate rhubarb, grapes, apples, European red wines and beers, although we have not been able to demonstrate the presence of benzoates or salicylates here. Other fruits and vegetables are allowed one at a time after a period on the basic diet. Inquiries concerning the amount of dyes and benzoates in drugs have been sent to all drug companies represented in Sweden. The information received reveals that one tablet can often contain 0.01–3 mg.

of an azo dye. Drugs free from these compounds have been listed, and are the only drugs allowed for these patients (Juhlin & Michaëlsson, 1973). Aspirin, as well as other anti-inflammatory analgesic drugs, are not allowed. If an analgesic drug has to be given, paracetamol might be tried, although cross reactions have been described for this drug as well (Smith, 1971).

Substances used for provocation

The doses, structural formulae, and colour indices (CI) of the substances used in the provocation tests are shown in Table 2. The substances were given in colourless gelatine capsules, with lactose added to complete the filling.

TABLE 2. Substances and doses used for provocation.

Substance	Dosage (mg)	Structural formula
Placebo (Lactose)	100	NaO_3S -  - $\text{N}=\text{N}$ -  - COONa
Tartrazine CI 19140 Colour: yellow	0.1,* 1, 2, 5, 10	HO -  - $\text{N}=\text{N}$ -  - SO_3Na
Sunset Yellow CI 15985 Colour: yellow-red	0.1,* 1, 2, 5, 10	NaO_3S -  - $\text{N}=\text{N}$ -  - OH SO_3Na
New Cocaine CI 16255 Colour: red	0.1,* 1, 2, 5, 10	NaO_3S -  - $\text{N}=\text{N}$ -  - OH NaO_3S - SO_3Na
Patent Blue V CI 42051 Colour: blue	5	$\text{Ca}_2\text{O}_3\text{S}$ -  - SO_3 $\text{C}=\text{N}^+(\text{C}_2\text{H}_5)_2$ $(\text{C}_2\text{H}_5)_2\text{N}$
Amaranth CI 16185 Colour: red	0.1,* 1, 2	NaO_3S -  - $\text{N}=\text{N}$ -  - OH SO_3Na
Sulphanilic acid	50	H_2N -  - SO_3H
Sodium benzoate	50, 250, 500	 - COONa
4-hydroxybenzoic acid	50, 100	HO -  - COOH
Aspirin	0.1,* 1, 5, 10 50, 500, 1000	 - COOH COOC_2H_5

* Initial doses which have been used for those with a history of asthma.

TEST METHOD

Provocation tests were performed when the patients had slight or no symptoms. After a light breakfast, the substance to be tested was given in the lowest dose at 8 a.m. If no objective reaction could be

noted, additional and increased doses were given at 1h intervals. When a positive reaction occurred the provocation was stopped and no further doses were given. Only one substance was given per test. The doses which were usually used are shown in Table 2. The patients with a history of asthma began with the lowest dose. As a rule, we used the placebo first and ended with aspirin. The provocation tests which were questionable and difficult to interpret were usually repeated on another day. The next test was not made before the previous reaction had disappeared. If asthmatic symptoms developed, adrenaline was given so that the reaction was not prolonged. No therapy was given for urticaria.

Both objective and subjective symptoms were carefully noted after each test. The symptoms which developed are seen in Table 3.

TABLE 3. Symptoms of a hypersensitivity reaction

Objective signs	Subjective symptoms
Urticaria	Itchy skin
Angio-oedema of the lips, eyelids or face	Eye-irritation
Reddening of the eyes	Nasal congestion
Sweating	Breathing difficulties
Increased tear secretion	Irritability
Nasal congestion	Upset stomach
Sneezing	Hot flushes
Rhinitis	Sensations of:
Hoarseness	Swollenness
Wheezing	Tiredness
	Drowsiness
	Thirst
	Stinging in the lips and throat
	Pressure across forehead
	Heaviness in the head

Some of the patients who had severe reactions after a provocation did not want to continue with tests as planned; therefore, it has not been possible to carry out tests with all compounds in every patient.

Evaluation of symptoms

The provocation results were judged as positive when the patient developed an unquestionable urticaria or angio-oedema after having had an inactive period before provocation. The reaction, especially angio-oedema, sometimes occurred within the first few hours after provocation, whereas urticaria often did not develop until 6-14 h afterwards.

Other objective symptoms which often appear during the first hours, but which can easily be overlooked, include erythema of the neck and face, nasal congestion, sneezing, hoarseness, and wheezing; these are listed in Table 3. In some cases they occurred without urticaria or angio-oedema, as shown in Table 4 under the heading; 'Other objective symptoms'. Such provocation results have been designated by us as 'probably positive'.

Subjective symptoms which have been difficult to verify are, for example, itching and a sensation being swollen or warm (Table 3). Most patients who developed urticaria also mentioned these symptoms. The number of patients who had no objective changes, but who mentioned one or several subjective symptoms, is shown in Table 4. Their provocation results have been classified by us

'probably negative'. Comparison of the results from a placebo is essential, however, and may be of help in the evaluation.

RESULTS

Thirty-nine of the fifty-two patients tested had positive reactions to one or several substances. The results of the provocations of these thirty-nine patients are summarized in Table 4. All except four of the patients reacted to aspirin with urticaria. The total amount of aspirin which produced urticaria was 1-10 mg in three patients, 20-200 mg in eleven, 400-700 mg in eleven, and 800-1500 mg in ten of them. The four patients without symptoms after aspirin had reactions to one or both benzoic acid compounds and to one or several dyes (Table 5). The histories and repeated provocation with a single dose of 1000 mg of aspirin gave no support for aspirin hypersensitivity in these patients.

Twenty-two of thirty-seven patients reacted with urticaria to sodium benzoate and twenty-one of them to 4-hydroxy-benzoic acid (Table 4). Sixteen developed urticaria to both substances while eleven had urticaria from one or the other. Seven patients had no objective symptoms from any of the benzoates. In all except three, the highest specified dose was given.

TABLE 4. Results of provocation tests. The figures show the number of patients. Of the fifty-two patients, thirty-nine reacted with urticaria to one or several provocations. None of the thirteen patients who did not have any objective signs are included

Substance used	Urticaria	Other objective signs (Table 3)	Only subjective symptoms	No reaction	Total no. tested
Placebo	0	0	4	35	39
Tartrazine	19	2	1	17	39
Sunset Yellow	10	0	6	11	27
New Coccine	9	1	6	9	25
Amaranth	1	1	3	2	7
Patent Blue V	0	0	2	17	19
Sulphanilic acid	0	0	2	6	8
Sodium benzoate	22	2	3	10	37
4-OH-benzoic acid	21	3	4	9	37
Aspirin	35	0	0	4	39

Tartrazine caused urticaria in nineteen patients (Table 4). About two-thirds of those reacting also had urticaria after other azo dyes and benzoic acid compounds (Table 5). Fifteen of the eighteen patients who were negative to tartrazine were tested with one or several other dyes. Seven of them reacted with urticaria to other azo-dye substances. In Table 5 the individual reactions have been listed for twenty-three patients tested with benzoic acid compounds and four dyes. No definite pattern for the reactions to the different azo dyes was observed. The degree of the reactions varied; in some cases the reactions were severe after 1-2 mg of dye, and even more pronounced than after 500 mg of aspirin. In all, twenty-seven patients reacted to some azo dye. In twelve of the patients, urticaria developed after 1-3 mg of tartrazine, while in the others 5-18 mg was needed. The doses of the other

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azo dyes causing urticaria were of the same magnitude. None of the patients reacted to Patent Blue, which is a non-azo dye, or to sulphanic acid, which is a metabolite of some azo dyes.

Placebo tablets gave no objective symptoms in any of the patients. Subjective symptoms were noted in four patients after the placebo. In patients with only subjective symptoms after the test substances, the number and types of symptoms did not in any case differ from those reported after the placebo.

Aspirin hypersensitivity was suspected from the medical history of three of thirteen patients with negative provocation tests. The three patients improved on a diet free from salicylates and dyes. In the other ten patients, we found no reason for the urticaria.

TABLE 5. Comparison of the individual reactions to provocations with placebo, aspirin and five common food and drug additives

Patient	Placebo	Aspirin	Sodium-benzoate	4-OH benzoic acid	Tartrazine	Sunset Yellow	New Cocaine	Patent Blue V
1	o	+	+	+	±	+	+	o
2	o	+	+	+	±	+	+	o
3	o	+	+	+	±	o	o	o
4	o	+	+	+	±	o	o	o
5	o	+	+	+	o	+	+	o
6	o	+	+	+	o	±	+	o
7	o	+	+	+	o	o	o	o
8	o	+	+	o	o	±	o	o
9	o	+	+	o	o	o	o	o
10	o	+	+	o	o	o	o	o
11	o	+	(+)	o	+	o	+	o
12	o	+	o	+	±	+	o	o
13	o	+	o	+	+	+	o	o
14	o	+	o	+	o	o	o	o
15	o	+	o	(+)	o	+	+	-
16	o	+	o	o	+	o	o	o
17	o	+	o	o	(+)	o	o	-
18	o	+	o	o	o	o	+	-
19	o	+	o	o	o	o	o	o
20	o	o	+	+	o	o	+	o
21	o	o	+	+	o	o	+	o
22	o	o	+	(+)	(±)	o	o	o
23	o	o	+	o	+	+	(±)	-
Total +	o	19	14	12	9	9	9	o
Total (+)	o	o	1	2	2	o	1	o
Total o	23	4	8	9	12	14	13	19

o, no reaction; (+), other objective signs; ±, provocation not done; +, positive reaction with urticaria or angio-oedema.

None of the thirty-three subjects used as controls developed urticaria. Two subjects, a mother and her son, with a history of moderate allergic rhinitis showed signs of rhinitis after tartrazine and Sunset Yellow, but not after repeated doses of aspirin. Subjective symptoms, but no objective ones, were noted in three of the controls. They occurred after placebo, sodium benzoate, tartrazine and New Cocaine.

DISCUSSION

Provocations were only done with some of the most common food additives. Several other accepted dyes have not yet been investigated; nevertheless, the number of patients who reacted with urticaria after provocation was quite large. An investigation of asthma patients in the Pulmonary Clinic of the University Hospital gave similar results (Rosenhall & Zetterström, 1973).

Neither the mechanism behind hypersensitivity reactions to aspirin nor the cross reactions in aspirin-sensitive patients to analgesic drugs, such as pyrazolone and its derivatives, are well understood. The results of provocation tests in our patients with urticaria demonstrate that these patients not only react to aspirin but also to benzoates and to commonly used azo dyes. One possible mechanism for cross reaction between pyrazolone and tartrazine might be that the azo-reductive cleavage of tartrazine in the intestines produces, in addition to sulphamic acid, 1-(4-sulphophenyl)-3-carboxy-4-aminopyrazolone (Ryan, Welling & Wright, 1959). This can later be hydrolysed to 4-sulphophenylhydrazine. We have not yet tested any of these compounds. For some of the azo dyes, a formation of aniline compounds can be shown (Walker, 1970). This could be an explanation for the cross reaction with the tested preservatives.

A variability in response to various analgesics in aspirin-sensitive patients has been reported by Smith (1971). We also found that the pattern for reactivity to the substances tested varies markedly from one patient to another. We have no explanation for this. It seems that no definite conclusions can be drawn from a single negative provocation. One might speculate that some tests have been performed too soon after a positive test, when the patient is still in a refractory state, or there might have been symptoms and signs which have been overlooked, or inappropriate test doses might have been used. Another explanation to the sometimes inconsistent pattern is that, despite the diet, the patient might have shown a possible reaction to some unknown antigen or might have been in a state of increased reactivity after a preceding provocation. Against such an assumption is the negative reaction to the placebo, Patent Blue and sulphanic acid.

The doses used for provocation are in the amounts which can easily be consumed during a day, with food or medicine. The choice of provocation dose is based upon our earlier experiences. Thus, 4-hydroxybenzoic acid gave a stronger reaction than sodium benzoate in the same dosage; therefore, the former has been given in lower doses in our tests and the results of the provocations are not completely comparable. In several countries both compounds are approved for use in foods in the same amounts.

Since some of the patients did not have any symptoms until 6 h after provocation, theoretically it might be more correct to have a 6 h interval between the doses; however, for practical reasons, shorter intervals were preferred.

The majority of the patients with a hypersensitivity to dye additives also reacted to aspirin. The reactions after aspirin were often pronounced; therefore, this test should be carried out at the end of the investigation, or with a separate provocation a couple of weeks before the others. Negative aspirin provocation does not, however, exclude a sensitivity to additives. Thus, four of our patients repeatedly reacted only to additives and not to aspirin. Some patients also had a stronger reaction to dyes than to aspirin. The fact that two controls also developed nasal congestion after tartrazine and Sunset Yellow but no symptoms after repeated provocations with aspirin, may indicate that hypersensitivity to additives without a parallel sensitivity to aspirin is more common than believed. The patients who were found to be hypersensitive to aspirin were often not aware of this. Only seventeen of our fifty-two patients had correlated their symptoms with aspirin consumption. One explanation may be that some of them had had urticaria a long time before they had taken any salicylic product and noticed a worsening even without aspirin consumption.

The high frequency of hypersensitivity reactions to food additives can possibly explain why the patient's urticaria often recurs. In many patients with recurring urticaria of a previously unknown cause, we have now, after positive provocation tests, been able to correlate the exacerbations of the urticaria with the intake of food and drugs containing certain additives. A dietary regimen is therefore recommended. Sixteen patients with positive provocations have been kept on a diet without dye additives or preservatives for 6 months, and thirteen of them have been completely free from urticaria, one has obviously improved, and two continued to have urticaria.

Information concerning the occurrence of these common additives in both food and medicine is difficult to obtain. Specifications of dyes and preservatives in medicines are lacking. The care of patients sensitive to additives is impossible, however, without this information. Prescription of unsuitable medicines has undoubtedly contributed to the continuation of the disease in several patients. Therefore, on the basis of information obtained after inquiries, lists of Swedish food and drugs which are free from additives have been published (Juhlin & Michaëlsson, 1973). Types of foods which are common in most countries and often contain azo dyes and preservatives are exemplified in Table 1. Lack of information has, in all probability, contributed to the delay in realizing that these substances had a damaging effect for a relatively large group of patients.

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Aspirin intolerance. III. Subtypes, familial occurrence, and cross-reactivity with tartrazine

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Evidence has been presented supporting the hypothesis that at least 2 different types of mechanisms may be involved in aspirin intolerance, one resulting in bronchospasm and the other producing urticaria/angioedema. Bronchospasm is the predominant symptom of aspirin intolerance in patients who have asthma. In contrast, the predominant symptom of aspirin intolerance in patients who have rhinitis is urticaria/angioedema. In the bronchospastic type of aspirin intolerance, there is a significant correlation with an increased frequency of nasal polyposis, and with a similar agreement of asthma and aspirin intolerance. These correlations were not present in the urticaria/angioedema type. Additional evidence for familial occurrence of aspirin intolerance is presented, and its relationship with subtypes of aspirin intolerance is discussed. In a double-blind, crossover study with normal control subjects matched by age and sex, 15% (6/40) of aspirin-intolerant individuals had significant adverse reactions to tartrazine challenge and not to the placebo. None of the 40 normal control subjects had any adverse reactions.

In our past publications,^{1,2} we presented the hypothesis that in aspirin intolerance two basic mechanisms may be present, one resulting in bronchospasm and the other producing urticaria/angioedema. We demonstrated that the predominant symptom of aspirin intolerance in patients with asthma was bronchospasm, while the predominant symptom in patients with rhinitis was urticaria/angioedema. In this investigation, we present further data to support this hypothesis. In addition, we present more evidence that aspirin intolerance may localize in certain families and offer an explanation as to one possible mechanism for this localization.

Cross-reaction between aspirin and tartrazine (FDC Yellow No. 5) was first reported in 1967³ and confirmed several months later by Samter and Beers.^{4,5} The frequency of this cross-reactivity following tartrazine challenge in aspirin-intolerant individuals is a matter of controversy since its report in the literature has varied from 7.5% to over 87%.^{4,7} As an aid in clarifying this situation, the initial purpose of this study was to determine the frequency of this cross-reactivity by challenging aspirin-intolerant patients and normal control subjects, who were matched by age and sex, with tartrazine and a placebo in a double-blind, crossover procedure using strict evaluating criteria.

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TABLE I. Major subtypes of aspirin intolerance in patients with asthma and/or rhinitis

Allergy diagnosis	Total No.	Symptoms produced by aspirin						
		No. with bronchospasm	%	P value	No. with urticaria/angioedema	%	P value	No. with both bronchospasm and urticaria/angioedema
Asthma with or without rhinitis	28	18	64.3		8	28.6		2
Rhinitis (alone)	8	0	0	<0.005	8	100	<0.005	0
Total	36*	18	50.0		16	44.4		2

*Four patients are not included in Tables I and II because they either did not have a primary diagnosis of asthma or rhinitis (2), or aspirin ingestion resulted in severe rhinorrhea only (2).

MATERIAL AND METHODS

Our recent epidemiologic study² helped localize aspirin-intolerant patients through direct interviews. An attempt was made to ask all those patients in the past study to participate in the present investigation. In addition, all new cases of aspirin intolerance acquired since that study either in the Rhode Island Hospital Allergy Clinic or in private practice were also asked to participate in the present investigation. Forty patients with aspirin intolerance in the present study includes all those who volunteered for this project. Twenty patients had participated in our initial study. For a control series, 40 normal individuals with no history of asthma, rhinitis, chronic urticaria, or aspirin intolerance were matched by age and sex to the 40 patients with aspirin intolerance.

Almost all of our normal group were volunteers from our hospital staff, including clerks, aids, nurses, and physicians. With the approval of this study by our hospital committee on human research, informed consent was obtained from all individuals who agreed to participate in this study.

All individuals were challenged in a double-blind manner with two types of empty gelatin capsules, one containing 0.22 mg of tartrazine dissolved in the gelatin shell and the other containing a gelatin shell with no dyes. Both types of capsules are of the same size and shape and are a standard product of a manufacturer (Parke, Davis & Co.). After placing these individuals on a special diet that eliminated all preservatives and color additives, for about 48 hr, a nurse challenged them first with one type capsule and at least 24 hr later with the other type capsule. In these challenges, capsules were placed directly in the patient's mouth with the patient's eyes closed. Neither the subject nor the evaluating physician knew what type of capsules were taken. All subjects received a 2-capsule challenge dose of tartrazine (0.44 mg) at one time except for 2 aspirin-intolerant individuals. One of these individuals had an adverse reaction to 1 capsule of tartrazine (0.22 mg) and, therefore, the double-capsule challenge (0.44 mg) was not attempted. The other individual did not wish to continue the double-blind study with the 2-capsule challenge, but completed the 1-capsule challenge. Most of our patients were initially challenged with the 2-capsule dosage without the preliminary 1-capsule challenge.

Pulmonary function tests, total vital capacity (TVC), forced expiratory volume in one second (FEV₁), peak flow rate (PEF), and an examination preceded each capsule challenge and were repeated 3 hr after each challenge. A positive reaction was accepted if the patient experienced objective signs of acute bronchospasm together with at least a 20% reduction in all 3 pulmonary function tests (TVC, FEV₁, and PEF). A positive reaction was also accepted if the patient experienced generalized pruritus, urticaria, or angioedema occurring within 3 hr after the capsule challenge. Patients were also questioned about any type of delayed reaction.

Our criteria for the diagnosis of asthma, rhinitis, and aspirin intolerance were the same as in our two past studies on this subject. A diagnosis of asthma was accepted if symptoms

TABLE II. Age onset, nasal polyps, and major subtypes of aspirin intolerance in patients with asthma and/or rhinitis

Symptoms produced by aspirin	Total	No. with similar age-onset of allergy (asthma-rhinitis) and aspirin intolerance*	%	P value	No. with nasal polyps	%	P value
Bronchospasm	18	16	89	<0.005	10	55.6	< 0.01
Urticaria/angioedema	16	4	25		2	12.5	
Both bronchospasm and urticaria/angioedema	2	2	100		2	100	
Total	36	22	61.1		14	38.9	

* in 1 yr of each other.

consisted of clinically reversible signs of wheezing, shortness of breath, and cough on a recurrent basis, not due to any other organic disease. A diagnosis of rhinitis was accepted if symptoms consisted of repeated nasal stuffiness, rhinorrhea, and frequent sneezing on a seasonal or nonseasonal basis. Cases of infectious rhinitis were excluded from this study. Vasomotor rhinitis was classified with those patients with rhinitis who had negative skin tests. Our criteria for intolerance to aspirin were acute bronchospasm, rhinorrhea, urticaria, angioedema, or shock occurring approximately within 2 hr of ingestion. Angioedema was included in the category of urticaria. A diagnosis of nasal polyposis was made by history, physical examination, or ENT consultation.

RESULTS

In the 40 patients with aspirin intolerance, there also was an additional diagnosis of asthma in 19, both asthma and rhinitis in 10, rhinitis alone in 9, and chronic urticaria in 2. Of the 38 patients with asthma and/or rhinitis, 36 had a history of reacting to aspirin by either bronchospasm or urticaria. In the remaining 2 patients, aspirin ingestion resulted in severe rhinorrhea only. The 2 patients with chronic urticaria alone as a primary diagnosis reacted to aspirin by experiencing a dramatic exacerbation of their urticaria.

The 40 patients with aspirin intolerance consisted of 34 females and 6 males with an average age of 42.6 yr and an age range of 17 to 73 yr. The 40 normal control subjects also included 34 females and 6 males. The average age of this group was 42.2 yr with an age range of 20 to 70 yr.

Aspirin intolerance was reportedly manifested by either bronchospasm or urticaria in 28 of our patients with asthma and in 8 of the patients with rhinitis alone (Table I). Bronchospasm alone was the predominant symptom of aspirin intolerance in patients with asthma, 64.3% (18/28), while none of the patients with rhinitis alone experienced bronchospasm following aspirin ingestion ($p < 0.005$). Conversely, urticaria/angioedema was the predominant symptom of aspirin intolerance in patients with rhinitis alone, 100% (8/8), while only 28.6% (8/28) of patients with a diagnosis of asthma experienced urticaria/angioedema ($p < 0.005$).

We mainly evaluated our data by subdividing it into the 2 major types of symptoms produced by aspirin ingestion, bronchospasm and urticaria/angio-

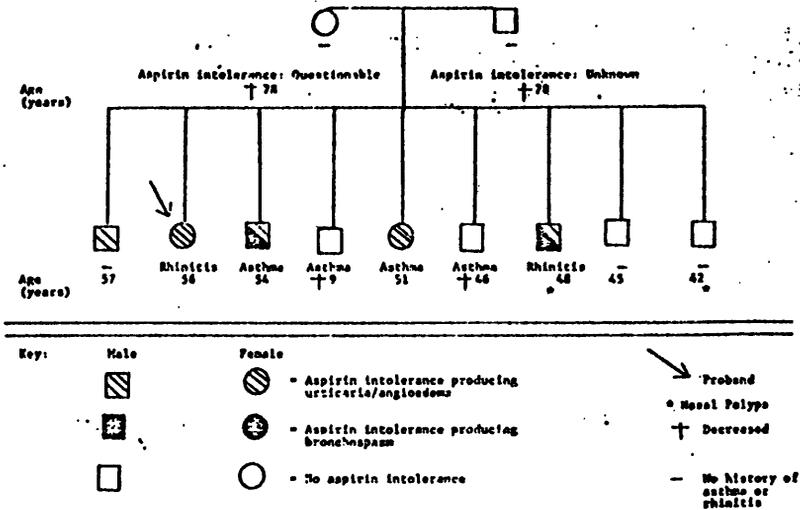


FIG. 1. Familial occurrence of aspirin intolerance (based on reliable information obtained from the proband after repeated questioning of her relatives).

edema. If aspirin ingestion produced bronchospasm, the age onset of aspirin intolerance was similar (within 1 yr) to the age onset of asthma in a majority of our cases, 89% (16/18). If the symptom produced by aspirin intolerance was urticaria/angioedema, then the age onset of aspirin intolerance was similar to the age onset of the primary diagnosis, asthma or rhinitis, in only 25% (4/16) of the patients. This difference in age onset is statistically significant ($p < 0.005$) (Table II). Further confirmation of these results revealed that the mean age onset of aspirin intolerance, 31.1 yr, in the group with bronchospasm was similar to the mean age onset of asthma, 32.1 yr, in this same group. However, the mean age onset of aspirin intolerance in the urticaria/angioedema group, 30.0 yr, was not as similar to the mean age onset of asthma or rhinitis, 34.3 yr.

Table II also demonstrates that the frequency of nasal polyps, 55.6% (10/18), is significantly greater ($p < 0.01$) in the group that responds to aspirin with bronchospasm than in the group that responds to aspirin with urticaria/angioedema, 12.5% (2/16).

There was essentially no difference in the frequency of positive allergy skin tests to a battery of inhalant allergens^{1,2} in either the bronchospastic or the urticaria/angioedema type of aspirin intolerance in patients with asthma or rhinitis.

A positive family history of aspirin intolerance was found in the immediate families of 7.5% (3/40) of our patients with aspirin intolerance. One asthmatic female whose aspirin intolerance symptom is bronchospasm has an asthmatic mother who also has acute bronchospasm following aspirin ingestion. Another asthmatic male whose aspirin intolerance symptoms is bronchospasm has an

TABLE III. Major subtypes of aspirin intolerance

	Bronchospastic type	Urticaria/angioedema type
Increased frequency in asthma	Yes	No
Increased frequency in rhinitis	No	Yes
Correlated with nasal polyposis	Yes	No
Similar age onset as asthma	Yes	No
Increased frequency in older age groups	Yes	No
Familial occurrence	Yes	Yes

asthmatic sister who developed urticaria/angioedema following aspirin ingestion. The third patient is a female who has rhinitis and has urticaria/angioedema as a result of aspirin ingestion. In her family, 5 out of 9 siblings have aspirin intolerance (Fig. 1). Three siblings have the urticaria/angioedema type of aspirin intolerance and 2 siblings have both the bronchospastic and the urticaria/angioedema type of aspirin intolerance. In addition, the mother of this family reportedly stated that aspirin made her "sick"; however, the type of symptoms produced by aspirin is unknown. Also one of the normal male siblings, 45 years of age, has an asthmatic son, 22, with the urticaria/angioedema type of aspirin intolerance. None of the 40 normal, matched, individuals in this study have a family history of aspirin intolerance.

Of our aspirin-intolerant individuals, 15% (6/40) reacted adversely to the tartrazine challenge and not to the placebo. None of the 40 normal control subjects reacted adversely either to the tartrazine challenge or to the placebo. The adverse reaction to tartrazine was similar to the type of reaction aspirin produced in these patients. In 3 out of 6 patients in whom tartrazine produced symptoms of generalized itch or urticaria, aspirin also produced urticaria. In the remaining 3 patients, tartrazine produced acute bronchospasm; in these patients aspirin also produced bronchospasm, except in one case in which aspirin produced both bronchospasm and urticaria. Cross-reactions between tartrazine and aspirin intolerance occurred essentially equally in each of the two major subtypes of aspirin intolerance.

The adverse reactions to tartrazine responded well to immediate treatment. The bronchospasm reactions were moderate and the urticaria reactions were mild to moderate. There was one questionable mild delayed reaction to the tartrazine challenge, and this was classified as a negative reaction. All of these adverse reactions occurred with the double-dose capsule (0.44 mg) of tartrazine except in 1 patient, whose primary diagnosis was chronic urticaria with no history of asthma or rhinitis. She reacted to 1 capsule of tartrazine (0.22 mg).

DISCUSSION

We reported previously that the frequency of aspirin intolerance is significantly greater in asthmatic patients (3.8%) than in rhinitis or normal individuals (0.9%).² Similar to our past reports,^{1,2} our present data also demonstrate that in aspirin intolerance the symptom of bronchospasm is found predominantly in patients with asthma while the symptom of urticaria/angioedema is found predominantly in patients with rhinitis. Our past reports

also demonstrated that the progressive increase of aspirin intolerance with advancing years was directly related to the bronchospastic type of symptomatology and not to the urticaria/angioedema type of symptoms.

For these reasons, we felt that there probably are at least two different mechanisms of aspirin intolerance, one producing bronchospasm, the other producing urticaria, angioedema. This hypothesis is supported by the additional evidence in the present study that the bronchospastic type of aspirin intolerance has a similar age onset as the asthma, and has a significantly greater frequency of nasal polyposis than does the urticaria/angioedema type of aspirin intolerance. It seems, therefore, that in the bronchospastic type of aspirin reaction, the development of aspirin intolerance may be related to the same disease process as the development of asthma. It is still speculative as to whether this disease process involves the kinins, prostaglandins, or other systems. It appears, however, that the asthmatic with a bronchospastic type of aspirin intolerance may represent a peculiar or different kind of asthma, with a high frequency of nasal polyposis, increased frequency in older age groups, and similar age onset of aspirin intolerance (Table III). This type of asthma should probably be classified as aspirin-asthma and should not include the urticaria/angioedema type of aspirin intolerance.

Occasional clustering of aspirin intolerance in a few families has been noted in the literature.^{8,9} A review of these cases reveals that the bronchospastic type of aspirin intolerance was present and usually only 2 members of an immediate family were affected. However, in one family our finding that 5 out of 9 children have aspirin intolerance appears to be unusually high (Fig. 1). Both the bronchospastic and urticaria/angioedema types of aspirin intolerance are present in this family. It is possible that the bronchospastic and urticaria/angioedema types of aspirin intolerance are transmitted by 2 separate genes and the chance occurrence of both genes in the same gene pool of one family may account for the exceptionally high number of siblings afflicted with aspirin intolerance. It is noteworthy that in our normal control group, there was no family history of aspirin intolerance. Whether separate genetic mechanisms may account for the different symptoms of aspirin intolerance, or whether a single genetic mechanism is the basis for the varied symptomatologic manifestations remains for speculation. It may be profitable to investigate the genetic aspects of aspirin intolerance in greater detail in future work.

Tartrazine (FD&C Yellow No. 5) is a color additive that has a widespread use in foods and medications. It has been estimated that the maximum ingested dose per capita is 16.3 mg per day.¹⁰ The first reported adverse reaction to color additives was in 1958 when Speer¹¹ reported that color additives caused asthma in 6 children. In 1959, Loekey¹² reported that tartrazine caused hives in 3 patients. Recently, interest was renewed in the frequency of cross-reactivity of tartrazine in aspirin-intolerant individuals.^{6,7} We are unable to confirm the exceptionally high frequency of cross-reactivity reported in these recent studies. However, this difference may be due to the altered dietary habits found in different countries. Our results of 15% cross-reactivity resembles the only other explicitly stated double-blind, large study in the literature, Samter and Beers,⁴

who reported 7.5%. However, these authors presented their data only in summary form. Most of the studies on this subject were not done in a double-blind manner.

The fact that the type of symptom produced by tartrazine was similar to that produced by aspirin may mean that the same type of biochemical abnormality may be present. This similarity of symptoms may also serve as confirmation of the partial cross-reactivity between these 2 chemicals. However, the molecular structure of tartrazine is vastly different than that of aspirin, and the exact mechanism of this cross-reactivity still remains largely unknown.

Our challenge dose of tartrazine was relatively low (0.22 mg to 0.44 mg). Other authors have used 1 to 5 mg, and Samter and Beer reportedly used 25 mg. However, a dose as low as 0.15 mg of tartrazine, as found in Premarin, 1.25 mg, has been known to cause adverse reactions to tartrazine.³ Samter and Beers, who used a 50 times⁴ larger dose than in our study, did not find a greater frequency of cross-reactivity. We emphasize that future studies evaluating adverse reactions of tartrazine and other food additives should employ a strict double-blind procedure and rigorous objective criteria.

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Allergic vascular purpura

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An instance of nonthrombocytopenic vascular purpura is reported due to tartrazine sensitivity. There were no abnormal hematologic findings present. Possible pathogenetic mechanisms involved in this condition are discussed.

Nonthrombocytopenic vascular purpura has been reported to be caused by allergy to drugs, insects, and inhalants.¹⁻¹¹ We wish to report an instance of vascular purpura resulting from sensitivity to a food additive, namely, tartrazine (yellow No. 5 dye).

CASE REPORT

Patient M. W. H., a 52-year-old married Caucasian female nurse, was first seen by us in 1968 through the courtesy of Dr. Philip Black. She had been troubled for the previous five years with acute intermittent bouts of profuse bleeding manifested by one or more of the following: menorrhagia, purpura, and/or bleeding from the ear, nose, gums, and bowel. These episodes were often accompanied by chills and fever. The bleeding was sufficiently severe at times to require hospitalization and blood transfusions.

There was no family history of bleeding. Past medical history was contributory only for bronchial asthma, perennial allergic rhinitis, and hay fever. Clinically significant positive intracutaneous tests were obtained to pollen, house dust, and molds but not to milk. There was no temporal correlation between these allergic respiratory manifestations and the bleeding episodes.

Physical examination revealed a young woman in distress only during the episodes of severe bleeding when her temperature ranged from 100° to 102° F. and her pulse and respiratory rate were increased. Blood pressure was 110/60. Purpuric lesions were found distributed on various body skin areas (Figs. 1 to 3). On one occasion bleeding into the soles of her feet was observed. No telangiectasia was present. Bleeding was seen at times from the ear canal and mucous membranes of the mouth, nose, and posterior pharynx. Cardiorespiratory examination was negative. Examination of the abdomen revealed no organomegaly or abdominal masses. Pelvic and rectal examination were normal except during the periods of bleeding. Neurological findings were unremarkable.

Extensive and repeated laboratory studies were carried out in 1968, 1969, and 1970 in various laboratories with essentially normal results. These included urinalysis and blood chemistry determinations such as creatinine, electrolytes, and serum protein electrophoresis. Bone marrow studies, multiple lupus erythematosus (LE) cell preparations, antinuclear factor, skeletal muscle, and skin biopsies were negative. Stools were markedly positive for occult blood during the active stage of the disease. Agglutination tests for *S. typhosa* (H), *S. paratyphi*, *S. schottmuelleri*, and *Brucella* were negative. Cold agglutinin titer was 1:32; antistreptolysin-O titer was 110 Todd units without rise. Serology was non-reactive. Gastrointestinal x-ray studies were negative. X-rays of the chest and sinuses and the electrocardiogram were normal. Intravenous pyelogram was negative. Blood cultures were sterile. Cervical scrapings were negative.

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FIG. 1. Petechias and purpura on mucous membrane of the mouth.

Hemostatic studies, carried out in the laboratory of Dr. Jessica Lewis, Department of Medicine, University of Pittsburgh, showed the following to be normal on three occasions: bleeding time (Duke and Ivy), tourniquet test, clotting time (glass and silicone), clot retraction, clot lysis, serum prothrombin time (SPT), prothrombin time (PT), recalcification time, partial thromboplastin time (PTT), plasma thrombin time (PL-T), and thromboplastin generation test (TGT). Recalcified plasma clots did not lyse in 5M urea. Also within the normal range were assays of factors I, II, V, VII, X, VIII, and IX. Platelet counts were between 200,000 and 216,000 per cubic millimeter. Platelet glass adhesive indices were normal. On three occasions the patient's platelets aggregated normally on addition of ADP (adenosine diphosphate, 0.001M), and on two of three tests aggregation was normal with added collagen.

Food diaries were instituted in January, 1968, in order to determine whether the ingestion of foods had any causal relation to the bleeding. The patient thus discovered that the ingestion of butter or margarine was invariably followed by bleeding. Provocative tests carried out with feeding butter or margarine always brought on bleeding into the skin and episodes of epistaxis with chills and fever which lasted for one to five days. Similar exacerbations were not precipitated by the ingestion of other dairy products. Further investigation revealed that tartrazine (FD&C yellow dye No. 5) is the food color additive in butter and margarine. Double-blind studies using coded capsules containing the dye or placebo precipitated bleeding in each and every instance only when the patient ingested dye-containing capsules. Scratch-patch tests were carried out with the dye in a 1:1,000 and later in a 1:100 dilution. No immediate wheal and flare reaction was obtained. However, there appeared at the test site in 24 hours a delayed response which consisted of erythema, induration, vesiculation, and pruritus occasionally accompanied by mild epistaxis and bleeding from the ears. Skin tests on controls were negative. Passive transfer tests (P-K tests) using the patient's serum and increasing concentrations of a solution of tartrazine were negative.

The Ouchterlony plate technique was employed for the determination of precipitins in the patient's serum against tartrazine, FD&C No. 5 dye. The center well contained the serum



FIGS. 2 and 3. Petechiae and purpura on various skin areas.

and the peripheral wells contained a 2 per cent solution of the dye. There were no demonstrable precipitin bands over a period of 7 days.

Furthermore, *in vitro* investigations failed to reveal that tartrazine (yellow No. 5) was toxic to this patient's platelets. The dye was prepared in concentrations of 1, 0.1, 0.01, and 0.001 per cent. The addition of $\frac{1}{50}$ part of these concentrations of the dye to normal or the patient's blood did not affect the subsequent clot retraction or SPT, PT, PTT, PL-T, or TGT.

Platelet-rich plasma from the patient and a normal individual was incubated with $\frac{1}{10}$ volume of the one per cent dye for 20 minutes with constant motion (in a platelet aggregometer, Chrono-Log Co.). No clumping or aggregation was observed, and subsequent addition of ADP produced normal aggregation in both samples.

DISCUSSION

The patient reported in this communication gave a history of repeated attacks of bleeding into the skin and from various mucous membranes. These episodes were related to the ingestion of foods containing tartrazine (FD&C yellow dye No. 5).

Various conditions were considered in differential diagnosis. A diagnosis of von-Willebrand's disease (hereditary vascular purpura or vascular pseudo-hemophilia) was made at one time elsewhere because of one suggestively abnormal factor VIII finding. This disease is characterized¹² by a prolonged bleeding time, a low antihemophilic globulin (AHG), low platelet adhesiveness, and a dominant autosomal hereditary history. This diagnosis was excluded because none of these findings was present on our own repeated examinations. The diagnosis of classical Schönlein-Henoch purpura was also considered. However, the abdominal pain, erythema, urticaria, serous effusions, renal and joint manifestations, and eosinophilia usually associated with this disease were absent in our patient.¹²⁻¹⁴

In a discussion of the pathogenesis of bleeding, it is desirable to consider the factors which normally control bleeding and then speculate on possible immunogenic causes. Bleeding is controlled by the coagulation components, the platelet factors,³ and the vascular component, i.e., the endothelium. As indicated above, we found no evidence of any coagulation or platelet abnormality. This then is essentially a vascular purpura associated with increased capillary fragility and permeability.

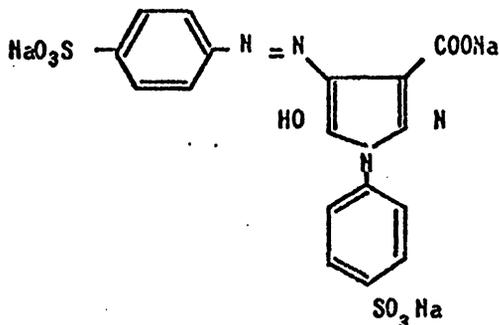


FIG. 4. Chemical formula of tartrazine (FD&C yellow No. 5). Trisodium salt of 3-carboxy-5-hydroxy-1-sulfophenyl azopyrazole, an aniline dye.

Instances of bleeding resulting from allergy to various substances have been reported. Snyder⁸ described a case of nonthrombocytopenic vascular purpura due to the ingestion of coffee occurring in an atopic individual who showed no abnormal hematologic findings. Many drugs, including quinine, belladonna, mercury, penicillin, meprobamate, aspirin, chloral hydrate, phenacetin, and oxytetracycline, have been reported to produce nonthrombocytopenic vascular purpura.^{9, 10, 11} We¹⁰ have reported nonthrombocytopenic vascular purpura accompanying a serum sickness type of penicillin reaction in three patients who had no abnormal hematologic findings. The purpura subsided upon cessation of penicillin therapy.

Experimentally, vascular purpura has been produced^{4, 5} by injecting dogs or guinea pigs with rabbit antisera against dog or guinea pig vascular endothelium. Stefanini and Mednicoff⁴ obtained precipitin reactions using sera of patients with anaphylactoid purpura and extracts of human aorta.

Tartrazine (FD&C yellow No. 5) is a pyrazole derivative (Fig. 4). It is the trisodium salt of 3-carboxy-5-hydroxy-1-sulfophenyl azopyrazole, an aniline dye. It includes heterocyclic rings and acidic groups. It is unfortunate that the Food and Drug Administration, while certifying some 11 coal-tar dyes, does not require packages to indicate the presence of the dye on the labeling. Artificial colors in foods and drugs may cause atopic reactions, such as bronchial asthma.¹² Urticaria has been reported from tartrazine (yellow No. 5) coloring agent following ingestion of Deronil, Decadron, or Paracortol tablets.

Lockey and Draude^{16, 17} have called attention to the presence of yellow No. 5 (tartrazine) in a great variety of products, including dry drink powders, carbonated beverages (Fang), colored candy, as a dye used for identification of solutions of drugs, in coloring drug capsules, and tablets such as Deronil, dexamethasone (Decadron), and prednisolone (Paracortol), toothpastes, cosmetics, hair-waving and hair-rinsing products, face powders, and many other articles.

The bleeding in this case was caused by the ingestion of tartrazine. The question then arises as to the pathogenesis of this bleeding. The completely negative hematologic studies here reported reveal no evidence of a toxic effect or of a hematologic disease responsible for the purpura. The presence of an atopic background in this patient does not necessarily imply an atopic or immunologic basis for her condition. Indeed, direct intracutaneous tests with the dye did not yield a wheal and flare reaction. Passive transfer tests were negative. The delayed positive skin test (scratch-patch test) was probably induced because of the local reaction on blood vessels. And yet, it is possible, though exact proof is lacking, that the dye, tartrazine, may in this case act as a hapten bound covalently to a protein carrier, such as the endothelial cells of small blood vessels. Circulating antibodies formed to this conjugate, it is postulated, react with the antigenic component of the endothelium or with an antigen which has become closely associated with the endothelium which is in constant contact with the circulating plasma. The complement system is then activated, leading to lysis of these cells. This results in increased blood vessel permeability, which in turn is responsible for the purpura and bleeding. Alternatively, circulating antigen-antibody complexes related to the dye might be deposited on the vascular endothelium. As is true of all allergic reactions, there is an acute onset on exposure, in this case the ingestion of the dye. Complete recovery occurs upon its withdrawal. Re-exposure causes reappearance of the bleeding, and the extent of the bleeding is dose dependent. The positive scratch-patch tests and provocative tests lend further weight to this supposition. However, Samter and Beers¹⁸ suggest the possibility that, as in the case of aspirin sensitivity, this dye may produce a direct stimulation of certain vulnerable peripheral chemoreceptors.

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Immunologic studies on aspirin

Clinical studies with aspiryl-protein conjugates

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Aspiryl-chloride was conjugated with human serum albumin, human gamma globulin, rabbit gamma globulin, and poly-L-lysine. These conjugates were evaluated in skin tests, indirect hemagglutination, lymphocyte culture, and in an immunophorescent test on tissues and sera of patients sensitive to aspirin. No evidence of specific pharmacologic reactions was found. Possible explanations for these results are discussed, and three possible immunologic mechanisms for adverse reactions to aspirin are offered.

The occurrence of asthma and hypotension following the ingestion of aspirin by certain patients has been documented by many authors.^{1,2} Some patients develop troublesome urticaria after taking aspirin.^{3,4} Many of these patients have no other manifestations of an allergic diathesis.⁵ A safe and reliable diagnostic method for aspirin sensitivity is not available. In previous publications^{6,7} it has been shown that aspiryl-protein conjugates are immunogenic.^{7,8} This report describes the use of aspiryl conjugates in testing of sera and tissues of patients sensitive to aspirin.

MATERIALS AND METHODS

Aspiryl conjugates

The aspiryl-protein conjugates were prepared as described previously.⁷ The conjugates used in this study were: aspiryl-human serum albumin (Asp-HSA), aspiryl-human gamma globulin (Asp-HGG), and aspiryl-rabbit gamma globulin (Asp-RGG). Aspiryl poly-L-lysine (Asp-P.L.) was prepared in a similar way: 250 mg. of poly-L-lysine (molecular weight 100,000, Pilot Chemicals, Inc., Watertown, Conn.) was dissolved in 25 ml. of distilled water and partially succinylated by the addition of 100 mg. of succinic anhydride. Then 75 mg. of aspiryl-chloride dissolved in ether was added and the mixture stirred until the reaction was complete. The conjugated Asp-P.L. was exhaustively dialyzed against phosphate-buffered

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saline, 0.15M, pH 8.6. The protein concentration of the conjugates was estimated by the Kjeldahl method. The number of aspiryl groups conjugated with each protein was determined spectrophotometrically by measuring absorbance at 295 m μ . The ratio of aspiryl groups to carrier molecule was: 15 to 20 for Asp-HSA; 10 to 15 for Asp-IgG; 21 to 30 for Asp- β_2 and 5 to 10 for Asp-PtL. The activity of all conjugates was examined by gel precipitation and tanned cell hemagglutination tests against rabbit antisera specific for the aspiryl groups. The tests were performed as described previously.¹ All conjugates gave strongly positive reactions in both tests.

Skin test

Freshly prepared conjugates of Asp-HSA, Asp-IgG, and Asp-PtL, diluted in 0.9% saline were used for direct skin tests. The concentrations of all conjugates used were 0.01 mg. per milliliter. Higher concentrations caused nonspecific skin reactions in some individuals. The skin test in the patients was performed by the intradermal injection of 0.1 ml. of 0.2 μ g and 0.2 μ g of each conjugate in 0.02 ml. The results were read after 20 minutes and 48 hours.

Immunofluorescent test

Tissue sections from nasal polyps obtained from 4 aspirin sensitive patients were stained for the presence of antibodies by an indirect immunofluorescent technique. The preparation of tissue sections and the general technique of the fluorescent test were described previously.¹ Two different aspiryl conjugates were used for this test. Tissue sections were incubated with Asp-IgG (10 mg. per milliliter) and, after washing, were covered with a fluorescently labeled rabbit antiserum to human IgG (Hyland). Another set of tissue sections was incubated with Asp-RGG (10 mg. per milliliter) and after washing covered with a fluorescently labeled rabbit antiserum to rabbit serum proteins. An additional set of sections was studied for the presence of complement with the use of a fluorescently labeled rabbit anti-serum to human β_2 (Hyland). All slides were examined immediately after preparation.

Lymphocyte culture

Lymphocytes of 6 patients sensitive to aspirin were examined *in vitro* with the use of different aspiryl conjugates. Heparinized blood of a patient was allowed to sediment and the supernatant containing the white cells was removed. The white cells were diluted in medium 199 (Grand Island Biochemical Company) to a concentration of one million to one million and a half nuclear cells per milliliter. Four milliliters of the cell suspension was distributed in culture tubes (Falcon Plastics). The conjugates Asp-HSA, Asp-IgG, or Asp-PtL were diluted in medium 199. Each was added (0.1 ml.) to separate duplicate sets of tubes to final concentrations of 2.5 μ g and 2.5 μ g per milliliter. Prior to each test, acetylsalicylic acid was dissolved and added to medium 199 at pH 7.4 and used in a final concentration of 2.5 μ g per milliliter. Control tubes without aspirin preparations and tubes containing 0.1 ml. of a 1:100 dilution of phytohemagglutinin-P (PHA-P, Difco) were included in each experiment. In addition, lymphocytes were incubated with a mixture of one of the aspirin compounds and PHA-P. The tubes containing PHA-P alone and PHA-P with one of the aspirin compounds were incubated for 3 days and the remaining tubes for 7 days at 37° C. Prior to harvest, 0.5 μ c of tritiated thymidine (New England Nuclear Corp., 0.7 c. per millimole) was added to each tube and the incubation at 37° C. continued for the final 16 hours. At harvest, the cells were centrifuged and washed twice in cold 5 per cent trichloroacetic acid (TCA). Two milliliters of 5 per cent TCA was then added and the nucleic acids extracted by heating the samples to 99° C. for 16 hours. An aliquot of 0.5 ml. of each sample was then added into 15 ml. of Bray's¹⁰ solvent and the radioactivity measured in a Packard Model 3000 scintillation counter.

Hemagglutination test

The sera of 17 patients were examined by a hemagglutination test with the use of acid-treated erythrocytes coated with Asp-RGG and Asp-IgG as described previously.¹

Table I. Description of patients and laboratory results

Patient	Age	Sex	Clinical disease*	Results of tests with aspiryl conjugate		
				Skin tests	Hemagglutination titer	Lymphocyte culture
D. C. J.	49	M	A, P, S	Negative	Under 10	S.I. = 14
S. M. J.	38	M	A, E, P, S	Negative	Under 10	S.I. = 1
W. M.	47	M	A, E, P, PR, S	Negative	Under 10	S.I. = 1
L. G.	57	F	A, E, P	Negative	Under 10	S.I. = 1
C. M.	59	F	A, P	Negative	Under 10	S.I. = 1
G. B.	63	F	A, E, P, PR	Negative	Under 10	S.I. = 1
Z. B. J.	30	F	CS, P, PR, S	Negative	Under 10	ND†
J. D.	33	F	A, CS, P, PR, S	Negative	Under 10	ND
A. P.	33	F	A, CS, E, P, PR, S	Negative	Under 10	ND
A. Y.	40	F	A, E, CS, P, PR, S	Negative	Under 10	ND
J. K.	43	M	A, P, PR, S	Negative	Under 10	ND
G. P. J.	50	F	A, CS, E, P, PR	Negative	Under 10	ND
D. M.	57	M	A, CS, E	Negative	Under 10	ND
E. P.	69	F	A, CS, E, P, PR, S	Negative	Under 10	ND
L. W.	20	F	PR, U	Negative	Under 10	ND
F. K.	33	M	A, CS, E, PR, S, R	Negative	Under 10	ND
C. W.	34	M	U	Negative	Under 10	ND

*A, asthma; AR, seasonal allergic rhinitis; CS, corticosteroid therapy; E, eosinophilia over 6 per cent; P, nasal polyps; PR, perennial rhinitis; R, macular rash from aspirin; S, sinusitis; U, urticaria from aspirin.

†Polyps studied by immunofluorescent technique.

‡S.I.: specific incorporation of tritiated thymidine = counts with antigen/counts of control; a ratio of one (1) indicates no stimulation of thymidine incorporation.

§Not done.

era prior to the test were absorbed with the carrier proteins. The coated erythrocytes were controlled by use of a rabbit antiserum to Asp-RGG absorbed with RGG and PGG.

RESULTS

A brief description of the 17 patients studied and the results of the immunologic tests performed are given in Table I. All but one patient was over 30 years of age. Ten of the patients were women. Only one patient had true seasonal allergic symptoms and was basically considered atopic. Fourteen of the patients had chronic perennial asthma and developed acute attacks of asthma upon ingestion of aspirin. Three nonasthmatic patients experienced repeated skin rashes after the ingestion of aspirin. Most patients had associated perennial rhinitis, nasal polyps, sinusitis, and peripheral blood eosinophilia. Eleven patients required a low dose (5 to 15 mg.) of prednisone to control nasal or conjunctival symptoms.

The skin tests with the aspiryl preparations did not produce any definite immediate or delayed skin reactions, and no systemic symptoms were induced by the procedure.

The immunofluorescent examination of the nasal polyps did not show specific staining either with the aspiryl conjugates or the antiserum to β_{2e} .

The lymphocyte cultures of the 6 patients studied showed no evidence of increased incorporation of tritiated thymidine over the control cultures. The lymphocytes of the same patients responded well to the action of PHA-P, indicating the potential for stimulation.

The tanned cell hemagglutination tests with the patients' sera showed positive reactions, indicating lack of antibodies to the aspiryl conjugates in sera. The rabbit antiserum to Asp-RGG reacted with the coated erythrocytes in a titer of 1:10,240.

DISCUSSION

Convincing evidence for an immunologic mechanism in adverse reactions to aspirin has not yet been found. Skin tests with aspirin performed in patients who are sensitive to aspirin are generally negative.¹¹ In addition, because hypertension and bronchospasm can be induced by such tests, they should be avoided. Previous efforts to demonstrate skin reactions to protein conjugates of aspirin have been unsuccessful. Peinberg and Malkiel¹² used a preparation of whole human serum conjugated with aspiryl chloride in direct and passive transfer tests, with negative results. Mathews, Lovell, and Sheldon¹³ used sera for skin tests which were obtained from normal persons who had ingested aspirin. The sera contained from 60 to 240 μg per milliliter of salicylates but were inactive in skin tests of aspirin-sensitive patients.

The immunofluorescent technique, to our knowledge, has not been utilized in tissue studies of patients sensitive to aspirin. Antibodies to the aspiryl conjugates were not detected in the patients' nasal polyps. In addition, the negative results with the use of the antiserum to β_{10} suggest that complement is not involved in the pathogenesis of the polyps.

The negative results of the lymphocyte cultures agree with those of Caron and Giraldo and associates¹⁴ but disagree with those of Halpern and associates. The latter authors reported uniformly positive results in lymphocyte cultures with the use of morphologic criteria to evaluate the results of the cultures. Their studies of Caron and Giraldo and associates and our own employed a radioisotope counting technique to evaluate the response of the cells, which is more objective than counting lymphoblast-like cells as was done by Halpern.

Gantner and Zuckner¹⁵ reported a suppression of response to PHA when aspirin was added to lymphocyte cultures. However, in the concentrations of aspirin and aspiryl conjugates used by us, there was no suppression of PHA response. We could not culture lymphocytes from those patients who were receiving prednisone treatment since corticosteroids inhibit lymphocyte stimulation.¹⁷

The failure to find agglutinating antibodies to erythrocytes coated with aspiryl conjugates contrasts with the positive results found by Weiner and associates¹⁶ in patients sensitive to aspirin or para-aminosalicylic acid (PAS). The antibodies found were peculiarly labile and disappeared after storage for 2 weeks at -10°C . Giraldo and associates¹⁴ found hemagglutinating antibodies in the sera of normal and aspirin-sensitive patients, but it is unclear if the patients' sera were absorbed with the carrier proteins used for their aspiryl conjugates.

If adverse reactions to aspirin have an immunologic basis, 2 explanations can be offered for our negative results. First, a metabolic product different from that of an aspiryl conjugate might be involved in allergic reactions. The

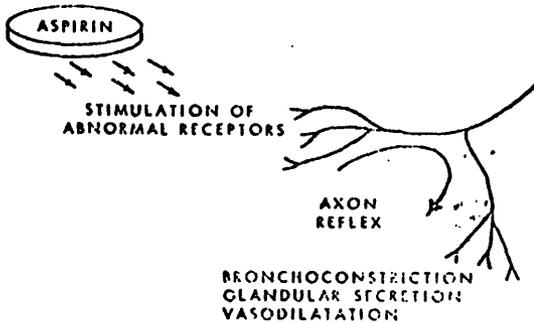


Fig. 1

Abnormal kinin receptor.

for testing patients sensitive to drugs with multiple metabolic products is well illustrated by the studies of Levine and associates²⁰ on penicillin allergy. Aspirin has multiple degradation products and can react with serum proteins in a number of ways.²⁰ One of such reactions is the formation of a salicylamide derivative, a pathway that salicylic acid cannot follow.²¹ Another is the acetylation of proteins, such as albumin, as shown by Hawkins and associates.²²⁻²⁴ Giraldo and associates²¹ found no positive responses with the use of aspirin-altered albumin in immunologic tests of aspirin-sensitive patients, but further studies of such products are awaited.

A second explanation for our negative results is the possibility that the conjugates used had lost their acetyl groups, since such antigens might not react with antibodies to acetylsalicylic acid. Acetylsalicylic acid can spontaneously hydrolyze to salicylic acid. In human beings, this process is accelerated by an enzymatic reaction so that only 25 to 50 per cent of ingested aspirin circulates after 30 minutes as the intact molecule.²⁴ We performed most of our tests within a few weeks of the preparation of the conjugates, but it is possible that the products used did not retain the acetyl group.

An alternative explanation of the negative results that could be offered is that nonimmunologic mechanisms are responsible for adverse reactions to aspirin. Senter and Beers²⁵ have proposed one intriguing hypothesis summarized in Fig. 1. These authors postulate that an underlying disease alters kinin receptors present in the lung, in capillaries, and in other tissues. Aspirin in sensitive patients stimulates these receptors and, in effect, causes symptoms similar to those produced by kinins. It is known that kinins can produce bronchospasm and vasodilation in man.^{26, 27} The induction of such symptoms in aspirin-sensitive patients by chemically unrelated compounds such as indomethacin, hydrazine yellow, and other drugs suggests that a nonimmunologic process may be involved.^{28, 29, 29}

Since no definite proof of an immunologic or nonimmunologic mechanism in aspirin sensitivity is available, we wish to offer two additional hypotheses.

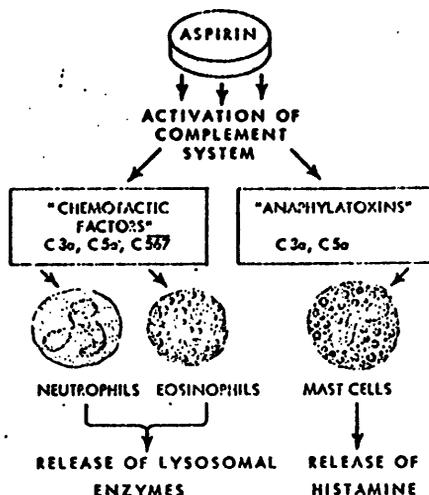


Fig. 2
Direct activation of complement.

One mechanism for the action of aspirin in sensitive patients might be the direct activation of complement components. It is known²⁰⁻²³ that substances such as endotoxin, dextran, and plasmin can directly activate complement components, releasing "anaphylatoxins" which then release histamine. No antibodies are required in these reactions. Activation of C3,²⁰ plasmin, or tissue protease produces chemoattractive factors that attract neutrophils and eosinophils.²⁴⁻²⁷ Thus, the possible activation of the complement system by aspirin could produce the eosinophilia and local tissue edema seen clinically without requiring an immunologic mechanism. This sequence of events is outlined in Fig. 2. The release of anaphylatoxins and histamine would lead to well-known immediate effects. The attraction of neutrophils and eosinophils might result in the release of lysosomal enzymes causing chronic tissue damage.

We are not aware of studies of serum complement levels during acute reactions to aspirin. Our immunofluorescent studies were done on chronically inflamed polyps and might not indicate events occurring during acute reactions. Further studies of the complement system seem worthwhile.

A second hypothesis we wish to offer concerns the effect of aspirin on enzymes and the pathways leading to its ultimate pharmacologic effects. Aspirin in physiologic amounts affects a number of enzyme systems.²⁸ Indomethacin, a drug that provokes symptoms in aspirin-sensitive patients, can activate a proteolytic enzyme in rat skin.²⁹ Two nonimmunologic diseases that simulate allergic states are known to be associated with deficiencies of enzyme inhibitors. In hereditary angioedema there is deficiency of an inhibitor to multiple

*Proposed nomenclature of complement: Bull. W.H.O. 39: 935, 1968.

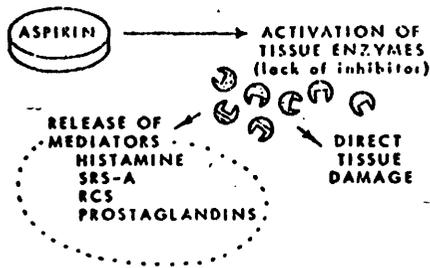


Fig. 3

Lack of enzyme inhibitor.

enzymes.^{40, 41} This results in the development of life-threatening angioedema due to the activation of kinins and complement.⁴² Hereditary emphysema or alpha-1 antitrypsin deficiency is another example of a disease associated with the lack of an enzyme inhibitor.⁴³ This disease sometimes presents as "allergic bronchitis." Based on these two examples, it may be suggested that adverse reactions to aspirin occur because of a deficiency of an enzyme inhibitor as shown in Fig. 3. The activation of hypothetical tissue enzymes could lead to tissue damage and the further release of chemical mediators.

Piper and Vane⁴⁴ in a recent provocative article described effects of aspirin on a number of mediators released in anaphylaxis. Their studies show a potentiation by aspirin of slow-reacting substance of anaphylaxis (SRS-A) and an inhibition by aspirin of the release of a new mediator of anaphylaxis, rabbit anti-contracting substance (RCS). Prostaglandins were also shown to be released subsequent to the action of SRS-A and the kinins. The role of all these mediators in adverse reactions to aspirin requires further study. Collier,⁴⁵ commenting on the work of Piper and Vane, notes that "aspirin and like-acting drugs block a route leading to or from the specific receptors for the agonists rather than blocking the receptors themselves."

We suggest that patients sensitive to aspirin may lack an inhibitor to an enzymatic reaction along this route. This lack might allow the release of kinins, the activation of complement, or the release of as yet unknown proteolytic enzymes. In future studies, it would be appropriate to search for nonimmunologic and enzymatic, as well as immunologic, mechanisms to explain adverse reactions to aspirin and other such chemical allergens.

The authors wish to express their appreciation to Dr. Donald R. Tourville for performing immunofluorescent studies. The authors thank Dr. Michael Schwartz of the Department of Pharmacodynamics, State University of New York at Buffalo, for help in the preparation of the acetyl-poly-L-lysine conjugate, and Miss Deborah Deaton for technical assistance.

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Tartrazine: Solid-Phase Radioimmunoassay
Studies of an Azo Dye Implicated in
Allergic Reactions
(Azo Dyes and Allergy)

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Tartrazine, an FDSC¹ approved synthetic organic dye, has been increasingly implicated in allergic reactions that range from urticaria to severe asthma (1,2,3,4,5,6). Recently, controversy has arisen over the claim that tartrazine and some other food and drug additives are responsible for some forms of hyperactivity in children (Zen F. Feingold, the Congressional Record of October 30, 1973, S. 19736-19742).

The haptenic properties of tartrazine and some structurally related food dyes have previously been examined by a quantitative hemagglutination procedure (7,8). This report examines the haptenic properties and relationships of tartrazine, aspirin, and a pyrazolone intermediate of tartrazine using a solid-phase radioimmunoassay procedure (9). The data derived from these studies may provide information on the possible etiological basis of tartrazine and aspirin sensitivity.

¹ The term FDSC refers to the Food, Drug, and Cosmetic Act of 1938 as amended by the Color Additives Amendments of 1960.

Materials and Methods

Haptens and reagents. Purified tartrazine (trisodium salt of 3-carboxy-5-hydroxy-1-p-sulphophenyl-4-p-sulphophenylazopyrazole), FD&C Yellow No. 5, was obtained from Allied Chemical, Morristown, New Jersey. Sulfanilic acid was obtained from Eastman Organic Chemicals, Rochester, New York. A purified preparation of sunset yellow (FD&C Yellow No. 6) was obtained from the Division of Color and Cosmetics, Bureau of Foods, Food and Drug Administration. Aspirin (acetylsalicylic acid) was obtained from Matheson, Coleman, and Bell, Norwood, Ohio.

The monosodium salt of 1-(4-sulphophenyl)-3-carboxy-5-hydroxy-pyrazole (SCHP), an intermediate in the synthesis of tartrazine, was prepared previously described (10). The theoretical values for nitrogen and sulfur in SCHP are 9.15 and 10.47%, respectively. The synthesized material contained 9.28 and 10.29%, respectively. Further, there was a greater than 99% agreement between the calculated and actual values for titration to neutral equivalence.

Production of antisera to tartrazine. Anti-tartrazine antibodies were obtained by rabbit immunization as described previously (7,8). The immunogen consisted of a conjugate of tartrazine and bovine serum albumin (BSA) prepared with bis-diazotized benmidium. A full description of the immunological properties of the antisera has been described (8).

Preparation and iodination of tartrazine and SCHEP conjugates for radioimmunoassay. Tartrazine and SCHEP were coupled to rabbit serum albumin (RSA) as previously described (8). Assuming a molecular weight of 70,000 for RSA, the tartrazine conjugate contained approximately 5 μ g hepten/ μ g RSA. The SCHEP conjugate contained approximately 9 μ g hepten/ μ g RSA. The protein moiety of the conjugates was iodinated with 4 μ Ci 125 I/ μ g protein by a modification of the chloramine-T method (9). This method of iodination was performed because of the technical difficulties associated with direct iodination of the haptens (unpublished data). Further, direct iodination of the haptens may have altered their reactivity with anti-tartrazine antibodies.

Solid-phase radioimmunoassay. Radioimmunoassay was carried out exactly as described (9). Various concentrations of inhibitors (in 1 ml) in phosphate-buffered saline (PBS) (0.07 M phosphate; 0.07 M NaCl, pH 7.2, and containing 1% RSA and 0.1% sodium azide, were added to anti-tartrazine-sensitized polystyrene tubes. The tubes were incubated at 37° for 18 hr, after which 0.001 μ g of 125 I-tartrazine-RSA or 125 I-SCHEP-RSA (in 0.1 ml) was added. The tubes were shaken vigorously, incubated at 37° for 4 hr, washed once with 2 ml of PBS, and counted in a Packard Auto-Gamma Counter (Model 5320).

Results and Discussion

The optimal concentration of anti-tartrazine antibody for sensitizing polystyrene tubes for radioimmunoassay was determined empirically. The salt-precipitated (9) antiserum pool used in the studies reported here was used at 1:50 dilution, representing 10 μ g immunoglobulin protein/ml.

Figure 1 contains the structures of tartrazine, SCHP, sunset yellow, sulfanilate, and aspirin. Solid-phase radioimmunoassay inhibition data with these haptens are presented in Fig. 2.

Inhibition data with 125 I-tartrazine-RSA and anti-tartrazine are presented in Fig. 2A. The most effective inhibitor in the system was tartrazine, followed by SCHP. Sunset yellow, which has the *p*-azobenzene-sulfonate structure, was a much less effective inhibitor, and sulfanilate, lacking the diazo group, was even less effective. These results suggest that the specificity of the anti-tartrazine antibody is strongly directed toward the SCHP moiety of tartrazine. Aspirin was non-inhibitory, even at a concentration that was 29,000 times greater than that of tartrazine, which resulted in approximately 60% inhibition of 125 I-tartrazine-RSA binding. Bis-diazotized benzidine-coupled RSA (8), 10 μ g/ml, had no inhibitory effect on the system, thus indicating that bis-diazotized benzidine specificities are not involved. Tartrazine, at the highest concentration employed in the present studies, had no inhibitory effect on an unrelated solid-phase radioimmunoassay system (9). Only 4% of the 125 I-tartrazine-RSA label was bound by anti-tartrazine-coated tubes in the absence of inhibitors. This result was probably due to the slow release of non-covalently bound tartrazine from the RSA.

Preliminary data indicate that this strong non-covalent binding and slow release continues indefinitely, even in the presence of 1 M NaCl (unpublished results). This effect is probably the reason that passive hemagglutination of tartrazine-coupled sheep-red cells by anti-tartrazine was not possible in previous studies (7,8). Nonetheless, the binding was sufficient here to define the haptenic specificities.

A similar pattern of inhibition (Fig. 2B) was also obtained with ^{125}I -SCHP-RSA as the radiolabel against anti-tartrazine. Tartrazine was again the most effective inhibitor, followed by SCHP. These results suggest that even though the SCHP moiety is probably dominant in the haptenic specificity of tartrazine, other functional groups probably also contribute to the specificity. Again, aspirin was non-inhibitory. Twenty-eight percent of the ^{125}I -SCHP-RSA label was bound by anti-tartrazine-coated tubes in the absence of inhibitors. The greater binding of ^{125}I -SCHP-RSA as opposed to ^{125}I -tartrazine-RSA was probably due to the lack of a strong non-covalent association of SCHP with RSA (unpublished data). Thus, a built-in inhibitor, slowly released from the conjugate, was not present in the conjugate to diminish the binding to antibody-coated tubes.

Numerous attempts to produce antibodies specific for aspirin (11) were unsuccessful, so this aspect of the haptenic relationship of tartrazine and aspirin was not examined.

efficiently, tartrazine and aspirin sensitivities seem to be associated (1,2,3,4,5,6). The lack of a haptenic relationship between the two substances, as studied here, suggests that mechanisms other than antigen-antibody reactions are responsible for aspirin and tartrazine sensitivity. This non-antigen-antibody aspect of aspirin sensitivity has been previously suggested (3). The data presented here provide immunochemical support for such a thesis.

Finally, tartrazine has been shown to undergo reductive scission in the gut after oral administration (12). An important product of this scission is 1-(4-sulfophenyl)-3-carboxy-4-amino-5-hydroxy-pyrazole, which differs from 1-(4-sulfophenyl)-3-carboxy-5-hydroxy-pyrazole (SCHP) only in that it contains an amino group. Further studies therefore seem warranted on the biological and haptenic properties of SCHP.

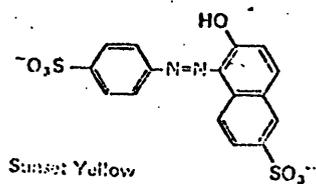
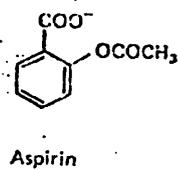
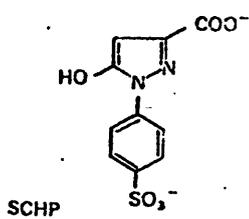
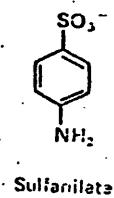
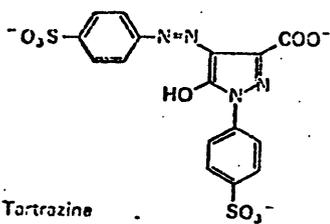
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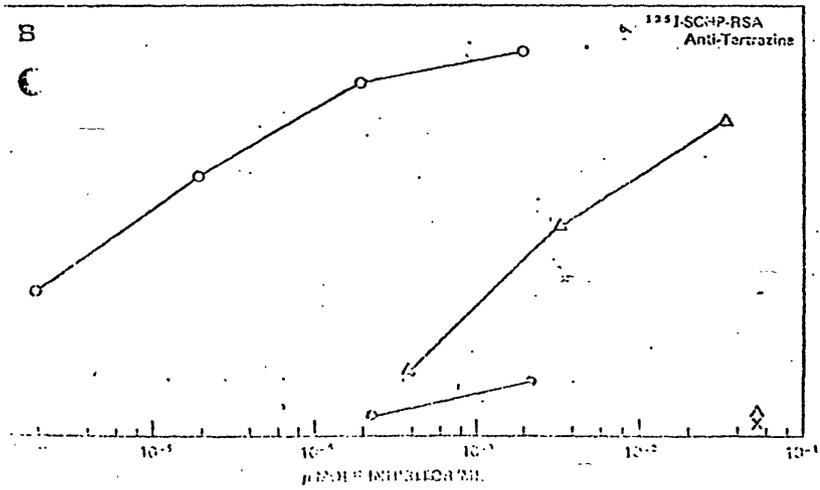
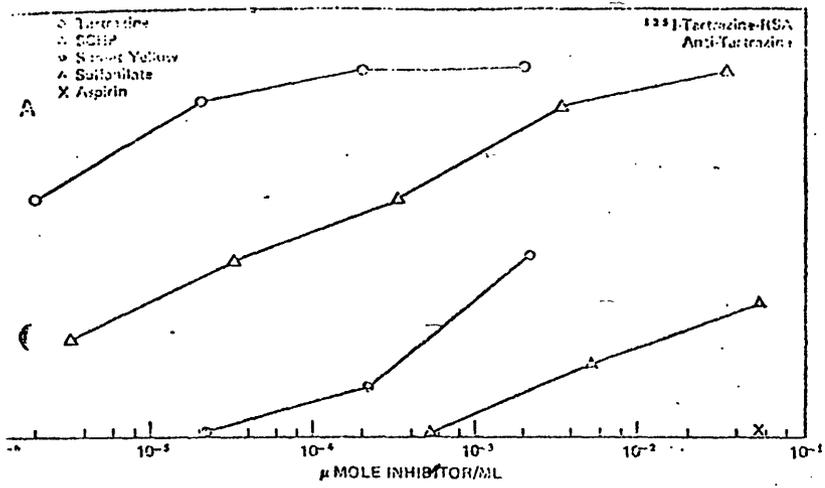
A solid-phase radioimmunoassay procedure was adapted for the haptenic study of tartrazine, an azo dye implicated in various forms of allergy. The specificity of antibody to tartrazine was directed strongly toward a pyrazolone intermediate of the molecule, 1-(4-sulfophenyl)-3-carboxy-5-hydroxy-pyrazole. Aspirin did not cross-react with anti-tartrazine, suggesting that the clinical association of aspirin and tartrazine sensitivity in patients is an non-immunological phenomenon.

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Fig. 1. Structure of heptosa

Fig. 2. Solid-phase radioimmunoassay inhibition of anti-tartrazine binding of (A) ^{125}I -tartrazine-RSA and (B) ^{125}I -SCHP-RSA.





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Intolerance to Aspirin

Clinical Studies and Consideration of its Pathogenesis

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SUMMARY Angioedema and rhinitis, nasal polyposis, and bronchial asthma of aspirin-sensitive patients are acquired diseases that develop, as a rule, after middle age in predominantly nonatopic patients. In many instances, nasal and bronchial symptoms precede the development of intolerance to aspirin by months or even by years. Salicylates other than acetylsalicylic acid fail to produce symptoms in aspirin-sensitive patients. Exposure to several chemicals, on the other hand, that are structurally unrelated to aspirin can induce comparable "aspirin-like" symptoms. The structural dissimilarity of these compounds is so pronounced that immunological cross-reactivity appears most unlikely. The substances that have been found to induce aspirin-like symptoms have one characteristic in common—they are strong minor analgesics and include pyrazolones and indomethacin as well as aspirin. Peripheral analgesics might act on peripheral chemoreceptors and initiate a series of reflexes that might produce either angioedema, or rhinitis and bronchial asthma, or all of these.

INTOLEANCE TO ASPIRIN has been known for more than 50 years. The first description, by Hirschberg (1), appeared only 3 years after the synthesis of the drug. Van Leeuwen (2) discussed its clinical significance in 1928. Several reports of death after ingestion of aspirin have appeared in the

literature (3, 4). Cooke (5) tested 3 of 9 aspirin-sensitive patients with salicylic acid, benzoic acid, antipyrine, sodium acetate, and methyl salicylate and noted the absence of untoward reactions. Prickman and Buchstein (6) analyzed the histories of 62 (40 female and 22 male) aspirin-sensitive patients: 4 had sinusitis; 11, hay fever; 21, vasomotor rhinitis and nasal polyps; and 43, asthma. Migraine occurred in 6 and urticaria (or angioneurotic edema) in 10 of their patients. As early as 1929, Francis (7) emphasized the risk of polypectomy precipitating bronchial asthma in the "aspirin-sensitive" patient.

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TABLE 1. Clinical Features of 182
Aspirin-Sensitive Patients

Data	No. of Patients
Sex: male	78
female	104
Onset before age 30	46
Duration of aspirin sensitivity:	
less than 5 years	70
more than 5 years	112
Respiratory symptoms only	154
Angioneurotic edema and urticaria only	18
Respiratory symptoms and urticaria	10
Family history of atopy	40
Positive skin reactions to seasonal and environmental inhalant allergens	19
Nasal and paranasal polyps	92
Polypectomies	72
First attack of bronchial asthma within 9 months after polypectomy	18
Bronchial asthma aggravated by polypectomy	30
Associated sensitivities	42*

* See breakdown in Table 2.

Gardner and Blanton (8) made an attempt to determine the prevalence of aspirin sensitivity in 467 patients—more than one half, asthmatics—and found only 5 patients who reported symptoms when asked: "Do you take aspirin?" and "What effect have you noticed from it?" They also polled a group of allergists about their experience with aspirin-sensitive patients: The returned questionnaires yielded a calculated prevalence of 0.2% which, we believe, is lower than the true prevalence of the disease.

Our studies, which are summarized in this report, indicate that intolerance to aspirin is reasonably common; that the evidence for an "allergic" response to aspirin, that is, for the formation of antibodies as a cause of this intolerance, is inconclusive; that the clinical triad of nasal polyposis, bronchial asthma, and life-threatening reactions to acetylsalicylic acid is a disease

entity, not a chance cluster of allergic symptoms, and represents, in fact, the prototype of a syndrome that has not been previously described and deserves recognition.

THE NATURAL HISTORY OF INTOLERANCE TO ASPIRIN

From 1954 to 1965, we have followed more than 1,000 "aspirin-sensitive" patients. In the beginning, new patients returned to the Allergy Clinic at weekly intervals for extensive studies and long-term observation in order to clarify the natural history of the disease and permit adequate classification. Since 1939 we have carried out prospective studies on 182 patients who were admitted to the medical wards of the University of Illinois Research and Educational Hospitals. Our conclusions are based upon our findings in this latter group of patients: Table 1 lists their essential clinical characteristics.

Onset and progression of the changes in skin and respiratory tract which are associated with intolerance to aspirin occur in a pattern that can be uncovered by history or observation in more than 80% of our patients, although Table 1 makes it evident that every patient does not necessarily present every potential component of the syndrome. Childhood, adolescence, and the beginning of adult life are not distinguished by any particular disease. During the second or third decade of life an intermittent "vasomotor rhinitis" develops in patients of either sex that is distinguished by profuse watery secretions and eventually followed by chronic nasal blockade and loss of intermittency.

The swelling of the nasal mucous membrane tends to become continuous and responds less and less to vasoconstrictors. Nasal and paranasal polyps develop: 92 out of 182 of our "respiratory" patients had nasal polyps. Polyps tend to be bilateral and respond poorly to therapy. Sixty-eight patients had multiple polypectomies, some as often as once a month: Polypectomy

does not control the tendency of the polyps to re-form.

The first episode of bronchial asthma is rarely related to a well-defined precipitating factor, but onset or aggravation of bronchial asthma follows nasal polypectomy in a suggestive number of patients.

Bronchial asthma in aspirin-sensitive patients occurs, as a rule, in middle age. Previous papers—for instance, Leigh and Rawsley's review (9) of bronchial asthma of late onset—suggest "that emotional precipitants are of notable importance in late onset asthma and that they are, perhaps, rather more important in the etiological complex of late onset asthma than of asthma originating in earlier life." Similarly, Rees (10) suspects that a special personality type predisposes to "intrinsic" bronchial asthma. We noted the common presence of great anxiety that appears to be associated with obstructive pulmonary disease of older patients (11), but we did not recognize any specificity.

During the initial stage, bronchial asthma of aspirin-sensitive patients is distinguished by [1] its impressive reversibility with bronchodilators and [2] by its response to maintenance doses of corticosteroids that are considerably smaller than the doses required for the control of immunologically induced bronchial asthma of comparable severity.

Nasal polyposis and bronchial asthma continue whether or not aspirin is ingested. Moreover, the first aspirin-induced reaction may not occur until respiratory manifestations of the disease have been present for years.

Bronchial asthma in aspirin-sensitive patients has been held to be severe, if not intractable (12). While it is true that some of our patients (26 of 182) showed progression, documented by ventilatory function tests during the period of observation, a surprising number of aspirin-sensitive patients—including patients who have suffered severely during the early stages of

the disease—may improve; and a few of our patients (16 of 182) have become free of asthma unless attacks are precipitated by aspirin. It is interesting, however, that no patient under our care has ceased to form nasal polyps once the presence of polyps had been established.

Since many of our patients had taken aspirin in the past with impunity, the initial reaction is usually unexpected and, in fact, quite often not attributed to the drug.

No correlation exists between the frequency with which patients have taken aspirin in the past and the severity of their reactions. By and large, the severity of the reaction is a function of a host and tends to remain the same. It is our impression, however, that patients who have noted comparatively mild reactions to aspirin but have avoided it faithfully for many years might have more severe reactions to the drug after a long and comparatively symptom-free interval.

Patients who are sensitive to aspirin suffer from respiratory symptoms, or urticaria, or both. Once they occur in a given tissue, reactions to aspirin are remarkably similar in quality but differ in quantity. The natural history—date of onset, severity of reaction, and progressiveness—varies from patient to patient. Urticaria, for instance, might occur only after ingestion of aspirin or may persist even though aspirin is avoided.

ATOPY AND ASPIRIN SENSITIVITY

The incidence of atopy in our group of patients is low. Each patient was carefully screened for signs of atopic sensitivity before hospitalization. Screening included skin tests to seasonal and environmental inhalants and to suspected foods. Atopy was assumed to exist when two of the following three criteria were fulfilled: [1] a positive family history of atopy; [2] an established correlation between exposure to an atopic antigen (allergen) and subsequent

development of clinical symptoms, regardless of skin tests; and [3] unequivocal positive skin reactions to atopic antigens to which the patient had been exposed, regardless of symptoms. Compared with an expected prevalence of up to 20% in a random population (13, 14), the aspirin-sensitive group has an incidence of atopy of less than 3%. A significant number of our patients, on the other hand, have a family history of atopy; 46 of 182 patients have children with confirmed allergic manifestations.

In the occasional patient who is clearly atopic before "aspirin sensitivity" develops, the clinical picture is not that of an atopic person who has simply acquired a new sensitivity. Without exception, the whole clinical syndrome shifts from intermittent symptoms, with usually clear-cut cause-and-effect correlation, to perennial symptoms without a pattern that can be recognized. For a brief time the previously identified allergens might continue to produce exacerbations of symptoms during exposure, but this changes rapidly. Clinical sensitivity to the previously significant allergens is lost—it is as though an entirely new, more profound disease has superseded the primary atopic state.

Serial throat and sputum cultures established that bacterial infection was of only minor significance in our series of patients. Pathogens were found less frequently (1:3) than in throat and sputum cultures, obtained during identical seasons, of non-selected asthmatic patients with cases of equal severity. We cannot state that initial infection does not play a role in bringing about the changes in skin and mucous membranes which eventually render them sensitive to aspirin, but we can state that extensive surgery of the nose and paranasal sinuses, carried out in eight of our patients before admission—Caldwell-Luc operation to remove foci of infection and establish better drainage—failed to alter the natural history of the disease.

THE REVERSIBILITY OF ASPIRIN-SENSITIVE BRONCHIAL ASTHMA

The respiratory tract of aspirin-sensitive patients does not present any unique functional behavior. Like patients with other forms of "intrinsic" bronchial asthma, aspirin-sensitive asthmatics are sensitive to intravenous administration of histamine and methacholine (Mechohyl[®]) (15). Particularly during the early stages of the disease, bronchial asthma of aspirin-sensitive patients responds readily to bronchodilators.

The reversibility of the early bronchial asthma of aspirin-sensitive patients is impressive. It seemed to us that it might be the bronchial equivalent of the initial intermittent rhinorrhea and might provide a clue as to the pathogenesis of the syndrome.

An attempt was made to compare the effect of histamine and methacholine on the vital capacity of aspirin-sensitive (nonatopic) asthmatics and non-aspirin-sensitive (clearly atopic) asthmatics of comparable severity. We infused methacholine chloride and histamine phosphate intravenously under electrocardiographic control and determined the amount required to induce a 40% reduction in the 1-sec vital capacity. Methacholine chloride was given in increments of 25 μ g, histamine, of 10 μ g (of histamine base). The results are summarized in Tables 3 and 4: The amounts of the drugs required to produce a 40% reduction in vital capacity in asthmatics with aspirin intolerance are significantly less (histamine, $P < 0.001$; methacholine, $P < 0.05$) than those in a similar group of non-aspirin-sensitive patients with bronchial asthma of equal severity. In fact, it was not possible to achieve a 40% reduction in 1-sec vital capacity in the majority of the "allergic" patients, because side effects—that is, violent headache after injection of histamine and first-degree heart block after injection of methacholine—made it necessary to discontinue the infusion before the end point was reached. Once, methacholine chloride produced rather severe bronchial asthma that was rapidly terminated by intravenous injection of atropine sulfate.

While acetylsalicylic acid is outstanding in its ability to precipitate severe reactions, associated idiosyncrasies occur and are listed in Table 2. Of foods that were impli-

cated, fresh pork, sweet corn, soft drinks, and cheese crackers predominate. None of the patients who reported reactions after ingestion of food gave positive skin reactions to the food under suspicion. Analysis of the suspected foods established the presence of multiple additives in all but two of the samples. Of preservatives, sodium benzoate was used most commonly; of coloring matter, hydrazine yellow—"tartrazine"—(Food, Drug, and Cosmetic yellow no. 5) turned out to be a component of all but one of the yellows in orange foods. The ability of hydrazine yellow to induce angioedema and respiratory symptoms in susceptible patients has been previously described (16, 17), but it had not been shown that reactions to hydrazine yellow and to aspirin coexist in the same patients: Figure 1 shows the structure of hydrazine yellow and of its metabolites (18).

The incidence of penicillin and sulfonamide sensitivity in this group is probably not any higher than in a random population, but morphine, morphine derivatives, and codeine produce striking and severe reactions in a limited number of patients. Reactions to aminopyrine and antipyrine—

TABLE 2. Associated Sensitivity in 132 Aspirin-Sensitive Patients

Sensitivities to:	No. of Patients
Foods	26
Alcohol (in various forms)	14
Tartrazine (Food, Drug, and Cosmetic yellow no. 5)	14
Seasonal and/or environmental inhalants	10
Perfumes and odors	12
Drugs	
Penicillin	11
Morphine, morphine derivatives, codeine	16
Antipyrine and aminopyrine	4
-caine compounds	5
Sulfonamides	3
Isoniazid	1

which are not commonly used at present—are comparatively rare but, if they occur, resemble reactions produced by acetylsalicylic acid.

SPECIFICITY OF INTOLERANCE TO ACETYSALICYLIC ACID

The assumption that patients who cannot take aspirin are allergic to it is probably due to the peculiar features of the

TABLE 3. Amounts of Histamine and Methacholine Required to Induce a 40% Drop in Vital Capacity in 11 Non-Aspirin-Sensitive Patients Suffering from Bronchial Asthma

Name	Sex	Age	Weight	Basal Vital Capacity		Histamine		Methacholine	
				1-sec	Total	Absolute	Per Kilogram	Absolute	Per Kilogram
		yr	kg	liters		μg		μg	
L. P.	M	40	52.3	1.60	2.00	100*	1.91	520	9.94
H. Z.	F	37	59.1	1.20	1.25	100*	1.69	400†	6.76
B. W.	F	50	50	1.00	1.20	10	0.20	75	1.50
R. T.	F	46	68.2	1.20	1.85	100*	1.46	350†	5.13
L. M.	F	13	55.5	2.0	2.90	70*	1.26	250†	4.50
P. S.	F	50	61.8	1.7	3.10	20	0.29	270†	4.36
C. U.	F	45	59.1	2.00	2.15	100*	1.69	250†	4.23
F. L.	M	35	91.8	1.40	1.45	100	1.08	450	4.90
V. Z.	F	33	56.8	2.30	3.00	90*	1.58	400	7.05
J. A.	M	22	100	2.50	4.00	100	1.00	600	6.00
R. L.	M	22	81.8	5.00	5.40			350	4.27
						Mean = 1.22 $\mu\text{g}/\text{kg}$		Mean = 5.23 $\mu\text{g}/\text{kg}$	

* No effect on vital capacity—titration terminated because of violent headache

† No effect on vital capacity—titration terminated because of heart block.

TABLE 4. Amounts of Histamine and Methacholine Required to Induce a 40% Drop in Vital Capacity in 11 Aspirin-Sensitive Patients Suffering from Bronchial Asthma

Name	Sex	Age	Weight	Basal Vital Capacity		Histamine		Methacholine	
				l. sec.	Total	Absol-ute	Per Kilogram	Absol-ute	Per Kilogram
		yr	kg	liters		µg		µg	
M. F.	F	62	86.4	1.10	2.20	20	0.23	250	2.77
J. J.	M	59	58.7	1.20	1.80	10	0.17	200	3.49
V. P.	F	45	65.9	1.50	1.90	30	0.45	240	3.49
F. P.	M	40	86.5	2.40	3.40	45	0.52	250	2.89
V. P.	F	47	70.5	1.40	2.20	10	0.14	100	1.42
J. S.	M	24	63.6	3.30	3.70	25	0.39	200	3.14
S. T.	M	41	65	2.90	3.50	50	0.76	400	6.15
V. G.	F	19	47.7	1.60	3.00	35	0.73	200	4.19
G. B.	M	47	99	2.90	4.25	20	0.20	300	3.03
E. P.	M	49	54.5	2.80	3.80	20	0.36	350	6.42
F. J.	M	51	75.7	0.95	2.30			250	2.91

Mean = 0.39 µg/kg Mean = 3.63 µg/kg

syndrome: The explosiveness of the reactions makes it attractive to compare the syndrome with anaphylaxis, which is similarly explosive.

It is interesting that a significant number of patients were not aware at the time of their admission that they were sensitive to aspirin and had to be given aspirin as part of their clinical survey. The decision to establish aspirin sensitivity by actual exposure was not made lightly. It requires anticipation of severe angioedema, bronchial asthma, cyanosis, asphyxia, and, possibly, coma, and should not be undertaken unless an anesthesia team is present to maintain open airways if necessary. On the other hand, it is obviously safer to administer aspirin to patients with suspected intolerance under controlled hospital conditions than to take a chance that they might obtain it, in one of many proprietary preparations, on the outside; but while safer, it is not safe.

Thirty-six patients who maintained that they could take aspirin without ill effects were given 0.3 g of acetylsalicylic acid: 34 developed reactions. Symptoms may appear almost at once; they begin in most patients within 20 min but occasionally

after an interval of up to 2 hr. The onset of the reaction is marked by profuse watery rhinorrhea and, often, by a vivid scarlet flush of head, neck, upper chest, and extremities. At times, the initial reaction is followed by nausea, vomiting, intestinal cramps, and diarrhea. Bronchoconstriction, wheezing (without impressive cough), and cyanosis occur, as a rule, within a few minutes after the nasal symptoms.

Pretreatment with corticosteroids did not prevent aspirin-induced bronchial asthma, but corticosteroids administered after the onset of symptoms seemed to shorten the period of recovery; the evaluation of their effectiveness is difficult, however, since they were used only in patients who failed to respond to nonsteroid therapy. As a rule, epinephrine (aqueous, 1:1,000, 0.4 ml intramuscularly) and aminophylline are sufficient to control reactions. Aminophylline is the drug of choice but must be given by syringe (500 mg in 20 ml, injected slowly), followed by intravenous infusion (500 mg in 250 ml 5% glucose, every 4 to 6 hr). Neither epinephrine nor aminophylline had any apparent effect on the rhinorrhea or on the cutaneous flush. Most patients recover from acute reaction within 2 hr, but a general feeling of malaise and excessive bronchial secretion might persist for several days. We induced several episodes but did not encounter shock or fatalities.

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INTOLERANCE TO ASPIRIN

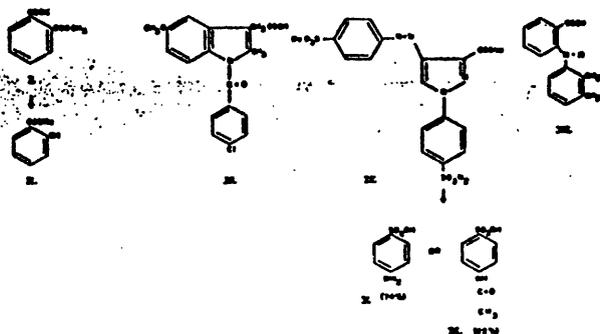


FIGURE 1. I. Acetylsalicylic acid. II. Sodium salicylate. III. Indomethacin. IV. Tartrazine (hydrazine yellow = Food, Drug, and Cosmetic yellow no. 5). V. Sulfanilic acid. VI. *p*-acetamidobenzenesulfonic acid. VII. Mefenamic acid. Compounds I, III, and IV induce reactions in aspirin-sensitive patients; compounds II, V, and VI are innocuous; compound VII is suspected.

Regardless of history, each of the 182 aspirin-sensitive patients was given, in rotation, at least 1 of the following drugs: [1] sodium salicylate, [2] salicylic acid esters with lengthening side chain in the ortho-position, [3] choline salicylate, [4] thioaspirin, and [5] *N* acetyl-*p*-aminophenol. Considering multiple tests, each drug was taken by 40 aspirin-sensitive patients: No untoward reactions occurred.

It is certain that intolerance to acetylsalicylic acid is not an intolerance to salicylates. Aspirin, however, is not the only chemical that causes symptoms in the skin and respiratory tract of aspirin-sensitive patients. We have previously mentioned that tartrazine is poorly tolerated by a significant number of patients who cannot tolerate aspirin. Recently, we observed that indomethacin—an indole derivative which has no structural relationship to acetylsalicylic acid—induces severe "aspirin-like" reactions in aspirin-sensitive patients. Since then we have confirmed this coexisting intolerance in 18 hospitalized patients who developed intense broncho-obstructive symptoms after administration of small doses of indomethacin. None of these patients had previously taken indomethacin;

and there is no reason to assume that they had an opportunity to develop a preexisting immunological sensitivity to the drug (19).

It should be noted that aspirin and indomethacin are effective minor analgesics, and the structural relationship between hydrazine yellow and aminopyrine is evident (Figure 1). In fact, it would not be surprising if hydrazine yellow—given in adequate doses—would turn out to provide effective relief for peripheral pain. By the same token, it is almost certain that the mode of action of one of the recently synthesized minor analgesics, mefenamic acid (Figure 1), is so similar to the mode of action of aspirin that it must be expected to produce aspirin-like reactions in aspirin-sensitive patients.

NONIMMUNOLOGICAL FACTORS IN THE PATHOGENESIS OF BRONCHIAL ASTHMA

Several investigators have suggested that bronchial asthma cannot be explained by an immunological sequence alone but might be associated with abnormal autonomic controls. Eppinger and Hess (20), Nakamuro (21), and Tiffeneau (22), for instance, have labeled bronchial asthma a

"vagotonic disease." More recently, an inadequacy of the beta-adrenergic-bronchodilator-receptors of the bronchial mucous membrane has been suggested as a possible explanation for the nonspecificity of bronchial asthma (23, 24). Neither sympathetic nor parasympathetic receptors, however, appear to participate in the pathogenesis of the bronchial asthma of aspirin-sensitive patients.

Even extrinsic bronchial asthma is a disease of multiple factors. The search for significant mediators of the immunological sequence, for instance, has yielded significant new information, but it is rarely certain which of the substances that have been identified—for example, histamine or the slow-reacting substance of anaphylaxis (SRSA) (25)—accounts for the clinical symptoms in the individual patient. Patients who suffer from aspirin-sensitive bronchial asthma are more closely "intrinsic" (to use Rackemann's terminology (26)) than in any other type of bronchial asthma (27, 28): More than 60% of our patients listed sensory stimuli, for example, exposure to draft, change in weather, contact with fumes and odors, as precipitating factors.

The clinical course of "extrinsic" bronchial asthma reflects the patient's exposure to inhalant allergens even though the severity of the symptoms might vary from patient to patient or within the same patient from season to season: The aspirin-sensitive patient, on the other hand, has "intrinsic" symptoms that occur "without rhyme or reason" (to use an expression that aspirin-sensitive patients seem to favor) and that are not correlated with seasonal or environmental exposure to allergens.

If the respiratory symptoms that precede the development of intolerance to aspirin in aspirin-sensitive patients are not the result of an immunological sequence, they must be due to the action of nonimmunological mediators or chemoreceptors that can cause vasodilation, secretion of mucus,

and bronchoconstriction. The analysis of our findings has led us to conclude that rhinitis and bronchial asthma of aspirin-sensitive patients are initiated by peripheral chemoreceptors that had not been known to participate in the control of respiration. It is likely that these receptors are kinin receptors since kinins—for example, the nonapeptide, bradykinin—have been identified in recent years as mediators of noxious stimuli in skin and respiratory membranes (29-31). Receptors that are stimulated by kinins may initiate an axon reflex, that is, wholly peripheral vascular response, or transmit stimuli to the dorsal root ganglion and beyond.

Our observations suggest an unusual responsiveness of the chemoreceptors of the nasal and bronchial mucous membranes of aspirin-sensitive patients. Unusual responsiveness alone, however, does not explain the unusual effect of aspirin on the skin and the respiratory tracts of these patients. Recent studies have clearly established that aspirin is an antagonist of bradykinin (32, 33). In aspirin-sensitive patients, on the other hand, it has a paradoxical effect that we attribute to a preexisting injury of the chemoreceptors in skin, or mucous membranes, or both (19): The antagonist has become an agonist and produces the stimuli that it ordinarily prevents.

If our interpretation is correct, it may become understandable why other analgesics which, like pyrazolones and indomethacin, act on the same receptors, induce comparable reactions.

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TARTRAZINE REVISITED

by Monte S. Cohen

TARTRAZINE, KNOWN ALSO AS CI 15950 YELLOW 23, C.I. 19130, hydrazine yellow, or FD & C. yellow Number 5, a pyrazole aniline dye, is the trisodium salt of 3-carboxy-5-hydroxy-1-*para*-phenyl-4-*para*-sulfophenylazopyrazole, and has an empirical formula of $C_{12}H_7N_3Na_3O_5$. Tartrazine is a bright orange-yellow powder freely soluble in water. The addition of sodium hydroxide imparts a red color to the solution.¹

Tartrazine is used extensively in the drug, food, and cosmetic industries as a dye for capsules and tablets as well as a dye for many foodstuffs. Tartrazine has been in common use in food since about 1910. Bright yellow is found in candies, cakes, and confections having a lemon-yellow appearance and in a great number of yellow and lime green foods. Tartrazine is combined

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with blue to yield a true lime color; the use has extended to carbonated and noncarbonated beverage drink powders such as 'Tang' and many remedies. Tartrazine is found in home food coloring and in Easter egg dyes. Tartrazine is a component of hair waves, hair dyes, face powders, lotions and other products and is used as a dye in and wool.

Tartrazine has been implicated in various type allergic reactions over the years. Admittedly, the occurrence of such reactions are rare; 18 patients exhibiting this reaction were reported as of August 1972; 25 such cases as of June, 1973² according to published literature. However, such allergic reactions are probable throughout. A listing of tartrazine should be required on drug and food packages; such information might be saving of food packages; a number of reactions occurred and not reported in the literature so the reported cases is a conservative figure.

It is also important to emphasize that allergic reactions are not limited to tartrazine; other approved food and drug dyes derived from coal tar as well as azo and aniline dyes have most certainly been implicated in causing allergic reactions,^{2,5} including Blue No. 1, Yellow No. 6, Red No. 4,⁷ Sunset Yellow, New, Cochine, and FD & C No. 3.⁸

Signs and Symptoms of Tartrazine and Other Dye-Induced Reactions

Severe, generalized pruritus,¹ itching of the tongue or uvula,² generalized urticaria,^{1,2,3,9} local edema of the lips, tongue, or uvula,¹ tingling of the mouth and tongue,² nausea,² vomiting,² severe headache,² nasal congestion,² tickling of the throat,² tight cough,² wheezing,² severe asthmatic attacks,² angioedema,² and vascular purpura² have been reported. A tickling of the throat, tight cough, and wheezing may persist for as long as 24 hours.² Heaviness of the chest was reported with Red No. 4.⁷ Such reactions have been reported with benzoic acid¹⁰ and its derivatives.¹¹ Symptoms of allergy occurred 6 to 14 hours after ingestion of dyes.² The one case of vascular purpura reported, to date, occurred in a 22-year-old caucasian woman.² Re-exposure caused a re-appearance of the bleeding; the extent of the bleeding was dose dependent and was sufficiently severe to require hospitalization and blood transfusions. The bleeding was manifested by purpura, menorrhagia, and/or bleeding from the bowel, gums, nose, or ear. Such episodes were sometimes accompanied by chills and fever. Complete recovery followed withdrawal of the substance.² Direct intracutaneous tests may give no wheal and flare; the passive transfer tests may be negative, and the positive reaction on the scratch-patch test may be negative. Immunologic studies, however, have demonstrated the presence of IgA, IgG, and IgM.² Reactions to tartrazine have occurred with varying amounts of the dye from trace amounts to 750 mg — 1 mg dissolved in 20 ml water² to 25 mg² to 1 ml of a 1:1000 dilution administered sublingually.² Parenteral adrenergic agents as well as oral ephedrine sulfate in a 25 mg dose are useful agents in alleviating the response.² Studies were performed to correlate reactions to tartrazine with persons allergic to aspirin. Michaelsson and Jullin¹² showed that the majority of patients exhibiting a hypersensitivity to dye additives also reacted to aspirin.² Erdemian and Cohen¹³ demonstrated that a significant number of patients reacting to tartrazine, parabens, sodium benzoate, or FD & C No. 3 exhibited reactions and cross reacted with aspirin. On the other hand, Jullin, *et al.*,¹⁴ and Sauter and Myers¹⁵ showed that the frequency of tartrazine reactions is fairly low among patients sensitive to aspirin.

Procedure and Inclusions of Data Collection¹⁶

The manufacturer's section of the 1974 *Physicians Desk Reference* was consulted as a base for writing a form letter to selected drug manufacturers and asking for a list of their drugs for internal use containing tartrazine in any amount. The following compilation is by no means complete, although the major manufacturers of drugs

in the United States are present herein and is the most complete compilation of tartrazine-containing drugs to date. Such listings are dated, in parentheses, as to when a specific manufacturer dated his letter with his drugs containing tartrazine to me. The drugs are tabulated as to trade name because of differences in formulation among trade names of a specific generic product. Cosmetic and food companies were not written to since a listing of brand name hairdolls and cosmetics containing tartrazine fall beyond the purview of this article. Such factors among many others, are obviously important when selecting products for a formulation or for a specific patient.

It cannot be overemphasized that such information presented herein is accurate as to the particular dates appearing after each manufacturer. As new guidelines on production methodology evolve by the FDA as well as the manufacturers themselves, the formulae of drug products may be changed with or without notice.

Conclusion

The potential hazards of excipients and other additives to different brands of generic products are real. The rationale that the rarity of such reactions is not of clinical importance is folly and dangerous. It is time that the Food and Drug Administration require manufacturers of drugs, foods and cosmetics to label excipients and additives in their products.

ABSTRACT

A compilation of tartrazine (FD and C No. 3) containing drugs from major pharmaceutical manufacturers in the United States of America appears.

Characteristics of tartrazine, spectrum of its use, incidence of allergic reactions and relationship with other food and drug dyes are discussed, as are the clinical manifestations of such reactions. The importance of excessive tartrazine disclosure, as well as the disclosure of other excipients and additives by the Food and Drug Administration is emphasized.

Drugs containing tartrazine dye

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The symptom complex of aspirin sensitivity, nasal polyps, and asthma has been recognized for many years as a syndrome found in a significant number of adult intrinsic asthmatics.¹⁻³ Recent evidence suggests that aspirin sensitivity with or without nasal polyps may also be present in patients with an extrinsic component to their asthma, in children and in some families.^{3,4} In fact, some severe asthmatics may be sensitive to aspirin without realizing it.³

How aspirin produces symptoms in this syndrome remains obscure. Several other unrelated drugs and chemicals have also been found to precipitate reactions in some aspirin-sensitive individuals. These include indomethacin,⁵ Na benzoate, mefenamic acid, aminopyrine, and F. D. & C. yellow No. 5 tartrazine dye.^{3,6} Management of these patients must include avoidance of aspirin and other cross-reacting drugs if they are felt to be contributory.

The exact frequency of cross-reactivity to tartrazine remains controversial, with reports varying from less than 10% to over 80% of aspirin-sensitive patients.^{5,7,10} Also unknown is the quantity of tartrazine necessary to provoke a reaction. Reports in the literature relate challenge doses ranging from 1 mg to as high as 25 mg.^{5,11} More recently, an investigator used challenge doses no higher than 0.44 mg to determine tartrazine sensitivity.⁸ We recently produced a severe reaction in a patient with 1 mg of tartrazine, a previously accepted dose to begin a challenge.

CASE REPORT

Our patient, a 27-year-old man, presented with a history of asthma of 1 yr duration. At the time he was seen he had daily wheezing, which required regular administration of bronchodilators. A history of severe exacerbations of asthma on several occasions following the administration of aspirin was obtained. Sinus films were normal, and the patient did not have nasal polyps. The personal and family histories were negative for atopy, and intradermal skin tests for common inhalant allergens were negative except for several moderate reactions to grass pollens, which were not felt to be clinically significant.

Because of the clinical impression of aspirin sensitivity and the concern that the patient's severe asthma might also be associated with tartrazine sensitivity, it was elected to give him an oral challenge of tartrazine. In a fasting state, with bronchodilators withheld for 6 hr and with adequate baseline pulmonary functions, the patient was given 1 mg of tartrazine in 6 oz of water. Within 2 min he complained of substernal burning and chest tightness. Severe dyspnea associated with marked wheezing was noted within 5 min. Subcutaneous 1:1,000 epi-

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ephrine 0.3 cc was given, but the patient's bronchospasm increased. Intravenous aminophylline 130 mg, Solu-Medrol 250 mg, and Benadryl 25 mg were administered, as well as additional subcutaneous epinephrine. The patient's symptoms failed to abate completely, and he was admitted to the intensive care unit where bronchodilator and corticosteroid therapies were continued. Six hours following the challenge he was asymptomatic. There was no recurrence of symptoms in the subsequent 24 hr, and the patient was discharged.

From this case report it can be seen that a low dose of tartrazine, an amount not infrequently encountered in medications, may provoke exceedingly severe reactions. Although most of the tartrazine used in this country is found in foods, tartrazine is in widespread use in the pharmaceutical industry. Tartrazine-containing drugs have definitely been shown to provoke reactions in sensitive individuals.¹² Tartrazine is a stable dye and is used in producing not only a bright yellow color but also many other colors such as maroon, rust, and turquoise. Quantities of tartrazine dye employed vary tremendously. For example, a 50-mg Cytan tablet contains 0.0018 mg tartrazine per tablet, while Questran contains 2.7 mg per tablet. The exact quantity of dye sufficient to cause a reaction is unclear. At this time it is difficult to estimate a tolerable quantity of dye which might be safely ingested by a sensitive individual. Considering the severity of reaction of our patient to 1.0 mg of tartrazine, it is doubtful that any amount of tartrazine could be tolerated by such an individual. Some drugs do contain only a minute quantity of dye, but no recommendations can be made regarding their safety.

The avoidance of tartrazine dye in medications is a significant problem to the physician. The presence of additives is not routinely listed on package inserts or reference materials available to the physician, including *Physicians' Desk Reference (PDR)*. Because of this we have compiled a list of drugs known to contain tartrazine dye. The list represents data compiled from drug companies responding to letters of inquiry sent in the fall of 1974 to all companies listed in the 1974 PDR. Those not responding received a second letter. From 198 companies queried there were 160 replies.

There was considerable variation in the replies we received. Many drug companies informed us that they were completely unaware of any problem with tartrazine dye. A few said that the incidence of tartrazine sensitivity in the general population was so low that this was not of importance to the drug industry. Several drug companies refused to give this information, stating that it was too great a task or that they had no way of obtaining this information on all of their products. Many companies stressed that although the list they sent was accurate on that day, addition or withdrawal of additives was subject to change without notice.

As more reports of sensitivities to drugs and drug additives appear in the literature, it becomes apparent that information about drug ingredients is essential. Every pharmaceutical company should have available complete listing of all of the contents of every product with which they deal, whether they manufacture all of the drug, part of it, or merely distribute it. In addition, this information should be included in package inserts and product information available to the physician. Notification of change of formulation of a drug, even if involving only an additive or preservative, should be required.

Recently, pharmacists and government officials have been advocating generic substitution legislation. In essence, this legislation would allow the pharmacist to substitute a drug generically equivalent to the brand name drug prescribed by the physician. Unless such legislation includes a provision for the physician to prevent any substitution if he so indicates, there will be no way to prevent a patient sensitive to an additive from receiving that additive. How can a pharmacist know that one brand has specifically been prescribed because the physician knows it does not contain a certain additive to which his patient reacts?

The following list of tartrazine-containing drugs is offered as an aid in managing tartrazine-sensitive patients until such time as changes in labeling are made. Drug companies whose products contain no tartrazine are listed first. This is followed by an alphabetical listing of manufacturers who responded with complete lists of dye-containing drugs. Drug companies that did not respond or supplied incomplete data are listed at the end. As was stressed by many of the companies, these data were accurate at the time their letter was sent and are subject to change without notice. In most cases a drug is listed by the company manufacturing it and may not be mentioned by the company distributing it.

DRUG COMPANIES WHOSE PRODUCTS DO NOT CONTAIN TARTRAZINE

Alcon Laboratories, Inc. (see Webeon)
B. F. Ascher & Company, Inc.
Astra Pharmaceutical Products, Inc.
Beutlich, Inc.
Brunswick Laboratories, Inc.
Burton, Parsons & Company, Inc.
Chiblochem
Calvin Chemical Corporation
Campbell Laboratories, Inc.
Comatic Laboratories, Inc.
Cutter Laboratories, Inc.
Doak Pharmaceutical Company, Inc.
Dome Laboratories
Elkins-Sinn, Inc.
Emko Company
Fellows Medical Manufacturing Company,
Inc. (Chromalloy Pharmaceuticals, Inc.)
Fluoritab Corporation
Fort David Laboratories, Inc.
Gerber Products Company
Geriatric Pharmaceutical Corp.
Glennco Laboratories, Inc.
Hollwig Pharmaceuticals
H. S. Herbert Laboratories

Hollister-Stier Laboratories
Hynson, Westcott & Dunning, Inc.
International Pharmaceutical Corp.
Keene Pharmaceuticals, Inc.
Mayrand, Inc.
Medicone Company
Medies Pharmaceutical Corporation
Metabolic Products Corporation
Metro Med, Inc.
Miller Pharmaceutical Company, Inc.
Owen Laboratories
Person & Covey, Inc.
Ror Chemical Company
Rystan Company, Inc.
Schmid Laboratories, Inc.
Serono Laboratories, Inc.
Star Pharmaceuticals, Inc.
Texas Pharmaceutical Company
Ulmer Pharmaceutical Company
Unimed, Inc.
Violin Corporation
Webeon Pharmaceuticals (Alcon Laboratories)

DRUG COMPANIES WHOSE PRODUCTS CONTAIN TARTRAZINE

Abbott Pharmaceutical Products Division
(Ross Laboratories)
Ascorbic Acid Filmtab Tablet 500 mg
Cefal Filmtab Tablet
Cobichine 0.5 mg Tablets

Cobichine 0.5 mg Tablets
Compoceillin-VK Filmtab Tablet 250 mg
and 500 mg
Compoceillin V Oral Suspension
Dayamin Capsules

Enduronyl Tablets
 Erythrocin Liquid-100
 Harmonyl 0.1 mg Tablet
 Hert Folic 500 Filmitab Tablet
 K-Lor Powder 15 mLq and 20 mLq
 Norisodrine Syrup with Calcium Lactate
 Ogen 0.025 Tablets and 5 Tablets
 Panwarfin Tablets 7.5 mg
 Paralione Capsules 300 mg
 Pedinmycin 400
 Placidyl 750 mg Capsule
 Sodium Phosphate-Biophosphate Solution,
 Flavored
 Trai 25 mg Filmitab Tablets
 Vi-Daylin with Fluoride Chewable Tablets
Aufre-Grant, Inc.
 Biocaps Tablets
 C₂ as Capsules
 N₂ Jorovan Tymcaps
 Nitrovas Tablets
 Prefort Capsules
 Zymogest Tablets
Arch Laboratories (Lewis-Howe Company)
 Lemon Tums Antacid Tablets
Armour Pharmaceutical Company
 Letter 0.05, 0.2, 0.3 mg
 Nicolid
 Nicolar
 Thyrolar ½, 2, 3
Arnar-Stone Laboratories, Inc.
 Belbarb No. 2 Tablets
 Emesert No. 2 Rectal Suppository
 Hasucode Strong Tablets
 Hasucode Tablets
 Isoolor Expectorant Elixir
 Isoolor Tablets
 Isoolor Timesubs (Capsule Shell)
 Isoolor Tablets 75, 150, 300 mg
Ayerst Laboratories, Inc.
 Atromid-S Capsules
 Beminal 500 Capsules
 Beminal Fortified with Iron and Liver
 Capsules
 Clusivol Capsules
 Clusivol Chew Tablets
 Inderal Tablets 10, 40, 80 mg
 Kerodex Cream
 Mediatric Capsules
 Mysoline Suspension
 PMB 200 Tablets
 Plegine Tablets
 Premarin Tablets 0.3 mg, 1.25 mg
 Premarin Vaginal Cream

Product discontinued.

Prenatin 1.25 mg with Methyltestoster-
 one, 10 mg Tablets
 Thiocinal A Forte Tablets
Barnes-Hart Pharmaceuticals, Inc.
 Nidolax
Beecham Laboratories
 Adrenascan Tablets
 Conar-A Suspension
 Cotrol-D Liquid, and Tablets
 Elixir Hybephen
 Hylephen Tablets
 Hycal (Line)
 Livitamin Chewable Tablets
 Livitamin Prenatal Tablets
 Menest Tablets 0.3 mg
 Salsedrox Tablets
 Theralax Tablets
 Tincture Phe-Mer-Nite
 Veterinary Mycodex Pet Shampoo
Biocraft Laboratories, Inc.
 Dalierene Ointment
Boehringer Ingelheim Ltd.
 Alupent Tablets 20 mg
 Dulcolax Tablets 5 mg
 Proludin Endurets 75 mg
 Proludin Tablets 25 mg
Bron Laboratories, Inc.
 Fergon Tablets
**Bristol Laboratories (Bristol-Myers Com-
 pany)**
 Azotrex Capsules
 Bristacycline Capsules
 Bristamycin Tablets
 Dynapen Oral Suspension
 Polycillin Capsules
 Polycillin-PRB
 Prostaphlin Capsules
 Salutensin Tablets
 Syncillin Tablets
 Tegopen Capsules
 Tetrex Capsules
 Tetrex BID Capsules
 Versapen Capsules
Burroughs Wellcome Company
 Artifel Syrup
 Euprazil Tablets
 Fedravit Tablets
 Lanoxin Elixir
 Lanoxin Tablets 0.125 mg
Carmick Laboratories
 Auen
 Bontil PDM

- Capital with Codeine
Hormonia
Minotal
- Central Pharmaceutical Company
Anabalm Green
APC (Greenies) Tablets
Asphar-G Green Tablets and Yellow
Tablets
Bilate Tablets
Bisilad
Cengesic Tablets
Cen-Asap Tablets
Cholineth Tablets
Codinal-DM
Coditate Tablets
Co-Xan
Neocylate Tablets
Niferex P. A. Tablets
Niferex Prenatal Tablets
Niferex with Calcium Tablets
Prednicen Tablets
Resereen Tablets
Rhinihist
Synate-M 1/2 strength Tablets
Synophedal Tablets
Synophylate 2 1/2 gr Tablets
Synophylate-GG Tablets
Triksulfazine Tablets
- Cetylite Industries, Inc.
Cetarsaine
- Ciba-Geigy Pharmaceuticals Division
Anturane 200 mg
Aprosaline Tablets 10 and 100 mg
Aprosaline-Esidx Tablets 25/15
Butazolodin
Butazolodin Alka
Butazolodin Film-Coated Tablets
DIB-TD 100 mg
Diambol Tablets 2.5 and 5 mg
Esidx Tablets 50 mg
Forthistal Syrup
Forthistal Lontabs
Forthistal Tablets 1 mg
Ismolin Tablets 10 mg
Metandren Linguets 10 mg
Metandren Tablets 25 mg
Proscoline Lontabs
Pyrilbenzamine Expectorant with Codeine
Pyrilbenzamine Expectorant with Ephed-
rine
Pyrilbenzamine Lontabs 50 mg
Pyrilbenzamine Tablets 25 mg
Rimectane
Ritaline Tablets 5 and 20 mg
- See-App-Ex
Serpasil Aprosaline No. 1 and No. 2
Serpasil Elixir
Serpasil Esidx Tablets No. 1 and No. 2
Sterazolidin
Tandemil Sugar-Coated Tablets
Tofranil PM 75, 100, 125, and 150 mg
Tofranil Sugar-Coated Tablets
Transentine/Phenobarbital Tablets
- Circle Pharmaceuticals, Inc.
Circavite-T Tablets
Tusquelin Cough Syrup
Uroptic Tablets
- Cole Pharmaceutical Company, Inc.
Baudy Lintment
Calciorbic Tablets
Sol-Eze Tablets
Xanthinax Tablets
- Cooper Laboratories, Inc.
Aminodur Dura-Tabs
Anodynes DHC
Cystex Tablets
Deconamine Tablets
Doxychol AS
Elixophyllin
Elixophyllin KI
Ergomar Tablets
Ferronord Tablets
Neo-Sedaphen
Nuna Dura-Tabs
Persistin Tablets
Vitron-C Plus Tablets
- Derna-Arts Laboratories (see Marion)
- Diata Products Company (see Eli Lilly)
- Dooner Laboratories, Inc.
Dularin Syrup
Dularin TH Tablets
Slo-Fedrin 30 Gyrocaps
Slo-Fedrin 60 Gyrocaps
Slo-Fedrin A30 Gyrocaps
Slo-Fedrin A60 Gyrocaps
- Dermik Laboratories, Inc.
Vanoxide and Vanoxid- HC Lotions
- Dorsey Laboratories
Asbron Elixir
Metaprel Tablets
Triaminic Expectorant
Triaminic Expectorant with Codeine
Triaminic Expectorant DH
Triaminic Tablets
Tusminic Tablets
- Dow Chemical Company
Novahistine Elixir
Novahistine Expectorant

- Novabistine I.P.
 Quile Tablets 10 and 25 mg
 Doyle Pharmaceutical Company
- Delmark Egg Nog Mix
 Delmark Milk Shake Mix, Vanilla Flavor
 Delmark Quick Creamy Pudding Mix,
 Vanilla and Butterscotch Flavors
 Delmark Quick Custard, Vanilla, Banana,
 Lemon, Coconut, and Butterscotch
 Flavors
 Delmark Quick Danish Souffle Mix
 Delmark Quick Tapioca Pudding Mix
 Dietary, Vanilla Flavor
 Merimix Real Egg Custard
 Meritene, Egg Nog Flavor
 Precision High Nitrogen Diet
 Precision-I.R. Diet, Orange, Lemon, and
 Lime Flavors
 Eaton Laboratories (Morton-Norwich Prod-
 ucts, Inc.)
- All Chloro-septic Products
 Furoxone Tablets 100 mg
 Jeenen Liquid Douche
 Nels Tablets
 NP-27 Liquid
 Pepto-Bismol Liquid (Green Mint)
 Endo Laboratories, Inc.
- Aron Capsules 25,000 and 50,000
 Crystalline Coumadin Tablets 7.5 mg
 Endecon Tablets
 Endoglobin Forte Tablets
 Endotussin-C Syrup
 Endotussin-NN Syrup
 Hycomine Compound Tablets
 Hycomine Pediatric Syrup
 Hycomine Syrup
 Mesopin Elixir
 Mesopin PB Elixir
 Mesopin PB Tablets
 Percodan Tablets
 Pycrogesic Tablets
 Remsol Tablets 5 mg
 Sodium Warfarin Tablets 7.5 mg
 Symmetrel Capsules
 Valpin Elixir
 Valpin PB Tablets
 Vifort Capsules
 Federal Pharmaceutical Corporation (see
 Ormont)
- Pisons Pharmaceutical Division (Lepfert)
 Intal Gelatin Capsules
 C. B. Fleet Company, Inc.
 Phospho Soda, Flavonol
 Phospho Soda, Regular
 Summer's Eye
 Summer's Eye, Herbal
 Fleming and Company
 Extendryl Chewable Tablets
 Flint Laboratories (Travenol Laboratories)
 Choloxin 2 and 6 mg
 Synthroid 0.1 and 0.3 mg
 Fuller Laboratories, Inc.
 Glycerol Phosphate Eucema
 Glenbrook Laboratories
 Bayer Children's Aspirin^{*}
 Bayer Decongestant Cold Tablets (adult
 only)
 Gray Pharmaceutical Company
 G. B. Prep Emulsion
 Guardian Chemical Corporation
 phlo-c-phind Tablets 0.25 gm green and
 0.5 gm orange
 Hoechst Pharmaceuticals, Inc.
 Doxinate Capsules 60 and 240 mg
 Holland-Rantos Company, Inc.
 Nylmerate Solution
 Hoyt Laboratories
 Fluorigard Mouthrinse
 Laride Drops
 Laride Tablets Lemon and Lime Flavors
 Phos-Flur Rinse Supplement Lime Flavor
 Thera-Flur Gel-Drops
 K'N Pharmaceuticals, Inc.
 Chlorpheniramine maleate
 Folic Acid
 Prednisolone Tablets
 T.D. Cold Capsules
 Ingram Pharmaceutical Company
 Thex Forte Capsules
 Ives Laboratories, Inc.
 Cyclospasmol Tablet 100 mg
 Isordil Sublingual Tablet 2.5 mg
 Isordil Translids Tablets 40 mg
 Johnson & Johnson
 Chew-C Brand Vitamin C Chewable Tab-
 lets, Lemon-Lime Flavor
 Micrin Brand Gargle and Rinse
 Kumar Laboratories

^{*}Company plans to discontinue use of yellow No. 5 dye soon.

[†]Product still in test market; company plans to discontinue use of yellow No. 5 dye when product is ready for regular market.

- Dimenhydrinate 50 mg
 Key Pharmaceuticals, Inc.
 Spastical Tablet (P) and SA
 Knoll Pharmaceutical Company
 Dilaudid Cough Syrup
 Tensolin Tablets
 Theokin Tablets
 Vita Metrazol Tablets
Lakeside Laboratories
 Cantil with Phenobarbital Liquid
 Cantil with Phenobarbital Tablets
 Cantil Tablets
 Dactil with Phenobarbital Tablets
 Dactil Tablets
 Daclilase Tablets Film-Coated and Sugar
 Coated
 Iron FA Tablets
 Iron Tablets
 Metalydrin Tablets 2 and 4 mg
 Metatensin Tablets 2 mg
 Norpramin 25 and 50 mg
 Pipital Tablets
Lanmett Company, Inc.
 A.C.P. Compound Tablets green
 Apacomp Tablets
 Aquex Tablets 4 mg
 Bethanechol Chloride Tablets 25 mg
 Ceetolan Concentrate yellow
 Dextro-Amphetamine Sulfate Tablets 5
 mg
 Dimenhydrinate Tablets 50 mg
 Efricon Expectorant
 Olaban Tablets 35 mg
 Pentylan with Phenobarbital Tablets No.
 1 and No. 2
 Phenetron Tablets 4 mg
 Phenobarbital Tablets $\frac{1}{2}$ gr
 Phenobarbital Tablets green $\frac{1}{2}$ gr and 15
 mg
 Sodium Butabarbital Tablets 30 mg
 Veltane Tablets 4 mg
Lederle Laboratories
 Achromycin V Syrup
 Aristocort Tablets 2, 4, and 8 mg
 Aureomycin HCl 250 mg
 Decloxylin Tablets 75, 150, and 300 mg
 Falvin Capsules
 Ferro-Mandelts
 Ferro-Sequels
 Fibbon OT Tablets
 Folvite Tablets 1 and 0.25 mg
 Polyron Capsules
 Geval Capsules
 Geval T Capsules
 Gevrite Tablets
 Hydromox B Tablets
 Methotrexate Tablets 2.5 mg
 Nitrol Oral Suspension
 Pathitamate Tablets 200 and 400 mg
 Peribamin Capsules
 Peritlinic Tablets
 Promemina Capsules
 Stresscops
 Stressubs 600
 Tri Hemie 600
 Triple Sulfas Suspension
 Varidase Oral Tablets
**Eli Lilly and Company (Dista Products
 Company)**
 Amesex
 Aminopylline and Amytal Pulvules
 Aminosalicic Acid Tablets 500 mg
 Ammonium Chloride Tablets $7\frac{1}{2}$ and 15
 gr
 Amytal Tablet 100 mg pink
 Anhydron Tablet 2 mg pink
 A.S.A. 5 and 10 gr
 Aventyl HCl Pulvules 10 and 25 mg
 Berothin-T Tablet cinnamon brown
 Berothin with Vitamin C Pulvules
 Betalin Compound Pulvules
 Biron Pulvules 150 and 300 mg
 Cascan Tablet N.F. 5 gr chocolate-colored
 Clopane Hydrochloride Solution 0.5%
 Cwo-Diazine
 Co-Elorine Pulvules 25
 Cologel Liquid
 Compren Pulvules
 Co-Pyrroul Pulvules
 Crystodigin Tablet 0.15 mg yellow
 Darvon-N with A.S.A.
 Darvon-N Suspension
 Darvon-N Tablet 100 mg
 Diethylstilbestrol Tablets 0.1, 0.25, 0.5,
 1.0, 5, and 25 mg
 Diglicisin Tablet sugar-coated green
 Dynedor Tablet 500 mg yellow
 Ephedrine and Amytal Pulvules
 Epragen Pulvules
 Ferro-Betalin Tablet chocolate-covered
 Ferrous Sulfate Tablets 5 gr red, green,
 chocolate
 Halbrane Tablet 1 mg yellow
 Hepicobin Tablet
 Hista Clopane Pulvules
 Histadyl and A.S.A. Compound Pulvules
 Histadyl and Ephedrine Hydrochloride
 No. 1 and No. 2 Pulvules
 Histadyl Pulvules 25 and 50 mg
 Hisono Chewable Tablets 125 mg and
 N.F. 250 mg
 Hisono Pulvules 125 and 250 mg

- Hiosone Tablet N.F. 500 mg
 I-Sedrin Plain Solution
 Keffex for Pediatric Drops 100 mg
 Keffex Pulvules 250 mg
 Lextron and Lextron Ferrous Pulvules
 Mi-Celbrin Tablets yellow
 Mi-Celbrin T Tablet orange
 Multicebrin Tablet
 Novacebrin Chewable Tablet yellow
 Novacebrin with Fluoride Chewable
 Tablet orange
 Novrad with A.S.A. Pulvules
 Novrad Pulvules 50 and 100 mg
 Ox Bile Extract 5 gr
 Pancreatin, triple strength 5 gr
 veril Phosphate and Amytal Tablet
 green
 Potassium Chloride Tablet 300 mg and 1
 gm
 Potassium Iodide 300 mg
 Prunecodine
 Quinine Sulfate Tablet 5 gr chocolate-
 colored
 Reticulex Pulvules
 Rhinitis Tablet full-strength chocolate-
 colored
 Sandril Tablet 0.25 mg green
 Seconal Sodium 100 mg
 Seconal Sodium Pulvules 30, 50, and 100
 Sedatussin
 Sodium Chloride Tablets 15½ gr
 Sodium Salicylate Tablets 5 and 10 gr
 Tes-Tape
 Thyroid Tablets 30, 60, 120, and 200 gr
 Trisicon and Trisicon M Pulvules
 ulnal Pulvules 50, 100, and 200 mg
 Tylandril Tablet green
 Ultram Pulvules 300 mg
 Ultram Tablet 200 mg light green
 V-Cillin K Pediatric for Oral Solution 250
 mg
 White Pine and Ammonium Chloride Com-
 pound
 White Pine Compound Syrup
 White Pine Compound Syrup red men-
 tholated
 Mullinckrodt Pharmaceutical Products Divi-
 sion
 Barbidonna Elixir
 Barbidonna No. 2 Tablets
 Covaminine Tablets
 Covangescic Liquid and Tablets
 Dalnite K1 Tablets
 K1-N Tablets
 Lufyllin EPG Elixir*
 Lufyllin GG Tablets*
 Obotan Tablets
 Pyridium No. 2 Liquid
 QIDpen VK for Oral Solution 250 mg/5
 ml
 QIDlet Capsules 250 and 500 mg
 Rynn-Tussadine Expectorant Tablets
 Rynatuss Pediatric Suspension
 Vertavis Tablets
 Marceon Laboratories, Inc.
 Osonate-Plus Oral
 Mavlon Laboratories, Inc. (Derm-Arts Lab-
 oratory)
 Os-Cal Forte Tablets
 Os-Cal Gescic Tablets
 Os-Cal Tablets
 Os-Feo Vini Tablets
 Pretta Tablets
 Triton Tablets 1 and 2.5 mg
 McKesson Laboratories
 Axon Cold Tablets
 Bay Rum
 Kesso-Mycin Tablets
 Lady Esther Dry Skin Cream
 Oral Mouthwash yellow and red
 Prednisolone Tablets
 McNeil Laboratories, Inc.
 Rutibel Elixir
 Buticaps Capsules 30 and 50 mg
 Butigetic Tablets
 Butisoprazide Tablets 25 Prestabs
 Butisol Sodium Tablets 50 and 50 mg
 Butizide Tablets 25 and 50 Prestabs
 Clistin-D Tablets
 Clistin Expectorant Syrup
 Clistin Tablets 12 mg
 Co-Tylenol Cold Formula Tablets
 Haldol Tablets 1 and 5 mg
 Naetisol Tablets
 Parafon Forte Tablets
 Reserparide Tablets 25
 Mead Johnson Laboratories
 Cytosan 25 and 50 mg
 Deca-Vi Sol green and yellow
 K-Lyte Line
 Megace 20 mg
 Natalins Rx
 Oracou 25 (placebo)
 Peristim Forte Capsules
 Questran
 Quilbron Capsules*

*Company plans to discontinue use of yellow No. 5 dye soon.

- Quilon Plus Capsules
 Quilon Elixir
 Quilon Plus Elixir
 Triol Syrup
- Merek Sharp & Dolme**
 Abdomet Tablets 250 and 500
 Albebor 250
 Benomid Tablets 0.5 gm
 Dendron Tablets 0.5 and 0.75 mg
 Eleerin Tablets 50 mg
 Elavil Tablets 25 and 50 mg
 HydroMiril Tablets 50 mg
 Hydropra Tablets 25 and 50 mg
 Trivil Tablets 4-25 mg
 Vivacil Tablets 10 mg
- Merrill-National Laboratories**
 Alysine Elixir
 AVC Suppositories
 AVC Suppositories with Dienestrol
 Copneol Anesthetic Troches
 Copneol Mouthwash/Gargle
 Copneol Throat Lozenges
 Clomid Tablets 50 mg
 Decapryn Syrup
 Decapryn Tablets 12.5 mg
 DV Suppositories
 Nitranitol Tablets with Phenobarbital
 Orenzyme Bittals
 Procalcine Lactate Capsules
 Sedudrops
 Tave Capsules 12, 25, and 72 mg
 Tennate Tablets 25 mg
- Miles Laboratory, Inc.**
 Chocks Bugs Bunny Multiple Vitamins
 Chocks Bugs Bunny Multiple Vitamins Plus Iron
 Chocks Multiple Vitamins
 Chocks Multiple Vitamins Plus Iron
 Flintstones Multiple Vitamins
 Flintstones Multiple Vitamins Plus Iron
 One-A-Day Multiple Vitamins
 One-A-Day Multiple Vitamins Plus Iron
- Mission Pharmaceutical Company**
 Equibol Tablets
- Organon, Inc.**
 Hexadol Tablets 0.5 mg
 Maxibolin Elixir
- Maxibolin Tablets
Ormont Drug & Chemical Company, Inc.]
 (Federal Pharmacol Corp. and Pan-
 american Pharmaceuticals)
 Dure-G Graduals
 G.I.I. Expectorant
 Lapay Graduals
 Lead Iron Forte Tablets
 Multiflor Tablets
 Venthera
Panamerican Pharmaceutical, Inc.* (see
 Ormont)
- Parke, Davis & Company**
 Adroyd Tablets 5 and 10 mg
 Alophen Pills
 Ambrolyl Hydrochloride Elixir
 Barbasol Filmseal
 Casarn Sagraola Extract Filmseal 3 and
 5 gr
 Celontin Capsules 150 and 300 mg
 Complex Capsules
 Combex with Vitamin C Capsules
 Coryza Rx "A" Tablets
 Cyclopar Capsules 250 and 500 mg
 Desical Capsules 325 mg
 Dilantin Infatabs 50 mg
 Dilantin Capsules 100 mg
 Erypar Filmseal
 Ferrous Sulfate Euplets
 Geriplex Capsules
 Humatin Capsules 50 mg
 Intrihex Capsules
 Midical Acetyl Suspension
 Midical Tablets 500 mg
 Milontin Capsules 250 and 500 mg
 Natabex with Fluoride Capsules
 Norlestrin Tablets 1 and 2.5 mg
 Norlutin Tablets 5 mg
 Norlutate Tablets 5 mg
 Nutritive Capsules
 Oxlopar Capsules 250 mg
 Palabac with Minerals Tablets
 Panteric Filmseal
 Parost Capsules 200 and 400 mg
 Pentobarbital Sodium Capsules 100 mg
 Phelantin Capsules
 Ponsel Capsules 250 mg

*Product expiring September, 1978, or later—no dye.

†Product expiring October, 1976, or later—no dye.

‡Product expiring May, 1979, or later—no dye.

§Product expiring May, 1976, or later—no dye.

¶Products manufactured for Panamerican Pharmaceuticals by Ormont Drugs do not contain yellow No. 5 dye but other products of that company may.

- Povan Tablets
 Procainamide Hydrochloride Capsules 250 mg
 Rhinitis Tablets full strength
 Taku Combox Capsules
 Taku-Diastase, Pepsin and Pancreatin Filmoen
 Thera-Combox H-P Capsules
 Thera-Combox Capsules
 Theridol Capsules
 Thyroid Strong Tablets
 Ventrex Capsules
 Ventrilox Capsules
 Vitamin C Tablets 100, 250, and 500 mg
 Penwalt Pharmaceutical Division (Pharmcraft)
 Agesic Capsules
 Aplin 10, 25, 50, 100 mg
 Cholan V. Tablets
 Tonamin 15 and 30 mg
 Metropine Tablets
 Sinarest Tablets
 Strascogest Tablets
 Tussionex Suspension and Capsules
 Zaroxolyn Tablets 10 mg
 Pfizer Laboratories Division
 Pfizerpen G Buffered Powder for Syrup
 Terramycin Pediatric Drops
 Terramycin Syrup
 Terramycin Tablets
 Vistarl Oral Suspension
 Vistrax Tablets
 Pharmacia Laboratories, Inc.
 Azulfidine En-Taba
 Pharmcraft (see Penwalt)
 P. S. Roxane Laboratories, Inc.
 Standinophen Elixir N.F.
 Belkoids Tablets
 Chlorpheniramine Maleate Tablets 4 mg
 Digoxin Tablets 0.125 mg
 Pentamethylol Tetranitrate Tablets N.F. 10 and 20 mg
 Penclobarbital Tablet 30 mg yellow
 Potassium Chloride Liquid 10%
 Prednisolone Tablet 5 mg
 W. P. Poythress & Company
 Antrocol Tablets
 Mudrane GG Tablets
 Mudrane GG No. 2 Tablets
 Mudrane Tablets
 Solfoton Tablets
 Purdue Frederick Company
 Gentlax 8 Tablets
 Senokap DSS Capsules and Tablets
 Reed & Curnick Pharmaceuticals
 Phasid
 Phazyne
 Sibonin
 Rexall Drug Company
 Anapae H Cold Tablets
 Antihistaminic Tablets 25 mg
 Asma Kets A.H. Tablets
 Balsam Conditioning Shampoo
 Bright Set Hair Setting Lotion—Hard to Hold
 Cura Nour Hormone Cream
 Cura Nour Moisture Cream
 Cura Nour Roll-On Deodorant, Antiseptic
 Chemere Cream Sachet
 Chemere Hand and Body Lotion
 Fast Home Permanent Wave—Silver
 Gel Wave Set—Hard to Hold
 Ger-Rite Tablets
 Greaseless Hair Tonic
 Hair Setting Lotion—Normal
 Herbal Conditioning Shampoo
 Mineralized "H" Complex Tablets
 National Tea Lady Kare Moisture Drop
 New Awakening Lemon Cream Rinse
 New Awakening Lemon Hand Lotion
 New Awakening Lemon Shampoo
 Pabizol without Pterogoric
 Park Lane Special Care Lotion
 Prednisolone 5 mg
 Rexall Lavender Pre-Shave Lotion
 Rexall Brite Conditioning Shampoo
 Rexall Lavender After Shave Lotion
 Rexall Minuteman Chewable Multivitamin
 Rose Hip Vitamin C Chewable 200 mg
 Safeway Chewable Vitamin C Tablets 100 mg
 Shopper Fair Balsam Hair Conditioning
 Shop Rite Decongestant Relief Tablets
 Shop Rite Dry Skin Cream
 Shop Rite Moisture Drops
 Riker Laboratories, Inc.
 Alu Tap
 Disipal
 Norgesic
 Rauwolfid
 Sombulex
 Veriloid
 A. H. Robins Company
 Adalax Tablets
 Adalax with Minerals Tablets
 Dimetane Elixir and Tablets
 Donnagel/Donnagel 141
 Donnatal Elixir
 Donnatal Extentabs

- Donnatal No. 2 Tablets
 Donnatal Plus Tablets
 Exna Tablets
 Pabalate Tablets
 Phenaphen Tablets
 Repoise Tablets 5, 10, and 25 mg
 Robinyein Tablets
 Robitex Capsules 250 and 500 mg
 Milmin Gel Tablets
 Sulta Tablets
 Tybatran Capsules 100, 250, and 300 mg
Roche Laboratories
 Heroen Tablets
 Dalmane 15 and 30 mg
 Elixir Alurate Verlum
 Larocee Tablets
 Larclopa 250 mg
 Libritals 5, 10, and 25 mg
 Librax
 Librium Capsules 5, 10, and 25 mg
 Natulune
 Menrium 5-2, 5-4, 10-4
 Roniacol Timspan
 Taractan Concentrate
 Taractan Tablets 10, 25, 50, and 100 mg
 Valium Tablets 5 mg
Korrig (Pfizer Pharmaceuticals)
 Antivert Chewable Tablets 25 mg
 Antivert Tablets 25 mg
 Atarax Tablets 25 and 50 mg
 Bonine Chewable Tablets
 Cartrax Tablets 10 and 20 mg
 Euarax-10 Tablets
 Heptuna Plus Capsules
 Lithane Tablets
 Marax Syrup*
 Navane Capsules 1, 2, 5, 10, and 20 mg
 Obron-6 Tablets
 Roeribec Tablets
 Vitamin B with C
 Viterra Tablets
W. H. Rorer, Inc.
 Ananase Tablets
 Emetrol
Ross Laboratories (see Abbott)
Rowell Laboratories, Inc.
 Cal-Ron OR
 Cal-Ron Freckles
 Iso-Tab
 Ro-Bile
 Ro-Sulfa
 Vio-Gerb
- Sandoz Pharmaceuticals**
 Belladonna Elixir
 Bellergal Spacetabs
 Cefergal 100 Tablets
 Cedilnol Tablets
 Fingresic Tablets
 Fiorinal Capsules
 Fiorinal with Codeine Capsules
 Mellaril Tablets 10, 100, and 150 mg
 Sansert Tablets
 Serenell Tablets, all strengths
 Torcean Tablets
Saron Pharmacal Corporation
 AL-R Capsules
 Azo-Sulfate
 Butabell HMB Elixir
 Fen-H 0.625 and 1.25 mg
 Gerinats-OTC
 Gerinats-T
 Gevizol
 Hi-Temp
 Maxi-E
 Mega-Vita
 Natalets-4*
 RBC Plus
 S-Aqua-D
 Saramp 250 mg
 Saramp Oral Suspension
 Sarolax
 S-Paincet
 S-Pain-Cpd-65
Savage Laboratories
 Pyrilgin Liquid
Schering Corporation
 Celestone Tablets
 Chlor-Trimeton Repetabs 8 and 12 mg
 Chlor-Trimeton Syrup
 Chlor-Trimeton Tablets 4 mg
 Cod Liver Oil Concentrate Tablets
 Coricidin Demilets
 Coricidin Medilets
 Coriforte Capsules
 Corilin Infant Liquid
 Demazin Syrup
 Demonil Tablets
 Disoprol Chronotab Tablets
 Dixoral Sustained Action Tablets
 Estinyl Tablets 0.02 and 0.5 mg
 Etrafon 2-10 Tablets
 Etrafon Tablets
 Gitaligin Tablets
 Gynotone Tablets 0.02 and 0.04 mg

*New formula (Marax DF Syrup) now being distributed does not contain yellow No. 5 dye.

- Mol-Iron Chromosule Capsules
 Mol-Iron Panhemie Capsules
 Mol-Iron Panhemie Chromosule Capsules
 Mol-Iron with Vitamin C Chromosule Capsules
 Naqua Tablets 2 and 4 mg
 Naquival Tablets
 Oreton Propionate Buccal Tablets
 Oreton Methyl Tablets 25 mg
 Permittil Chronotabs Tablets 1 mg
 Permittil Tablets 0.25 mg
 Prantal Repetabs Tablets
 Rola Tablets
 Trilafon Syrup
 Trilafon Tablets 2, 4, 8, and 10 mg
Searle Laboratories
 Drine
 Bantnine Tablets
 Bantnine with Phenobarbital Tablets
 Dartal Tablets 10 mg
 Dramamine Liquid
 Dramamine Tablets
 Keophyllin Tablets
 Pro-Bantnine with Dartal Tablets
Smith Kline & French Laboratories
 Benzadrine Spansule Capsules
 Benzadrine Tablets 5 and 10 mg
 Combid Spansule Capsules
 Compazine Spansule Capsules
 Compazine Tablets 5 and 10 mg
 Dexanymyl Elixir
 Dexanymyl Spansule Capsules No. 1 and No. 2
 Dexnymyl Tablets
 Dexamdrine Elixir
 Dexamdrine Spansule Capsules 5, 10, and 15 mg
 Dexamdrine Tablets
 Dibenzylamine Capsules
 Ecotrin Tablets
 Eskalith Capsules
 Eskatrol Spansule Capsules
 Fescol Spansule Capsules
 Fescol Tablets
 Fortespan Capsules
 Paredrine Tablets
 Prydon Spansule 0.4 and 0.8 mg
 Prydonal Spansule Capsules
 SK-Ampicillin Capsules 250 and 500 mg
 SK-Erythromycin Tablets
 SK-Estragen Tablets 0.3, 0.625, 1.25, 2.5 mg
 Stelazine Concentrate
 Thorazine Tablets 10, 25, 50, 100, and 200 mg
 Tuss-Oracole Liquid
 Ventrol Tablets
E. R. Squibb & Sons
 Amnestrogen 1.25 and 2.5 mg
 Dumogran Tablets
 Dumone Tablets
 Mystecellin F 125 and 250 Capsules
 Naturetin Tablets 2.5 and 5 mg
 Nydraxil Syrup
 Pentids 400 for Syrup
 Pentids 800 Tablets
 Prolixin Tablets 2.5 and 5 mg
 Theragrau-M Tablets
 Valadol Chewable Tablets
 Vectids 125 Oral Solution
 Vesprin Tablets 50 mg
Stuart Pharmaceuticals
 Buchadin
 Chewable Sorbitrate 5 mg
 Difose Plus
 Feracece Chewable Tablets
 Feracece HP
 Mulvidron Softab Tablets
 Mylanta Tablets
 Mylanta II Tablets
 Normocel Tablets
 Probee Tablets
 Probee T Tablets
 Sorbitrate Tablets oral 5 and 10 mg
 Stuart Amino Acids and B₁₂ Tablets
 Stuart Prematal with Folic Acid Tablets
 Stuart Therapeutic Multivitamin Tablets
 Stuartine
 Stuartine Tablets film-coated
 Stuartinal 1 + 1
 Theron Tablets
Syntex Laboratories, Inc.
 Norinyl 1 + 80
 Nor-Q-D
S. J. Tutug & Company
 Lotussin Cough Syrup
 Pentafin Tablets 20 mg
 T. D. Therat Capsules
Upjohn Company
 Alphadol 0.75 and 1.5 mg
 Casakol
 Celefortis
 Celestinic
 Calical
 Conycin
 Dildex
 Diostate D
 Emeracel
 E-Mycin 250 mg
 Ferrous Sulfate USP

- Gel-tabs Vitamin D 125-mg
 Halobestin 2, 5, and 10 mg
 Indacell with Vitamin B₆
 Macbate
 Me-hol Modules 2 and 4 mg
 Minicap
 Motrin 200 and 400 mg
 PAC Compound with Codeine 80,
 PAC Compound Green
 Pamine PB Elixir and Tablets
 Pamine PB half-strength
 Panmycin HCl 250 mg
 Panmycin Tablets 250 and 500 mg
 Phenobarb and Belladonna No. 1 and
 No. 2
 Phenolax
 Polykol 250 mg
 Pyroxate
 Pyroxate with Codeine Phosphate ¼ gr
 Reserpoid 0.1, 0.25, and 1 mg
 Rigtabs
 Special Formula No. 2
 Super D Perles
 Unicap
 Unicap M
 Unicap Therapeutic
 Unigestic H
 Uracl Mustard 1 mg
 Vitamin E 75 I.U.
 Zymafolio (disc)
- USV Pharmaceutical Corporation**
 Aquasol E Capsules 30, 100, and 400 I.U.
 Azolid-A Capsules
 Duo-GVP Capsules
 Femicin Tablets
 Histaspan Capsules 8 and 12 mg
 Histaspan Plus Capsules
 Meltrol 50 mg Capsules
 Nitrospan Capsules
 Perispan Capsules 50 and 80 mg
 Pertofrane Capsules 25 and 50 mg
 Vi Aquamin Capsules
 Vi Aquamin Therapeutic Capsules
 Voramil Tablets
- Vale Chemical Company, Inc.**
 Acedyne
 Aminopylline 1½ gr yellow
 Antacid yellow
 Digitoxin 0.2 mg
 Ephedrine and Sodium Phenobarbital
 Escate C
 Glycotuss-DM
 Isona B green
 Laphol
 Magnulin
- Minto-Chlor Syrup with Codeine Sulfate
 Newcet
 Newcet, Junior
 Neuroval Elixir
 Sevrotose No. 3 yellow
 Nitrin
 Nyrul
 Phenobarbital ¼ gr green and yellow
 Phenobarbital ½ gr green
 Phenobarbital and Atropine
 Rhinogestic
 Rhinogestic, Junior
 Rhinogestic GG
 Serpate
 Sodium Butabarbital ½ gr green
 Sodium Salicylate 5 gr yellow
 Sulfisoxazole
 Thiamine Chloride Solution
 Trioval
 Valacet Capsules
 Valacet, Junior
 Valacet Tablets
 Valdrene Tablets
 Valolar
- Walker Corporation & Company, Inc.**
 Ah Trist
 Aspir B
 Aspirin
 Brondilate
 Calathesia
 Child's Drikof
 Child's Ongestol
 Cystitabs
 Fenbane
 Limitite
 Neo Vinsol
 Pentacine
 Quiazone III
 Silikulin
 Spasmo Forte
 Thedrinal
 Tristalin
 Uceytime
 Walen Gen
- Wallace Laboratories**
 Deprol Tablets
 Meprospan 200 and 400 Capsules
 Milpath 200 Tablets
 Miltrate 200/20 Tablets
- Winpole Laboratories (Denver Chemical
 Manufacturing Co.)**
 Acazyme Tablets
 Hical Tablets
 Croo-Terpin
 Croo-Terpin Plus

VOLUME 58
NUMBER 4

- Dr. Hand's Teething Gel
Dr. Hand's Teething Lotion
Ephed-Organidin Elixir
Siroff
Tussl-Organidin DM
Warner-Chilcott Laboratories
Anusol-HC Suppositories
Euthroid 1/2, 1, and 3 gr
Gelasil—pineapple and spearmint
Mandelamine Tablets and Granules
Papase
Peritrate 10 and 20 mg
Peritrate with Phenobarbital 10 and 20 mg
Peritrate SA
Peritrate SA with Phenobarbital
Siantab with Codeine
Tedral Anti H*
Tedral SA*
Utiibid
Warren-Ford Pharmaceuticals, Inc.
Cal-O-B
Evac-Q-Tab
Homel Powder
Knochlor-Eff
Knochlor 10% Liquid
Kaon Elixir, lemon-flavor
Modane Mild and Regular
Teels
Winthrop Laboratories
Acholol Pepsin Capsules Hard Gelatin
Bilepaque Capsules Hard Gelatin
Hytakerol Capsules Soft Gelatin
Kabal 1/4 and 1/2 gr
Koroin
Mytelase
Plaqueuil Sulfate Tablets 400 mg shellac-coated
Trancopal Caplets 100 and 200 mg
Wyeth Laboratories
AMT Tablets
Beillin Oral Suspension 150,000 and 300,000 U
Cyclospasmol Tablets 100 mg
Epagesic Tablets
Epanitrate-20 Tablets
Ovrette Tablets
Pen Vee Suspension 180 mg
Pen Vee K for Oral Solution 125 and 250 mg
Phenergan Expectoant, Pediatric
Phenergan VC Expectoant, Plain
Phenergan VC Expectoant with Codeine
Phenergan Syrup
Polyononin Plain Tablets
Prokeltazine Maltate Tablets 12.5 and 25 mg
Parodign Tablets 0.15 mg
Serepax Tablets 15 mg
Sparine Hydrochloride Syrup 10 mg
Spartine Hydrochloride Tablets 10 and 25 mg
Unipen for Oral Solution
Zactane Tablets 75 mg
Zactrin Compound-100 Tablets
Zactrin Tablets
Zenith Laboratories, Inc.
Chlorpheniramine Maltate Tablets 4 mg
Chlorpromazine HCl Tablets 10, 25, 50, 100, and 200 mg
Conjugated Estrogen Tablets 1.25 mg
DAS Tablets 5 mg
Dimenhydrinate Tablets 50 mg
Folic Acid Tablets 1 mg
HBB Tablets
Hi Potency Vitamin B Complex with Vitamin C
Hydralazine Hydrochloride Tablets 10 mg
Hydrochlorothiazide with Reserpine Tablets
Phenclimetzazine Bitartrate Tablets
Probenecid Tablets 0.5 gm
Sodium Butobarbital Tablets 30 mg
Vitamin B Complex with Vitamin C
Zenivite M

DRUG COMPANIES NOT RESPONDING OR PROVIDING INSUFFICIENT INFORMATION

- Arco Pharmaceuticals, Inc.
Beach Pharmaceuticals
Bowman Pharmaceuticals
Boyle & Company Pharmaceuticals
Brown Pharmaceutical Company, Inc.
Cauright Corporation
Coast Laboratories, Inc.
Dattel Pharmaceutical Company, Inc.
Deke Chemical Company, Inc.
Drug Industries Company
P. E. Elder Company, Inc.
Everett Laboratories, Inc.
Ferndale Laboratories, Inc.
Gilbert Laboratories

*New formulation eliminates tartazine.

Hyrex-Key Pharmaceuticals
Inwood Laboratories, Inc.
Jamieson-McKames Pharmaceuticals, Inc.
Kenwood Laboratories, Inc.
Kremers-Urban Company
Laser, Inc.
Lemmon Pharmaceutical Company
Mallard Inc.
Marlop Pharmaceuticals, Inc.
Medical Products Panamericana, Inc.
Meyer Laboratories, Inc.
Nion Corporation

Norgine Laboratories
Nutrition Control Products
Obetrol Pharmaceuticals
O'Neal, Jones & Feldman, Inc.
Orbit Pharmaceutical Company, Inc.
Ortho Pharmaceuticals
Reid-Provident Laboratories, Inc.
Standard Process Laboratories, Inc.
Stayner Corporation
Westfield Laboratories
Western Research Laboratories
Zemmer Company

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MEMORANDUM OF CONFERENCE
April 24, 1975

VISITOR: Max Santer, M.D.

Professor of Medicine
Grant Hospital of Chicago
Chicago, Illinois

FDA: Personnel:

Mr. H. R. Gittes
Mr. R. J. Ronk
Mr. G. L. McCowin

HFF-152
HFF-330
HFF-334

SUBJECT: FD&C Yellow No. 5 ✓
Proposal for epidemiological survey

CAP 23

Dr. Santer stated that he had reviewed an RFP for conducting an epidemiological survey of FD&C Yellow No. 5. He considers the proposal as being unworkable since it will probably not provide good data concerning the true incidence of individuals sensitive to FD&C Yellow No. 5.

He had found that those individuals sensitive to aspirin or FD&C Yellow No. 5 had a predisposition. The problem of aspirin sensitivity appears to be related to an alteration of collagen. He noted that from 5 to 30% of aspirin sensitive individuals are also sensitive to FD&C Yellow No. 5. However, he mentioned that those individuals who are sensitive to aspirin are also sensitive to a large number of related analgesics. He believes that it would be profitable to investigate the cause of sensitivity to FD&C Yellow No. 5 rather than conducting a survey among physician's patients to determine who has a reaction to it. He mentioned his findings that the form in which FD&C Yellow No. 5 is taken has a definite effect upon whether a reaction occurs. He found that its presence in food does not appear to be as likely to provoke a reaction as when it is used in drugs. He speculated that one reason may be that the individual taking drugs may have a disease condition which predisposes him being sensitive. He mentioned asthma as being one instance where drugs with FD&C Yellow No. 5 should not be prescribed. He said that, of 8 million asthmatics in the country, up to 400,000 could be aspirin sensitives.

As relates to the use of the color in food, he felt that the low potential for a reaction could make avoidance of such foods by aspirin sensitives an unnecessary measure.

Mr. Ronk supplied him with copies of the SCOGS reports on Gum Arabic, Gum Tragacanth, and Gum Stearculin. He asked for his opinion on the approach discussed in the preamble to the regulation for these gums. Dr. Santer stated that drugs should be given the first consideration since these are the uses where reactions are most likely to occur. As an example, he mentioned that FD&C Yellow No. 5 should be removed from use in drugs for asthmatics and those suffering from hives.

Gerard L. McCowin

MEMORANDUM OF CONFERENCE

June 12, 1975

VISITORS: Dr. Max Samter Grant Hospital of Chicago
Chicago, Illinois

FDA Personnel:

Dr. A. C. Kolbye	HFF-100
Dr. C. J. Kokoski	HFF-152
Dr. D. L. Archer	HFF-124
Dr. F. Cordle	HFF-103
Mr. R. J. Ronk	HFF-330
Mr. J. J. McAuliffe	HFF-334
Mr. G. L. McCowin	HFF-334

SUBJECT: FD&C Yellow No. 5, Tartrazine
Hypersensitivity

Dr. Samter discussed briefly the occurrence of allergic responses. He noted that many such responses can be due more to suggestion than actual physiological reasons. He mentioned that there are very few instances of true allergic reaction to food. Although such responses may occur in the infant, allergic reactions to food often disappear by the age of two. He added that occasionally some people do continue to be allergic to certain foods. He discussed in detail the various causes for allergic responses noting:

1. A person may be allergic to a substance but never show symptoms. As an example, approximately 20 percent of the people are atopic and allergic to ragweed. However, only 15 million people show symptoms of ragweed allergy whereas approximately 60 million are positive on skin testing.
2. A person allergic to one substance may not necessarily be allergic to other substances normally considered to be allergens.
3. There are other substances which will break-up the heparin-histamine complex in the mast cell and release the histamine into the system.

He discussed with Dr. Archer the relevance of cytotoxicity testing for sensitivity. His opinion was that such testing was not suitable for diagnosing a reaction as allergic.

In discussing Dr. Feingold's findings, he noted that there were inadequate controls for the study. He further commented that many such children show improvement largely because of the increased attention given them.

He noted that there were three types of sensitivity responses:

1. Allergic responses (immunological)
 - a. Humoral
 - b. IgE-antigen-antibody formation and release of histamine. This leads to an immediate reaction.
 - c. Cellular sensitivity. This is a delayed type of reaction of lipoproteins rather than a direct action of the cells with the antigen; i.e. poison ivy.
2. Non-immunological responses
 - a. Mast cell degranulation, releasing histamine by a replacing action.
 - b. Enzyme activation where histamine is released.

He mentioned that there was no problem with showing the occurrence of an allergic response. This can be tested through the Rauech skin test for detecting antigens.

3. "Bypass" reactions

These are non-allergic responses where IgE compliments are formed. These compliments may have the effect of causing a lysis of the cells.

Since our last meeting, Dr. Samter said they have been requesting that people be referred to them who were considered to be allergic to food. They have found that out of 35 patients referred to them, only 3 could be shown to be actually sensitive to food. He mentioned that, accordingly, any investigation of sensitivity to food additives must include a food additive clearance and consultation center. Such a center would gather the data necessary for substantiating claims of sensitivity to food additives. The observations from the various subjects could be evaluated by computers. After collation and evaluation of the data, it would be necessary to verify the type of reaction involved. This would require identification of whether they are allergic or non-allergic responses.

Dr. Samter stated that tartrazine sensitivity appeared to be the result of a disease condition which leads to an intolerance to the color. He noted that aspirin sensitivity in the respiratory tract is due to an antigen-antibody formation. He added that it appeared that the urticaria and angioedema caused by aspirin appeared to be an antigen-antibody reaction. He noted as evidence of this that an individual who has a respiratory

effect to aspirin will not necessarily be sensitive to salicylate while one who has a skin reaction to aspirin very likely will also be sensitive to salicylate. He noted that many people do not become sensitive to aspirin until they have suffered an attack of asthma. The asthma may lead to a connective tissue (collagen) disease and the formation of polyps. They have formed the following theory:

1. Asthma is a disease of the "air conditioning" system of the body, e.g., cold air must be warmed, moisture added, and intake slowed down before the air can be breathed into the alveolae. In asthmatics, there is a difficulty in reversing this system, e.g., the bronchial system will not dilate once it has become constricted.
2. The aspirin sensitivity is a response to receptor sites in the nose. These receptor sites are probably kinase receptors. When there is pain or other impulse at the nose this results in the release of kinase which causes the typical asthmatic action of the "air-conditioner."
3. Aspirin is a peripheral pain reliever and an antagonist to the effects of kinase. If the receptor sites are damaged, the aspirin will cause the opposite effect and will lead to constriction of the bronchial tubes. This also occurs with other peripheral pain relievers.

He noted that tartrazine would appear to be a superior pain reliever. He mentioned that metabolism of the tartrazine results in release of the pyrazol fraction.

He added that apparently the sensitivity response to tartrazine depends largely upon the form of the food containing the tartrazine. The possibility for a reaction appears to depend upon the likelihood of the tartrazine reaching the receptor site. They ordinarily don't see people reacting to tartrazine when it is with food as a color additive. He also mentioned that they had never seen any tartrazine sensitive individuals who were not also sensitive to aspirin. He noted that tartrazine appears to have relatively mild effects. Only around 20% of aspirin-sensitive individuals get reactions with tartrazine.

He next discussed the relationship of tartrazine to bleeding time. He mentioned that small amounts of aspirin will interfere with platelet aggregation resulting in a lengthening of bleeding time. Tartrazine will also lead to increased bleeding times in sensitive individuals, but much larger amounts of tartrazine are required. This phenomena seems to support the theory that aspirin and tartrazine sensitivity are related to collagen diseases of the connective tissue. He noted that effects are not always observed in the tartrazine sensitive individual upon exposure to tartrazine.

MEMORANDUM OF CONFERENCE

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The above comments on tartrazine were limited to respiratory problems. He added that tartrazine could also have a true allergic response in causing dermal effects in some few individuals, as is the case with most substances.

Dr. Kolbye summed up the meeting by mentioning the several questions that he believes face the Agency:

1. Do food allergies relate to hyperkinesis?
2. Are there any other food problems that relate to hyperkinesis, e.g., lead and learning disorders?
3. Are food additives related to any other allergic responses, e.g., tartrazine?

Gerard L. McCowin

FDA's PROPOSAL ON SACCHARIN

TAB J

FRIDAY, APRIL 15, 1977

PART III



DEPARTMENT OF
HEALTH,
EDUCATION, AND
WELFARE

Food and Drug Administration

SACCHARIN AND ITS
SALTS

Proposed Rule and Hearing

federal register

19996

PROPOSED RULES

DEPARTMENT OF HEALTH,
EDUCATION, AND WELFARE

Food and Drug Administration

[21 CFR Parts 145, 150, 172, 180, 189,
310, 430, 510, 585, and 700]

[Docket No. 77-7008]

SACCHARIN AND ITS SALTS

Proposed Rule Making

AGENCY: Food and Drug Administration

ACTION: Proposed rule.

SUMMARY: The Commissioner of Food and Drugs is proposing to revoke the interim food additive regulation under which saccharin and its salts (saccharin) are currently permitted as ingredients in prepackaged foods, such as soft drinks, and as tabletop nonnutritive sweeteners. The Commissioner is also inviting comments on a proposal to accept and promptly review new drug applications for the marketing of saccharin as a single-ingredient drug, available without a physician's prescription. If approvable under the requirements of section 505 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 355), such products would be required to bear a conspicuous warning about the risk of cancer. The Commissioner is also proposing to prohibit the use of saccharin in cosmetics that are likely to be ingested, to amend the standards of identity that provide for the use of saccharin and to prohibit the use of saccharin in animal drugs and animal feed.

The Commissioner's determination that saccharin must be banned as a food additive is based on a series of scientific studies conducted in accordance with currently accepted methods for determining whether compounds can cause cancer. The most recent of these studies, conducted by Canadian scientists under the auspices of the Canadian Government, confirms what earlier American studies have suggested: that saccharin poses a significant risk of cancer for humans. Under these circumstances, conscientious concern for the public health requires that FDA prohibit the continued general use of saccharin in foods.

This conclusion is also dictated by the so-called Delaney clause of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 348(c)(3)), which prohibits the use in food of any food additive which has been shown by ingestion or other appropriate tests, to cause cancer in laboratory animals.

The Delaney clause does not apply to human drugs, however, and it therefore does not prohibit the approval of a drug that has been shown to cause cancer in laboratory animals if the drug provides medical benefits that outweigh the potential risk. For many individuals, including diabetics who must limit their intake of sugar and other carbohydrates, the availability of a nonnutritive sweetener may serve a legitimate medical need. The Commissioner is therefore proposing to permit the submission of new drug applications for the marketing of saccharin as a single-ingredient OTC

drug, which applications must be accompanied by legally sufficient evidence of the effectiveness of saccharin for its labeled indications.

DATES: Comments on this proposal may be submitted by June 14, 1977. Published elsewhere in this issue of the *Federal Register* is a notice of an informal hearing before the Commissioner to be held on May 18 and 19, 1977 to hear oral comments on this proposal.

ADDRESS: Written comments should be sent (preferably in quadruplicate) to the Hearing Clerk (HFC-20), Food and Drug Administration, Rm. 4-85, 5600 Fishers Lane, Rockville, Md. 20857.

FOR FURTHER INFORMATION CONTACT:

GENERAL: Ronald J. Wylie, Compliance Regulations Policy Staff (HFC-10), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, Md. 20857, 301-443-3480.

FOODS: John J. McAuliffe, Bureau of Foods (HFF-334), Food and Drug Administration, Department of Health, Education, and Welfare, 200 C St. SW., Washington, DC 20204, 202-472-5690.

HUMAN DRUGS: Paul Fehnel, Bureau of Drugs (HFD-30), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, Md. 20857, 301-443-3640.

COSMETICS: Heinz Eiermann, Bureau of Foods (HFF-440), Food and Drug Administration, Department of Health, Education, and Welfare, 200 C St. SW., Washington, D.C. 20204, 202-245-1530.

VETERINARY DRUGS: Edward Ballitch, Bureau of Veterinary Medicine (HFV-231), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, Md. 20857, 301-443-3338.

SUPPLEMENTARY INFORMATION:

I. SACCHARIN AS A FOOD INGREDIENT

A. HISTORY OF THE USE AND SAFETY OF SACCHARIN

Saccharin is a nonnutritive, artificial sweetener that is approximately 350 times sweeter than sugar. Following the discovery of saccharin in 1879, commercial interest was initially shown in its possible usefulness as an antiseptic or as a preservative to inhibit fermentation in foods, but from the beginning, questions about its safety existed. In 1896, workers in Europe noted no effects in human subjects who had been given single doses of saccharin up to 5 grams. In 1898, a French scientist reported no harmful effects in diabetics who ingested 5 grams per day for 5 months. During the succeeding decade, several reports both endorsing and criticizing the use of saccharin in diabetics noted evidence in some patients of loss of appetite, nausea, and pressure in the stomach. In the meantime, attempts to use saccharin in

the treatment of intestinal infections, chronic gastritis, cystitis, and numerous other diseases proved unsuccessful. By 1907, however, canners of fruits and vegetables in the United States had developed an interest in using saccharin to sweeten their products. In 1923, a Board of Scientific Advisors to the Secretary of Agriculture, appointed by President Theodore Roosevelt, concluded that 0.3 gram/day of saccharin was safe and that higher levels of intake, especially above 1 gram/day, caused disturbances of digestion.

In numerous toxicological studies in experimental animals during the period 1920 to 1950, no findings were reported that raised serious questions about the safety of saccharin as then used. In Europe, during World Wars I and II, the consumption of saccharin greatly increased, with no apparent adverse effects among consumers, though no adequate epidemiologic studies were conducted at that time.

Saccharin use today is widespread. Approximately 6 to 7.6 million pounds of saccharin were used in the United States in 1976. It is used in food and beverages, cosmetics, drugs, animal feed, and industrial processes. Food and beverage uses by far the most extensive, accounting for over 70 percent of the saccharin used.

The soft drink industry accounts for about 74 percent of the saccharin consumed in food and beverages in the United States. Other dietary uses, which account for 14 percent of the saccharin consumed, include powdered juices and drinks, other beverages, sauces and dressings, canned fruits, dessert toppings, cookies, gums, jams, candies, ice cream, and puddings. About 12 percent of the saccharin consumed is as a sweetener in place of nutritive sweeteners (e.g., sugar) in coffee and tea and on cereal.

Although saccharin's predominant use is in foods, it is also used in drugs—both prescription and OTC—especially those intended for pediatric use and for use by diabetics. Saccharin is also found in a variety of cosmetics, including lipsticks, dentifrices, mouthwashes, after-shave lotions, moisturizing skin preparations, hair tonics, skin cleansers, bubble baths, colognes, face powders, and douches. Saccharin is also used to a limited extent in animal feed and animal drugs.

One of the first chronic toxicity studies of saccharin was reported by Fitzhugh et al. in 1951 (discussed below). The findings of that study were inconclusive and there continued to be debate among scientists about the safety of saccharin. Accordingly, in 1955 the Committee on Food Protection of the National Academy of Sciences reviewed the literature bearing on the safety of saccharin and concluded that the "maximum probable tolerance level for saccharin in the human diet is at least as great as 1.0 gram per day." The National Academy of Sciences (NAS) committee further concluded that the substitution of saccharin for the average daily consumption of sugar in the United States would amount to about 0.3 gram of saccharin, and that

"The maximal amount of saccharin likely to be consumed was not hazardous."

Because of greatly increased use of saccharin and cyclamate, another non-nutritive sweetener, as well as drastic changes in the patterns of their consumption during the 1960's, in 1967 FDA requested the National Academy of Sciences again to evaluate the safety of these nonnutritive sweeteners. In response to this request, an ad hoc committee was formed under the NAS Committee on Food Protection. In 1968, the committee issued an interim report in which it concluded that the intake of 1 gram or less per day of saccharin by an adult should present no hazard. However, the committee also recognized at that time that the existing carcinogenesis studies on saccharin, judged by current standards, were inadequate, and it therefore recommended that contemporary studies be undertaken.

During the late 1960's, saccharin was being widely used competitively or in combination with cyclamate. Consequently, when the use of cyclamate was banned by FDA in 1969, it was anticipated that the daily intake of saccharin by users of nonnutritive sweeteners would increase substantially. An ad hoc subcommittee of the NAS Committee on Food Protection was once again requested by FDA to review all available toxicity data on saccharin in the light of the projected sharp increase in use.

The NAS subcommittee issued its final report in July 1970. It arrived at conclusions regarding the safety of saccharin very similar to the assessments of 1955 and 1958. The subcommittee again recommended that chronic toxicity studies, designed according to modern protocols, be completed. It further recommended that: (a) epidemiologic studies should be carried out with emphasis on the diabetic segment of the population and in relation to pregnancy; (b) comparative metabolism studies should be done in man and in animals; and (c) toxicologic interactions with other selected chemicals should be explored.

Although the then existing studies raised some questions about whether saccharin could cause cancer, no firm conclusions could be reached on the basis of those data. In 1972, because of the questions about the safety of saccharin, FDA removed saccharin from the list of substances generally recognized as safe (GRAS) and imposed limits on the use of saccharin to discourage general use by consumers and to inhibit an increase in its use by the general population. At that time, FDA also issued an interim food additive regulation to permit continued limited use of saccharin pending completion of studies to resolve the questions concerning the safety of saccharin. In issuing the interim regulation, FDA concluded that the continued limited use of saccharin did not constitute a significant risk to public health.

B. HISTORY OF SCIENTIFIC AND MEDICAL INQUIRY INTO THE CAUSES OF CANCER

Sir Percival Pott's description, almost 200 years ago, of the relationship between exposure to soot and cancer of

the scrotum in chimney sweeps is usually cited as marking the beginning of studies in environmental carcinogenesis (Ref. 1). It was not until the late 19th century, however, that the association between exposure to aromatic amines and the production of bladder cancer among workers in the German dye industry was established, and only in the early part of this century that the production of skin cancer by X-radiation and radium became evident.

Modern research on chemical carcinogenesis dates from the classic studies of Yamagiwa and Itchikawa (Ref. 2). They successfully induced cancer by applying coal tar to the ears of rabbits and thereby produced the first experimental animal analogy of a type of chemically induced human cancer. The work of these Japanese investigators in 1915 was quickly followed by similar investigations in many laboratories and culminated in the isolation from coal tar of the carcinogenic polycyclic hydrocarbon benzo(a)pyrene by Kenaway and Cook (Ref. 3). But it was only in 1938 that Busper experimentally produced bladder cancer in dogs by administration of β -naphthylamine (Ref. 4).

The known causes of human cancer include physical, chemical, and biological agents. According to Boyland (Ref. 5):

Reasonable estimates are that not more than 5% of human cancer is due to ionizing and less than 5% to radiations. Some 90% of cancer in man is therefore due to chemicals, but we do not know how much is due to endogenous carcinogens and how much to environmental factors. An expert committee (WHO, 1968) has concluded that at least half of all cancer in man is due to environmental factors. It should therefore be possible to prevent a great deal of human cancer by finding and removing chemical carcinogens from the environment.

In 1960, Dr. G. B. Milder prepared for a committee of the United States Congress a summary of the current state of scientific knowledge about the causes of cancer (Ref. 6). Despite major subsequent advances in our understanding of the role of microsomal enzyme metabolism in the action of carcinogens, in molecular biology, in virology, in our knowledge of the immunological aspects of cancer, and in the development of *in vitro* models for carcinogenesis, the summary of the causes of cancer prepared by Dr. Milder more than a decade ago is still essentially correct:

(1) Although cancer can be caused by extraneous agents, not all members of the exposed population will develop cancer. Those who are most susceptible can be identified only by experience.

(2) Even a powerful carcinogen requires weeks or months to elicit cancer in mice or rats and probably requires years in man.

(3) No change need be recognizable in the organ or tissue destined to become cancerous before the cancer itself appears.

(4) Experiments in the laboratory do not predict unequivocally the reaction of humans to the same agent. On the other hand, those few chemical and physical agents known to produce cancer in man, with the possible exception of inorganic arsenical compounds, have elicited cancers in animals.

(5) No one at this time can tell how much or how little of a carcinogen would be required to produce cancer in any human being, or how long it would take the cancer to develop.

(6) The effect of certain chemical carcinogens can be markedly increased by other compounds with little or no carcinogenic power.

(7) The accumulated evidence suggests the irreversibility of the cancerous response once it has been initiated and further suggests a cumulative effect.

(8) The most potent carcinogens, by their very strength, are almost sure to be discovered clinically. It is assuredly the less potent carcinogens that seem most important in human cancer and provide the real problem for evaluation. A major objective of experimental carcinogenesis is, therefore, the bioassay for the presence of weak carcinogens.

(9) Chemical configuration alone cannot be used to predict the ability of a new compound to produce cancer.

(10) Possession (by a substance) of a biological effect, known to be associated with a particular type of cancer production, may be of importance in assessing potential carcinogenicity. Examples are: estrogenic activity, gonotropic activity, production of liver cirrhosis.

The special attention given to the prevention of cancer is reflected in the Food Additive Amendment of 1968 and Color Additive Amendments of 1960. In principle, both laws recognize that all substances have a potential for harm and that, conversely, there are conditions under which most substances may be used safely. However, both laws also provide that under no conditions are cancer-producing substances to be considered safe. This Congressional expression of concern about cancer-producing agents indicates the need to know about the cancer-producing potential of food additives.

USE OF ANIMAL TESTS TO IDENTIFY RISKS TO HUMAN HEALTH

Testing for acute toxic effects in animals has long been and remains today the primary basis for evaluating the safety of food for humans. Now, however, scientists also test substances in animals to assess their long-term, or chronic effects, including their potential to cause cancer.

The first chronic animal studies were conducted in the late 19th century, after it was found that certain diseases were associated with lack of certain essential dietary constituents. For example, vitamin C deficiency, which leads to scurvy and niacin deficiency, which causes pellagra, were extensively studied in animals after scientists discovered that these diseases could be mimicked in animals. After it became apparent that laboratory animals were useful in studying nutritional diseases, scientists quickly concluded that animal experience might also be useful in predicting the long-term effects in man of ingestion of small amounts of chemicals. In the early 1930's, FDA scientists initiated some of the first long-term, or lifetime chronic feeding studies on substances to which humans are exposed. These studies—on lead arsenate pesticides—led, in 1940, to the establishment of limitations on the use of lead arsenate.

Since these early days of toxicology, the use of tests in laboratory animals to predict the long-term chronic effects of chemicals in man has been accepted by virtually all scientists and is today used by every technologically advanced country in the world. In the United States, many Federal agencies, in addition to FDA, such as the Environmental Protection Agency and the National Cancer Institute, rely on these animal tests to assess the safety of a variety of compounds. In 1964, the National Academy of Sciences/National Research Council (the Academy) published a report entitled "Principles and Procedures for Evaluating the Safety of Intentional Chemical Additives in Foods." This report updated pamphlets published in 1951 and 1952 on the safe use of chemicals in foods. The 1964 report and subsequent publications by the Academy describe the widely accepted approach of animal tests for evaluating the safety of chemicals added to foods. The World Health Organization has also espoused the use of animal tests to assess the safety of food ingredients.

The difficulty of identifying chemicals that may cause cancer has been considered many times in the last 15 years, and distinguished expert committees of the World Health Organization, Food and Agricultural Organization, National Academy of Sciences/National Research Council, and Department of Health, Education, and Welfare, as well as FDA, have published reports setting down principles and guidelines. Again the accepted test model is the chronic test in laboratory animals. As Berenbaum (Ref. 8) has pointed out, our existing knowledge does not provide a basis for firm agreement on the optimal conditions for carcinogenicity testing, but merely allows the setting down of minimal requirements for animal tests for carcinogenicity. These minimum accepted requirements include: (1) more than one species of animal should be used to demonstrate lack of carcinogenicity; (2) continuation of testing for the "practical" lifetime of the animals to establish a negative finding; (3) use of test doses close to the pharmacologically active range, several orders of magnitude above the actual use level; (4) maximization of numbers of animals on test, recognizing the practical limitations on population size; (5) use of routes of administration analogous to those by which humans will be exposed; and (6) whenever possible, commencing exposure during pregnancy and continuing exposure in the offspring for a lifetime. The three principal tests of saccharin on which the Commissioner is basing the accompanying proposals generally meet these basic criteria.

Even with the best test system, it must be recognized that a positive result only labels a substance as a suspect human carcinogen; at the same time, a negative result does not necessarily exclude the possibility that the substance is carcinogenic for man. Furthermore, it should be remembered that absolute demonstration of noncarcinogenicity, even in the species tested, is impossible. As J. Cornfield has indicated:

Expression of results as confidence limits rather than as a test of significance is to be preferred, since even when the lower confidence limit is below zero and no positive evidence exists, the upper limit may well be above zero, and this will serve as a constant reminder that failure to uncover positive evidence of carcinogenicity is not the same as a positive demonstration of noncarcinogenicity (Ref. 9).

Questions are frequently raised about the significance of carcinogenesis observed in animal experiments based on the belief that the high dosages to which animals are customarily exposed have no relevance in the assessment of human risk. Indeed, such questions have been raised about the findings in the WARP, FDA, and Canadian studies that saccharin causes bladder cancer. The Commissioner therefore believes that it is important to clarify this crucial issue.

It should be recognized that, generally, only high dosages will produce tumors in animals under the experimental conditions that must customarily be employed. In setting up model experimental systems, scientists have no choice but to use relatively small numbers of animals in comparison to the human population likely to be exposed. In order to obtain meaningful, consistent, and reproducible results, studies must be designed to produce a significant number of cancers in the animals under test.

Even a low incidence of cancer as 10 percent in a group of 100 experimental animals, which would approach the limit of reproducibility, would exceed any acceptable human risk. An incidence of 0.01 percent would represent 20,000 out of the total U.S. population of 200 million, and would certainly be considered unacceptably high. But to detect such a low incidence in experimental animals using dosage levels comparable to those administered to humans would require literally tens of thousands of animals. For this reason, scientists administer large doses to relatively small groups of experimental animals and then extrapolate the results to estimate the risk of cancer at low dosages.

Several methods for making such calculations of risk have been employed, but based on present knowledge and experience, the Commissioner believes the proper conservative approach is to assume a direct proportionality between the size of the dose and the incidence of tumors. For example, if a daily dosage of 1 gram per kilogram (kg) fed to experimental animals over a 2-year period produces a 10 percent incidence of tumors, FDA would assume that there would be a 1 percent incidence with 0.1 gram per kg dose, or a 0.1 percent incidence with a 0.01 gram per kg dose. Using this method of calculation, the agency would estimate, conservatively, that if a substance produces 10 percent incidence of cancer in the rat at a dose of 1 gram per kg, it would produce a 0.01 percent incidence, representing 20,000 persons out of a total population of 200 million, if ingested by man at a dose of 1 milligram per kg.

It is important to recognize that such calculations may indicate only a minimal risk. Experimental assays are conducted

under controlled dietary and environmental conditions, using animals of a homogeneous genetic background, while humans live under diverse conditions and are genetically heterogeneous, and are therefore likely to include subpopulations of unusual susceptibility.

Another popular misconception about the use of high dosages in animal carcinogenesis testing is the belief that any substance will induce cancer in animals if fed at sufficiently high levels. Excessively high levels of most substances can induce toxic effects in animals, but only a small number of such substances can produce cancer. This fact is illustrated by a study of 120 pesticides and industrial chemicals reported by J. R. M. Innes, et al. (Ref. 10). The compounds were selected on the basis of toxicity evidence suggesting potential harm to man, widespread use, or chemical structure suggesting possible carcinogenicity. In this study, both sexes of 2 hybrid strains of mice were orally administered maximum tolerated doses of the 120 test compounds starting at the age of 7 days. The authors found that administration of only 11 of the compounds unequivocally induced a significantly elevated incidence of tumors.

D. CARCINOGENICITY TESTING OF SACCHARIN

The first long-term study to evaluate the chronic toxicity of saccharin in the diet of rats was reported at FDA in 1951 by Fitzhugh, Nelson, and Prawley (Ref. 11). Various levels of saccharin were fed, some as high as 5 percent of the diet, to 10 male and 10 female rats per dosage level. At the conclusion of the study, the authors reported:

No pathological effect whatever could be attributed to saccharin at levels of 1.0 percent or less. At 5 percent only one effect was noted, in the latter part of the experiment, namely an increased incidence of the ordinarily uncommon condition of abdominal lymphosarcoma. In the 5 percent group there were seven animals with lymphosarcoma; this number is not out of line with the incidence in comparable groups of rats, but the fact that in four of the seven rats abdominal as well as thoracic lymphosarcoma were present is unusual, since ordinarily the ratio is about 1 to 15-20. Three of these four combinations occurred in animals on experiment one hundred and two or more weeks.

In 1969, FDA pathologists reevaluated the findings from the Fitzhugh study (Ref. 12). They concluded:

The only effect of treatment during life was retardation of growth at 5 percent. In regard to pathological changes, our diagnoses of individual lesions were almost identical to those of Dr. Nelson. However, there were differences of opinion as to the role played by saccharin. Dr. Nelson stated in his 1951 report and also in the paper previously mentioned that the only pathological effect of saccharin was an increase in the incidence of the ordinary uncommon condition of abdominal lymphosarcoma. While we were not impressed by this, our examination of the written data and the microslides led us to conclude that saccharin had induced 2 lesions, and possibly a third: (1) Papillary excrescences from the papilla and papillo-crectal junction of the kidney in 13 of the 17 rats with kidneys sections microscopically

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at 5 percent, 3/18 at 1 percent, and 1/16 at 0.5 percent. The papillary excrescences were the result of edema, vascular congestion, leukocytic infiltration, and fibroblastic proliferation of the stroma, stratification of the normally simple cuboidal epithelium to the stratified cuboidal or transitional type, calcium deposition, and in a few instances, pleothrombosis. (8) Increased cellularity of the bone marrow at 5 percent. (9) Walls we have presented evidence which suggests that saccharin may have increased the incidence of the malignant lung tumor, lymphosarcoma, which occurs spontaneously in the rat and was very common in FDA rats at the time this study was performed, the data are inconclusive. A consideration of data, the presence of the renal changes, and the lack of knowledge as to whether the urinary bladder was affected strongly suggest the need for another two-year experiment.

A second long-term test of saccharin by oral administration to rats was completed in 1959 by Lessei (Ref. 13). As in the earlier Fishough study, rats were fed saccharin for 24 months at levels up to 5 percent of their diet. Twenty male and 20 female rats were used per group. Lessei included a positive-control group to determine the susceptibility of his rats to the development of this type of type, which he felt resembled the lymphosarcoma noted by Fishough et al. Lessei found this type of tumor, and several other tumors noted in both controls and treated animals; however, he did not find the incidence of tumors in the rats to be altered by the presence of saccharin in the diet even at the highest level (5 percent) fed.

In 1969, a re-study of the urinary bladders of some rats from the Lessei study was undertaken; however, all of the rats were not examined and the procedure used in the fixing of the urinary bladders would not be regarded as adequate by qualified experts. On gross observation of the rats, bladder abnormalities were noted at all feeding levels. Five males and three females at the 5-percent level exhibited these abnormalities. The author concluded that saccharin promoted the formation of bladder stones which in turn led to the bladder lesions (Ref. 14).

In 1970, at the request of FDA, the previously described studies and other data on the safety of saccharin were evaluated by NAS/NRC. At the conclusion of its review, the Academy made the following recommendations:

Long-term studies designed according to present-day protocols and including adequate investigation of effects on reproduction should be completed in at least two species. In view of the concern about effects on the kidney and urinary bladder, special attention should be given to pathological examination of these organs.

Based on the data available in 1970, the Ad-Hoc Subcommittee on Nonnutritive Sweeteners of the NAS Committee on Food Protection accepted 1 percent as a "no-effect" level of saccharin in the diets of rats and mice (Ref. 15).

Between 1970 and 1975, additional life-time chronic feeding studies of saccharin were conducted in which the compound was fed to laboratory animals either at a single- or multiple-dose level. These

studies were carried out in a variety of laboratory animals including rats, mice, and hamsters. Two of these modern studies yielded notable and troubling results. In both of these studies, diets containing saccharin were fed to male and female rats from weaning. As the proper age, these rats were bred and their offspring carried to lifetime. Thus, these offspring were exposed to saccharin in their diets from the time of conception until death. These two studies were conducted by FDA and in the laboratories of the Wisconsin Alumni Research Foundation (WARF).

The FDA study fed doses of 0.01, 0.1, 1, 5, and 7.5 percent saccharin to the laboratory animals. There were 50 males and 50 females in each dose group and 100 control animals (animals not fed saccharin). The study was terminated when the number of survivors in a test group fell to 20 percent of the starting number. Serial sacrifices were performed at 14 and 18 months. Of the 25 males fed the saccharin diet at the 7.5-percent level which were examined, 7 developed bladder tumors. No tumors were found at lower saccharin levels, but 1 of 25 rats examined fed the control diet developed a bladder tumor. Of the female rats, bladder tumors were found in 2 of 31 examined animals fed the 7.5-percent diet. None were found in the control females, nor in female rats fed the 5-percent or lower levels of saccharin.

The WARF study followed essentially the same protocol as the FDA study, except there were 20 males and 20 females per group and the study was terminated at 100 weeks. In the WARF test, bladder tumors were found in 7 of 15 male rats fed the diet containing 5 percent saccharin. No bladder tumors were found in the female rats at any level of saccharin feeding.

In the FDA study, the rats fed the higher dose levels (5.0 and 7.5 percent) tended to grow less well than did controls or those fed lower levels of saccharin; a body-weight deficit of about 15 percent prevailed throughout the test period. All other measurements of well-being were normal, however, including survival and organ weight/body weight ratios. In the WARF test, the high level (5 percent) saccharin-fed rats lagged behind the other groups during the period of rapid growth, but as adults revealed no difference in body weight. Indeed, the control group was the lightest among the males on test, but the weight range among the various groups was remarkably narrow.

The high dietary sodium (Na⁺) level introduced by feeding high levels (5 to 7.5 percent) of sodium saccharin (about 11 percent Na⁺) was taken into account in the FDA study by adding an equivalent level of Na⁺ as Na₂CO₃ to the diet of a group of rats fed the basal (no saccharin) diet. However, no test was made of Na⁺-free saccharin as opposed to soluble saccharin. This is an important issue, since, for example, the metabolic disposition of saccharin may be altered by higher Na⁺ levels; the question is not accounted for by the Na₂CO₃ control, nor

is it clear whether carbonate is an appropriate anion for this particular study.

As previously explained, the rationale of animal testing for possible carcinogenic hazards to man contemplates maximizing the sensitivity of the bioassay system, requiring administration of the highest tolerable dose along with appropriate lower doses. Because saccharin has a low toxicity, dose levels as high as 5 to 7.5 percent of the diet were fed in the FDA and WARF studies. As of 1975, tumors had been associated with saccharin feeding only at these high levels and in only two of many studies—those conducted by FDA and by WARF. This result raised uncertainty as to whether saccharin itself was the carcinogen, or whether the bladder tumors were induced by an impurity in the saccharin (orthothiosemicarbonyl) which was present at a detectable dose when high levels of saccharin were fed.

In addition, the high levels of saccharin fed were thought to raise the problem of calculus formation (Ref. 16). Calculus were associated with the occurrence of bladder tumors in the study by Elcks et al. (Ref. 17). Orthothiosemicarbonyl is a carbonic anhydrase inhibitor which can increase urinary pH, predisposing to calculus formation. Clayson found that bladder tumors due to certain sulfonamides were eliminated by feeding NH₄Cl to give an acid urine (Ref. 18). Furthermore, saccharin alone may cause bladder calculi (Ref. 19). This was thought to be potentially important, because there is evidence that bladder stones may play a determining role in the appearance of bladder tumors in the rat. Occurrence of bladder stones and increased urinary pH associated with saccharin feeding were not investigated in the FDA or in the WARF study. It was thought that this phenomenon may be critical in the embryo or newborn rat that is exposed to saccharin.

It should be emphasized that in both the FDA and WARF tests the offspring (F₁) generation of rats, i.e., those that were conceived after the parental generation had been placed on the saccharin-containing diets, were held and observed for manifestation of toxicity. The relatively high sensitivity of experimental animals to transplacental exposure to carcinogens has become obvious in recent years (Ref. 20). A number of carcinogens have been shown to be effective at very low levels by the transplacental route. Frequently, exposure of the pregnant female is associated with the relatively early appearance of tumors in the offspring. Despite these important implications, information about transplacental carcinogenesis is limited. For example, the dose level to which the fetus is exposed is often unknown, nor is there an understanding of the importance of developmental state, metabolic capacity, immune competency, and factors related to fetal pharmacology.

Even with these uncertainties, however, the F₁-F₂ feeding procedure is considered to be an appropriate and essential test because saccharin may be consumed by pregnant women as well as in-

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dividuals of all ages. The technique of in utero exposure in lifetime testing has been recommended by an expert on carcinogenesis of the FDA Advisory Committee on Protocols for Safety Evaluation, *J. Tox. and Appl. Pharmacol.*, 20:419-438 (1971). The panel recognized that exposure of an animal to a chemical early in life, even during pregnancy, may be important in influencing expression of carcinogenesis later in life. The panel stated that "since one of the important purposes of the chronic toxicity tests is the detection of carcinogenic potential, it would seem desirable to begin the exposure as early in life, i.e., as close to conception as possible."

The International Agency for Research on Cancer of the World Health Organization (IARC Scientific Publications No. 4) has also endorsed the need to consider in utero exposure in the study of carcinogenesis potential. The IARC report noted that "experimental studies have indicated the increased susceptibility of neonatal animals to the carcinogenic insult. The logical development of studying the effect on the rodent fetus of maternal exposure to a chemical carcinogen has made it clear that this pathway could well be operative in the human fetus."

Unfortunately, in neither the WARF study nor the FDA study were the rats in the parent (F₁) generation continued for long-term carcinogenicity testing of saccharin; thus no comparative data on the susceptibility of F₁ and F₂ rats in an internally controlled experiment were obtained. Therefore, at the conclusion of these studies, doubt remained about whether the concurrence of transplacental exposure and of bladder tumors was causally related.

Because of the continuing questions about the carcinogenicity of saccharin, in June 1972, FDA once more called upon the Academy to review the results of all experiments on the issue. To be able to provide FDA with a complete and up-to-date report, the Academy delayed completing its review until several studies, including the FDA study, then underway, were completed.

The Academy's report was received by FDA in December 1974. The report's primary conclusion was that the data then available had "not established conclusively whether saccharin is or is not carcinogenic when administered orally to test animals." This conclusion was based in part on the uncertainty about the role of orthotoluenesulfonamide (OTS) in the induction of tumors. The Academy recommended that additional research on saccharin be conducted to determine whether saccharin is a carcinogen. The Academy recommended further that FDA reconsider the question when a substantial portion of the additional data became available.

E. CANADIAN STUDY

The recently reported Canadian study was initiated in February 1974 under the sponsorship of the Department of Health and Public Welfare of the Canadian Government (Toxicity and Carcinogenicity

Study of Orthotoluenesulfonamide and Saccharin, Project—E405/405E). Two generations of test animals (the F₁ and F₂ generations) were fed OTS and OTS-free saccharin to evaluate the toxicity and carcinogenicity of these compounds. The study on saccharin was the third experiment in which rats were exposed to saccharin during their period of development in the uterus and then throughout their entire life span. Both the earlier FDA and the WARF studies had shown an increased incidence of bladder tumors in male rats, but had left unresolved the question whether the tumors were caused by saccharin itself or by OTS. The Canadian study was designed to clarify this question by testing the OTS by itself as well as by testing purified saccharin containing only minimal amounts of the impurity. The Canadian study was thus designed to resolve the uncertainties noted by the NAS in its 1974 report.

Six groups of 50 male and 50 female rats were included in the study: a control group, 3 dose levels of OTS at 2.5, 25, and 250 milligrams per kilogram per day, a group receiving 1 percent saccharin (2,500 milligrams per kilogram per day) in the diet, and a group receiving 250 milligrams per kilogram OTS per/day and 1 percent ammonium chloride in the drinking water. The doses of OTS incorporated the amount of OTS ranging from 0.6 to 27 milligrams per kilogram OTS per day, which may have been consumed by animals in the FDA and WARF studies on saccharin. OTS, a weak carbonic anhydrase inhibitor, may have a tendency to produce a slightly alkaline urine, possibly resulting in an increased incidence of bladder stones. Therefore, ammonium chloride was added to the drinking water of one OTS group to prevent this effect by producing a more acidic urine. The rats were observed daily, their weights and food consumption were recorded weekly, and 20 males of each generation of controls, saccharin treated, and high-level OTS treated animals with and without ammonium chloride had urine examined at 8-month intervals for microscopic calculi and parasite eggs.

The results of the Canadian study have been evaluated by expert pathologists, including scientists from FDA and other institutions in the United States, from Great Britain, and from other European countries, as well as from Canada. The findings indicate unequivocally that saccharin causes bladder tumors in the test animals. Specifically, 7 male and no female rats in the F₁ generation developed bladder tumors. Twelve male and two female rats in the F₂ generation developed bladder tumors. Thus, of a total of 200 rats fed saccharin, 21 developed bladder tumors.

In sharp contrast, of 100 control animals—those not fed saccharin or OTS—only 1 developed a tumor. Moreover, the low incidence of tumors in the animals fed OTS clearly resolves the earlier speculations, based on the FDA and WARF studies, that OTS and not saccharin may have been responsible for the cancers in the test animals. No evidence

of bladder parasites was found in any of the rats. Microscopic crystals were found in the urine but the distribution did not seem to be related to treatment. Two grossly visible bladder stones were found in rats bearing tumors, one receiving saccharin and the other receiving OTS, while six were found in animals of various groups without bladder tumors. There was no significant increase in bladder tumors in any of the groups treated with OTS.

F. ASSESSMENT OF HUMAN RISK

An important question raised about the animal studies on saccharin is their relevance to human beings. Public reaction to recent publicity about the Canadian study suggests considerable misunderstanding about the nature of toxicity testing in animals and the interpretation of results. For example, it has been widely publicized that the dose of saccharin found to be carcinogenic in rats is about 1,000 times that ingested by a human in a single diet beverage (when both doses are adjusted for the difference in body weight between rats and humans). Since this amount of saccharin would clearly never be ingested chronically by any person, some have suggested that these results have no pertinence whatsoever to human risk. In the judgment of FDA, this conclusion is not valid for the reasons to be described in this section.

Before dealing with the saccharin data specifically, however, the principles of appraising the risk of chemical carcinogenic substances should be explicitly stated. Those principles are as follows:

1. Certain substances can be shown in validly controlled animal experiments to increase the incidence of benign and/or malignant tumors. This result does not occur with all chemicals, only with certain ones.
2. Those substances that cause benign or malignant tumors in one species often also do so in other species. Therefore, any substance that causes such tumors in any species must be considered a potential carcinogen in man.
3. Chemical carcinogens, like other toxic substances, generally demonstrate a dose-response relationship, i.e., the greater the dose the greater the tendency to produce tumors, and vice versa. The predominant opinion among experts in the field of carcinogenesis is that the dose-response principle extends to very low doses of the carcinogen—that is, that there is no dose, however small, at which one can be certain there is no risk. In other words, there is no threshold dose below which a carcinogen may be considered safe in the absolute sense.
4. Estimation of the risk of a low dose of a carcinogen in animals requires that one test the carcinogen at a dose high enough to produce tumors in the group of animals tested and then calculate what the risk is likely to be at a very small dose. The intent of animal testing is not only to identify potential risks such as carcinogenesis but also to estimate whether such an effect is likely to occur with a frequency, e.g., of 1 in 100, 1 in

1,000, 1 in 10,000, 1 in a 100,000, 1 in a million, etc. Since the actual measurement of a single event once in a 1,000 times, requires several thousand animals, it is evident that direct measurement of low frequency events cannot feasibly be done because of limitations on cost, the difficulty of handling large numbers of animals, etc. The problem is thus currently solved, albeit imperfectly and not without difference of opinion among experts, by conducting tests with a feasible number of animals at high doses and extrapolating the results to low doses.

5. The method of extrapolation of results obtained at high doses to low doses should be a "conservative" method, i.e., it should err in the direction of overestimating risk rather than understating it. Two accepted methods that meet this principle are the linear extrapolation method and the Mantel-Bryan procedure. In the dose range under consideration, the two methods give similar results for saccharin. The linear extrapolation method has been used in the FDA calculation on saccharin because it is easier to explain and understand.

6. The results of animal tests and their extrapolation to low doses provides an estimate of the risk of developing a tumor in the species tested. If one is to assume that such results are directly applicable to man, one must assume that one lifetime in the test animal is equal to a lifetime in man and that the test animal and humans are equally sensitive to the carcinogen. These assumptions are clearly open to debate, but in the absence of data to the contrary, the opinion of most experts is to assume that they are applicable. In the case of some carcinogens, wide variation among species in their sensitivity to the chemical has been demonstrated. The current view of experts is that these differences are due, at least in part, to species differences in the way the carcinogen is metabolized. In the case of saccharin, the drug is metabolized little, if at all, in either the rat or man. This fact supports the assumption that results from testing in rats are applicable to human risk assessment. The FDA risk estimates are then based on the principle that risk estimates in the rat are directly applicable to man.

Current scientific methods are not capable of determining the exact risk to humans of a chemical found to be carcinogenic in animals. However, techniques are available for estimating the upper limits of the risk. The Food and Drug Administration estimates that the lifetime ingestion of the amount of saccharin in one diet beverage per day results in a risk to the individual of somewhere between zero and 4 in 10,000 of developing a cancer of the bladder. If this risk is transposed to the population at large and if everyone in the United States drank one such beverage a day, this would result in anywhere between zero and 1,500 additional cases of bladder cancer per year. These estimates are identical to the estimates recently

presented publicly by representatives of the Food and Drug Administration and of the National Cancer Institute (NCI) in hearings before the Health Subcommittee of the House Committee on Interstate and Foreign Commerce. The approach used in their calculation is described in the following paragraphs.

In the Canadian study, a 34 percent incidence of bladder tumors (12 of 60) was noted in the second generation male rats fed saccharin in a dose of 8 percent of the diet. This was the most sensitive group in the study to the carcinogenic effect of saccharin. Thus, in the absence of evidence that factors involved in its sensitivity are not relevant to the human population, this group is used to estimate the upper limit of human risk. There were no bladder tumors in an untreated control group of comparable size. Although the observed incidence of bladder tumors was 34 percent, the upper limit of risk in this study at the 98 percent confidence level is 28 percent. A 5 percent dietary level of saccharin in the rat is equivalent to 3,500 milligrams/kilogram/day of saccharin. If a 60-kilogram human (approximately 132 pounds) were to ingest 150 milligrams/day of saccharin (i.e., 2.5 milligrams/kilogram/day over a lifetime, he or she would thus receive the equivalent of one one-thousandth of the rat dose per day. This dose is approximately that contained in one large diet beverage drink (12½ ounces) per day.

Since rats fed 3,500 milligrams/kilogram/day may have as high as a 36 percent incidence of bladder tumors, ingestion by rats of one one-thousandth of that dose could yield, by linear extrapolation, an incidence of 0.036 percent or 4 cases per 10,000.

The lifetime risk of bladder cancer in humans in the United States is 1.5 percent; that is, of every 10,000 persons, it is expected that 15 will develop bladder cancer sometime during their lives. Extrapolating from the Canadian rat study, and if one assumes a direct correlation between the estimate of maximum risk of saccharin in rats and in humans, if a human ingests 150 milligrams/day of saccharin for a lifetime, he could increase the risk of bladder cancer by 0.036 percent for a total risk of approximately 1.54 percent. That is, of every 10,000 persons, 154 might develop bladder cancer if they all use 150 milligrams/day of saccharin and if the assumptions are valid.

The risk from use of 150 milligrams/day of saccharin over a lifetime can be assessed in another fashion. The annual case rate of bladder cancer in the United States is given by the NCI as approximately 30,000. If everyone in the United States ingested 150 milligrams of saccharin per day (e.g., from one large diet drink) over a lifetime, and if the other assumptions are correct, there could be approximately an additional 1,500 cases per year (or an increase in risk of 4 percent over the basal risk). If only half the population ingested 150 milligrams of saccharin per day over a lifetime, an

additional 600 cases per year could occur (or an increase in risk of 2 percent over the basal risk).

The estimated increase risk from this moderate use of saccharin cannot be detected in human epidemiological studies. Such studies usually can only detect increased risks of 200 to 300 percent (i.e., 2 to 3 times the baseline rate) or greater. Even the best feasible epidemiologic study is not likely to detect an increased risk of only 2 to 4 percent over background incidence. Thus, for example, the author of one epidemiological study of bladder cancer in consumers of artificial sweeteners (Kessler, I. L. J. Urology, 115:143-146, 1976) noted that "The sample sizes used here would permit the detection of an 80 percent increase in bladder cancer owing to nonnutritive sweetener use . . ." This study would not, then, have detected any increase in bladder cancer due to saccharin consumption if the risk is at the level suggested by the Canadian study in rats. As discussed previously, cancer has a long latent period, requiring 5 to 30 years before it can be detected. Although saccharin has been used in food for over 70 years, it is only in the past 15 to 30 years that its use has become substantial. Thus, it is probably too early to ascertain from human epidemiological studies the number of bladder cancers associated with saccharin consumption. This conclusion was reached by the authors of one of the epidemiological studies on saccharin (Armstrong, B. and E. Doll, Brit. J. Prev. Soc. Med., 23:333-40, 1974) who pointed out that "If the minimum time necessary to see a significant number of bladder cancers induced by saccharin were more than thirty years . . . it would be too early to see an effect of saccharin consumption on mortality rates."

In a third epidemiological study on saccharin consumption (Armstrong, B. et al., Brit. J. Prev. Soc. Med., 30:151-157, 1976) only about 600 of the diabetics studied had consumed saccharin for more than 35 years. This number is far too low to detect the level of risk from saccharin consumption suggested by the experiments in rats. The fact that these epidemiological studies in patients with diabetes who used saccharin for prolonged periods revealed no detectable increase in bladder cancer is therefore compatible with the available animal data. A common feature of all three epidemiological studies is their comparative insensitivity, which could permit a sharply increased incidence of bladder cancer attributable to consumption of saccharin—on the order of more than 20,000 cases per year in the American population—to go undetected.

By contrast, the risk of lung cancer from cigarette smoking (which FDA has no authority to regulate) is now readily detectable in human epidemiological studies. However, even though cigarette smokers have been shown to incur a risk of developing lung cancer that is 500 to 2,000 percent greater than the risk of lung cancer incurred by nonsmokers, it depends on how much they smoke, it

took many years to recognize and document the increased risk. The first suggestions of an association between cigarette smoking and lung cancer were not made until the late 1930's (Ruller, F. H. Z. Krebsforsch. 49:57-84, 1939). Several epidemiological studies reported an association between cigarette smoking and lung cancer in the early 1950's, but widespread acceptance of the relationship did not occur until publication of the 1964 report to the Surgeon General entitled *Smoking and Health*.

The Food and Drug Administration thus considers the animal data and the human epidemiological data on saccharin to be compatible. The estimated excess risk to the individual of developing bladder cancer from lifetime use of, e.g., 150 milligrams of saccharin per day, is believed to be somewhere between zero and 4 per 10,000. The estimated population risk in the United States, assuming such use by each individual, is somewhere between zero and 1,200 cases per year.

Although the risk from consumption of saccharin is small compared to that of other health hazards, e.g., cigarette smoking, saccharin is only one of a potentially large number of hazards present in our environment. The Commissioner believes that reduction of prolonged, general exposure to a number of weakly carcinogenic substances in our environment as they are discovered may be essential to reduce the total incidence of cancer.

G. LEGAL BASIS FOR ACTION

Press reports of the announcement of FDA's intention to withdraw approval of saccharin as an ingredient in foods and beverages have given the impression that the Commissioner is acting reluctantly, based exclusively on the Delaney anticancer clause of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 348 (c) (3) (A)) and, further, that the agency's action was triggered solely by the findings of the Canadian study. Neither impression is accurate.

As should be clear from the foregoing discussion, questions about the safety of saccharin have persisted almost from the date of its introduction. Serious doubt about its potential for causing cancer in laboratory animals arose much later, but this concern, too, is not new. Before the Canadian study, two scientifically sound and generally well-conducted studies had suggested an association between saccharin and bladder cancer in animals exposed to high doses of the sweetener. The Canadian study unequivocally confirms this association and lays to rest speculation that the causative agent may have been OTS. There can no longer be any doubt that saccharin causes a sharply increased incidence of bladder cancer in test animals.

The discussion in the previous section makes clear that the human risk of cancer indicated by these findings is significant and cannot be ignored. The Commissioner believes that conscientious protection of the public health is not consistent with continued general use in foods of a compound shown to present

the kind of risk of cancer that has been demonstrated for saccharin—regardless of the asserted benefits of its use for some individuals in the population.

Section 409(c) of the act (21 U.S.C. 348(c)) requires that any food additive must be found to be safe for human consumption before it can be approved or, in case of an additive already approved, continue to be used in foods. Based on the accumulated evidence of hazard associated with ingestion of saccharin, culminated by the Canadian study, the Commissioner concludes that the finding required by the statute can no longer be made, and that the interim food additive regulation approving the use of saccharin should be repealed.

FDA has previously prohibited the use in food of ingredients found to cause cancer in laboratory animals to which the Delaney clause was not applicable. For example, in January 1950, before enactment of the Delaney clause, FDA prohibited the use in food of two artificial sweeteners as "poisonous substances." This conclusion was based in large part on the finding of liver tumors in rat studies. In May 1968, FDA prohibited the use in food of the flavoring agent, oil of calamag, based on a finding of carcinogenicity in animal studies. Oil of calamag had been used in food on the determination that it was generally recognized as safe; thus, the Delaney clause did not apply. There are a number of other examples. In short, although FDA has acted on a number of occasions to remove carcinogenic substances from the food supply during the past 25 years, only two previous actions—both involving minor indirect food additives—have been based on the Delaney clause.

Those actions, like this one, were based on certain well-recognized postulates about chemical carcinogenesis: (1) there is reason to believe that those substances which cause cancer in animals may also cause cancer in man; (2) animal tests, despite inadequacies, provide the best evidence currently available about the potential of chemicals to cause cancer in humans; (3) there is no reliable basis for concluding that there is a completely "safe" level of a carcinogen, i.e., a threshold level that will not cause cancer in some members of the population; and (4) cancer appears to be an irreversible process, in both test animals and in man.

It is of course true that the present law would afford the Commissioner no choice but to prohibit the marketing of saccharin as an ingredient in foods even if he were not persuaded that the scientific evidence independently warranted such action. The Delaney anticancer clause specifies that "No additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal . . ." (21 U.S.C. 348 (c) (3) (A).) There can no longer be any question that saccharin does cause cancer when ingested by laboratory animals, in this instance in tests that the Com-

missioner would in any event regard as appropriate for the evaluation of carcinogenicity.

Therefore, under both the general safety requirement of the Food Additives Amendment of 1958 and the Delaney anticancer clause, the Commissioner concludes that saccharin may no longer be approved as a food additive. This proposal is issued to comply with the procedural requirements of section 409(h) of the act. The Commissioner welcomes comments on any facet of this proposal, including the reasonableness of his judgment about the safety of saccharin under the law. He feels constrained to point out, however, that the wisdom of the Delaney clause is not at issue in this proceeding. FDA could not ignore that provision even if the Commissioner were persuaded that the risks to human health were less than they appear. He further notes that under the provisions of the law relating to food additives, FDA is not empowered to take into account the asserted benefits of any food additive in applying the basic safety standard of the act.

The Commissioner does recognize, however, the potential medical value of permitting saccharin to remain available for individuals who may depend on a nonnutritive sweetener to maintain a diet free from sugar, provided such products can meet the standards of the drug provisions of the act. This subject is addressed in the following part of this preamble, on which the Commissioner specifically invites comments from specialists in the treatment of diabetes and obesity.

II. USE OF SACCHARIN IN DRUGS

A. HISTORY OF DRUG USE OF SACCHARIN

In addition to being used in foods, saccharin has been used in drugs for a number of years as a sweetening agent to improve the taste of oral drug products. Thus saccharin is used extensively in such drugs as pediatric liquid preparations, chewable tablets, and mouthwashes and toothpastes with drug claims. When used as a sweetener in a drug product, it is usually used in conjunction with a nutritive carbohydrate sweetener, such as sucrose or sorbitol, to mask the bitter aftertaste often experienced with saccharin. Saccharin is a pharmaceutical aid in liquid pediatric products where palatability is important to induce small children to take the medication. The volume of sucrose needed to provide acceptable levels of sweetness in some of these products has posed problems of incompatibility in the formulation in certain products.

The quantity of saccharin used as a flavoring agent in drug products covers a wide range. For example, of 12 penicillin V potassium products for oral suspension that were examined, the concentration of saccharin ranged from a low of 5.2 milligrams per teaspoonful to a high of 42.8 milligrams per teaspoonful. If a pediatric liquid oral preparation contains 40 milligrams of saccharin per teaspoonful (one dose) and the maxi-

imum daily dose is 2 teaspoonfuls four times a day, a child could consume 320 milligrams per day of saccharin from this one drug. Obviously, if other products containing saccharin were also being consumed, the daily intake of saccharin would be much higher. It should also be noted that drug products can be used for both the treatment of acute and chronic conditions. Thus, if a drug product containing saccharin is administered daily for the treatment or prophylaxis of a chronic condition, such as rheumatic fever, the patient could be exposed to a daily amount of saccharin equivalent to that contained in one or more diet soft drinks.

Saccharin is also marketed in tablet, powder, and liquid forms as a so-called "tabletop sweetener" for use in conditions in which nutritive carbohydrate sweeteners in the diet must be avoided. Certain of these products meet the statutory definition of a drug in that they are recognized by the U.S. Pharmacopoeia or the National Formulary. In addition, they are at one time or another been tacitly recognized by FDA as drugs. In recent years, however, such products have been marketed and regulated as food additives.

In light of the recent Canadian study's unequivocal demonstration that saccharin causes malignant bladder tumors in test animals, the Commissioner has examined the use of saccharin in drug products, both as an inactive ingredient and as an active ingredient. In his judgment, the safety considerations involving the use of saccharin in drug products differ from those regarding its use in foods. Moreover, the Delaney clause does not apply to drug products. An ingredient that is clearly unjustified for general use in foods for humans may be suitable for use as a drug when there is a legitimate medical need that outweighs the risks of possible adverse effects. The Commissioner is thus permitted under the drug provisions of the law to evaluate the risk of using saccharin compared to the benefits of its use as a drug ingredient.

B. SACCHARIN AS AN INACTIVE INGREDIENT IN DRUG PRODUCTS

With respect to the use of saccharin as a pharmaceutical aid, the Commissioner has tentatively concluded that the risk of such use in most drug products is not outweighed by the benefits, and thus, saccharin will not be permitted as an inactive ingredient unless it affords an overriding benefit. The Commissioner therefore proposes to add new § 310.514 to Part 310 (21 CFR Part 310) of the new drug regulations, declaring that any drug product for human use containing saccharin as an inactive ingredient is a new drug and is misbranded unless such product is specifically exempted from the regulation. The Commissioner bases this proposal on the fact that the use of saccharin in most drug products as an inactive ingredient produces no direct therapeutic benefit. Thus, the possible risk associated with the use of saccharin for such purpose is medically unjustified.

This is particularly true because individuals do not have the opportunity to choose whether or not to take such a risk if saccharin were to remain available as an inactive ingredient in drug products.

In § 310.514, the Commissioner proposes that any holder of an approved new drug application for a drug product containing saccharin as an inactive ingredient be required to submit to FDA within 9 months of the date of publication of the final regulation, a supplemental application providing for a new revised formulation removing saccharin as an ingredient. The revised formulation may not be marketed before the receipt of written notice of approval of the supplemental application by FDA. Any sponsor of a "Notice of Claimed Investigational Exemption for a New Drug" (IND notice) for a drug product containing saccharin as an ingredient shall amend the IND notice within 9 months of the date of publication of the final regulation to revise the formulation removing saccharin as an ingredient. Under the proposal, the Commissioner would initiate action to withdraw approval of an application or terminate an IND notice if any current holder of an approved new drug application or sponsor of an IND notice fails to submit a supplemental application or to amend an IND notice as set forth, and within the time periods provided for, in § 310.514.

A period of 9 months for the submission of supplemental applications is being proposed to allow manufacturers time to reformulate their products and perform the stability and bioavailability studies, where necessary. Depending upon the type of product, i.e., tablet or liquid, and the amount of saccharin currently in the product, reformulation to maintain palatability may pose problems. For example, attempts to raise the content of nutritive sweeteners to mask the bitter taste of a drug is limited by such physical factors as solubility. Further, because of the increased nutritive sweetener content, a preservative may have to be added. Likewise, as of July 7, 1977, firms will also have to comply with the bioavailability requirements as set forth in §§ 320.31 and 320.32 (21 CFR 320.31 and 320.32) of the regulations. Similar provisions, applicable to antibiotic drug products, are set forth in a new § 430.300 that the Commissioner proposes to add to Part 430 (21 CFR Part 430) of the regulations.

Because of the potential need for specially formulated drugs for diabetics or for special situations in which saccharin may be necessary for the product as a pharmaceutical aid, the Commissioner is also proposing a specific provision under which a petition may be submitted to FDA requesting that a specific use of saccharin as an inactive ingredient be permitted. To support such a petition, the person requesting the exemption must submit the following information: (1) the amount of saccharin in the drug product; (2) is saccharin included as a pharmaceutical aid, an

adequate showing that there are no technically feasible alternatives to saccharin, or an adequate showing that the drug product containing saccharin provides a substantial health benefit that would not be available without the use of saccharin, for example, the product is one specifically formulated for diabetics; and (3) copies of the proposed labeling specifying the saccharin content.

Whether or not the drug product is subject to the requirements for an approved new drug application or for antibiotic certification, under the proposal, a drug product containing saccharin as an inactive ingredient shall, unless exempted, not be manufactured after 15 months and shall not be initially shipped into interstate commerce 18 months from the date the final regulations are published in the FEDERAL REGISTER. INITIAL introduction into interstate commerce of a drug product for purposes of this regulation means the first shipment of the final dosage form of the drug product into interstate commerce pursuant to a sale or consignment to an independent party. Since these dates are applicable to all drug products, firms submitting supplemental new drug applications or amendments to antibiotic drug files should assure that they are complete when they are submitted.

C. SACCHARIN AS A SINGLE-ACTIVE-INGREDIENT DRUG

Saccharin has been available for many years in single-active-ingredient products for use by individuals who must control their caloric intake. These products consist of tablets, liquids, or powders containing saccharin as the primary sweetening ingredient, and some are popularly known as "tabletop sweeteners." For the most part, these products have been regulated by the agency as food additives, and most recently as special dietary foods (see 21 CFR 106.79, formerly 21 CFR 128.7 prior to reclassification published in the FEDERAL REGISTER of March 15, 1977 (42 FR 14392)).

These products have also historically been recognized as drugs. The Referee Board of the United States Department of Agriculture, while considering the safety of saccharin in foods in 1912, stated, "The Food and Drug Act provides that any substance which is intended to be used for the prevention, cure, or mitigation of disease is a drug, and a product containing saccharin and plainly labeled to show that the mixture is intended for the use of those persons who, on account of disease, must abstain from the use of sugar, falls within the class of drugs." This statement by a board of scientific advisors indicates that, even as early as 1912, saccharin was recognized as a drug when offered for sale for use by persons with a medical need to limit nutritive sweeteners in their diets.

The United States Pharmacopoeia has recognized saccharin as a pharmaceutical aid since at least 1924. The current edition of the National Formulary recognizes saccharin calcium, saccharin sodium, and saccharin sodium tablets. By

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virtue of the recognition of those products in the official compendia, and depending on their labeling, they may fall within the definition of "drug" in section 201(g) of the act (21 U.S.C. 321(g)).

Saccharin was reviewed in the mid-1960's under the new drug provisions of the act as an active ingredient of a new drug product in combination with a cyclamate salt, but the new drug application for this product is no longer approved. In addition, as recently as August 27, 1976 (40 FR 38179), FDA published an amended notice requesting data and information on saccharin for review by its OTC Miscellaneous Internal Products Panel. This publication was a part of the agency's ongoing review of OTC drug products for human use currently marketed in the United States. Saccharin was included in the listing of ingredients under the product categories of sweeteners and weight control products. The Commissioner notes, however, that in response to the August 27, 1976 notice, no submissions of any type were made for any product containing saccharin as an active ingredient. The Bureau of Drugs of FDA thus has no request before it at the present time from any manufacturer to market saccharin either OTC or by prescription, under the OTC review or as a new drug.

Although single-ingredient tabletop sweeteners containing saccharin in the form of tablets, liquids, or powders have been subject to regulation as foods, the Commissioner believes that such products may be considered as drugs, depending upon the claims made for them. The essential criterion for determining whether a product is a drug is whether it meets the definition in section 201(g) (1) (B) and (C) of the act, as "articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of a disease in man or other animals" and "articles (other than food) intended to affect the structure or any function of the body of man or other animals."

Once determined to be a drug, a product must meet the standards of the drug provisions of the act, among them the safety and effectiveness requirements of either section 201(p) or 505. Section 201(p) states that a drug is a new drug if it is "not generally recognized, among experts qualified by scientific training and experience to evaluate the safety and effectiveness of drugs, as safe and effective for use under the conditions prescribed, recommended, or suggested in the labeling thereof." If a new drug, the law requires among other things "substantial evidence that (it) will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the proposed labeling thereof" (21 U.S.C. 355(d)(5)). Such substantial evidence means "evidence consisting of adequate and well-controlled investigations, including clinical investigations, by experts qualified by scientific training and experience to evaluate the effectiveness of the drug involved, on the basis of which it could fairly and responsibly be concluded by such experts that the drug will

have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the labeling or proposed labeling thereof" (21 U.S.C. 355(d)(6)).

Finally, if a drug is otherwise marketable, the Commissioner must determine whether it should be considered as a prescription or OTC drug. The applicable standard (21 U.S.C. 353(b)(1)(B)) requires that a drug must be dispensed by prescription if, "because of its toxicity or other potentiality for harmful effect, or the method of its use, or the collateral measures necessary to its use, (it) is not safe for use except under the supervision of a practitioner licensed by law to administer such drug."

The Commissioner recognizes that saccharin is the only product available on the market for use as a nonnutritive sweetener and that such use may be important to the proper dietary management of individuals who must control their intake of nutritive sweeteners. These include individuals with conditions such as diabetes, obesity, reactive hypoglycemia, and carbohydrate-induced hyperlipemia. Whether such use is properly construed as a drug use under the law depends upon the claims made by the manufacturer and is reasonably open to debate. The Commissioner is prepared to consider the possibility that such single-ingredient sweeteners may be marketable as drugs, even if the same formula might not be approvable as a food additive. The Commissioner believes, however, that the proper context for considering such a use under the drug laws is in reviewing new drug applications for individual products.

In reaching this tentative conclusion, the Commissioner has specifically excluded the possibility of reviewing the matter further as part of the OTC review. This review is fundamentally intended to identify those conditions under which specific ingredients can be generally recognized as safe and effective for OTC use within the meaning of section 201(p) of the act. There is no realistic prospect, however, that such a determination can be made for saccharin as a drug. Saccharin has no history of marketing in the United States as a drug approved for effectiveness under the Drug Amendments of 1962; general recognition of effectiveness under these conditions would seem to be precluded, even though effectiveness *vel non* may be demonstrated. Similarly, the Canadian study represents new evidence reflecting on the safety of the product which the Commissioner considers sufficient to remove it from the market as an approved food additive. In the face of this new evidence, general recognition of safety does not appear to be a reasonable possibility. For these reasons, the Commissioner concludes that saccharin is not a suitable ingredient for review by the Miscellaneous Internal Products Panel of the OTC Review, and the call of August 27, 1976 for submission of information on sweeteners to this panel is hereby rescinded.

The Commissioner believes that the new drug application is a more appropriate mechanism for considering the issues related to the marketability of saccharin-containing sweeteners for use by individuals who for medical reasons must limit their intake of nutritive sweeteners. He therefore invites comment on a proposal to add a new § 210.514(b) to the regulations which would permit the submission of new drug applications for such products. This authorization would be limited to consideration of tabletop sweeteners in packaging appropriate for use by individual patients. The agency will not entertain under this proposal new drug applications for any products that are clearly foods sweetened with saccharin, e.g., diet soft drinks, canned fruits, etc.

The proposed regulation requires that any manufacturer wishing to ship a single-active-ingredient sweetener containing saccharin in interstate commerce would have to meet the following conditions after publication of the final regulations:

1. Within 180 days, submit a new drug application for the product, meeting the requirements of § 314.1 of the regulations.

2. Within 120 days, label the product with the following interim indications statement: "For use as a noncaloric sweetener when a sugar-restricted diet is medically indicated, as in patients with diabetes" and with a warning statement concerning the risk of cancer. The Commissioner proposes the following warning statement, and solicits additional suggestions: "Warning: Saccharin causes bladder cancer in animals. Use of saccharin may increase your risk of cancer."

Any manufacturer not meeting these conditions would be subject to regulatory action.

The Commissioner proposes that those manufacturers whose products meet the requirements of this section will be permitted to market their products while their new drug applications are under review. Such marketing is permitted for a marketed drug which is newly declared as a new drug provided the Commissioner determines it is or may be medically necessary (*Hoffman-LaRoche, Inc. v. Weinberger*, 425 F. Supp. 890 (D.D.C. 1975)). The Commissioner has determined that the continued marketing of saccharin as a single-ingredient drug meets this criterion, at least for purposes of permitting further consideration of the data and information in new drug applications, since saccharin is the only remaining sweetener on the market for patients on sugar-restricted diets.

The foregoing determinations should in no way be construed as committing the Commissioner to approve any new drug applications submitted either for the interim indication proposed or any other indication. Approval will depend on whether the products as labeled meet the definition of a drug and whether the evidence presented in these applications meets the criteria for approval set forth in the statute and in the regulations.

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The Commissioner tentatively concludes that, if saccharin-containing sweeteners are labeled as drugs and if they are deemed to be otherwise approvable under the new drug provisions of the act, they may be marketed OTC. This conclusion is based on the lack of toxicity (other than that of cancer, for which it will be labeled), the lack of other collateral measures necessary for its safe use, which would require a prescription, and the long history of safe OTC use of the product without a physician's prescription. The Commissioner invites comments on this tentative conclusion.

III. USE OF SACCHARIN AS A COSMETIC INGREDIENT

Saccharin is currently used as an ingredient in a number of cosmetic products, principally to affect taste. Many of these products, such as dentifrices (toothpastes) and mouthwashes, as well as lipsticks, are likely to be ingested under normal conditions of use. Although the risk of exposure to significant amounts of saccharin from any of these products may not be large, the use of saccharin affords no benefit sufficient to warrant the acceptance of any increased risk. The Commissioner therefore proposes to determine that the use of saccharin in any cosmetic product that is likely to be ingested and which is manufactured more than 30 days after the date of publication of a final regulation will result in the product being deemed to be adulterated under section 801(a) of the act (21 U.S.C. 801(a)).

IV. USE OF SACCHARIN IN STANDARDIZED FOODS

Saccharin is listed as a mandatory ingredient in nine standards of identity for artificially sweetened fruit products. In addition two standards, 21 CFR 146.111 and 146.121 (formerly 21 CFR 27.128 and 27.103, prior to recodification published in the *Federal Register* of March 15, 1977 (42 FR 14302)) list as a mandatory ingredient "one or more of the artificial sweetening ingredients listed in and complying with Parts 170 through 129 of this chapter."

The Commissioner proposes to amend those standards of identity for artificially sweetened fruit products that require saccharin to be used as the artificial sweetener by deleting the reference to saccharin and replacing it with more general language requiring the use of "one or more of the artificial sweeteners listed in and complying with Parts 170 and 189" of Chapter I of Title 21 of the Code of Federal Regulations.

When the ban on saccharin as a food additive takes effect, the marketing of the foods covered by the 11 standards will be unlawful. The Commissioner has opted to amend the standards rather than revoke them to conserve agency resources.

If the standards were revoked and an artificial sweetener was subsequently approved for use by FDA, the process of establishing standards for artificially sweetened fruit products would have to

begin anew. By keeping the standards on the books, the Commissioner will avoid unnecessarily expending scarce agency resources. The Commissioner emphasizes, however, that his election of the amendment approach rather than revocation should not be taken to be an implied prediction that FDA will soon approve to another artificial sweetener as a replacement for saccharin. The amendments are being proposed as a matter of administrative convenience, not as a harbinger of future approval of any artificial sweetener.

V. USE OF SACCHARIN IN ANIMAL DRUGS AND ANIMAL FEED

The use of saccharin as an ingredient in animal drugs or animal feed for food-producing animals requires a demonstration that no residue will be found in food from the edible products derived from those animals, either by an assay designated in accordance with the proviso to the anticancer clauses of the act (sections 409(c)(3)(A), 512(d)(1)(E), and 706(b)(6)(B)) if it is a carcinogen, or by an assay designated under sections 409(b)(2)(D), 512(b)(7), and 706(b)(8)(A) if, in accordance with the general safety provisions of the act. No such assay has been submitted, nor, to the knowledge of the Commissioner, does such an assay exist. Accordingly, the Commissioner proposes to ban saccharin for all uses in food-producing animals.

Since saccharin is also an ingredient in some animal drugs and feeds intended for use in non-food-producing animals, the Commissioner proposes to disapprove this use as well. Saccharin provides no therapeutic benefit to animals and has not been shown to provide any overriding benefit to a measurable animal treatment population. For these reasons, the Commissioner concludes that any risks to animals from the use of saccharin in such drugs outweigh any theoretical benefit alleged from its continued use.

VI. COMPLIANCE POLICY

An important aspect of this proposal is quite obviously, the compliance policy that FDA intends to adopt as part of the final regulations on saccharin. Matters of interest to consumers and manufacturers and users of saccharin alike are: When will the ban take effect? Will it apply to manufacture or shipment of saccharin containing foods? Is a recall contemplated? When must new drug applications be submitted? This section summarizes FDA's intended compliance policy when final regulations are issued.

A. SACCHARIN USED IN FOOD

Under section 409(e) of the act (21 U.S.C. 348(e)), the final regulation revoking the interim food additive regulation for saccharin (21 CFR 180.37) shall be effective on publication in the *Federal Register*. The Commissioner intends, in the final regulation, to prohibit the addition of saccharin to any food (e.g., soft drinks) after the effective date of the final regulation. Foods that have been fully processed and packaged for sale to consumer or institutions on the

effective date of the final regulation would be permitted to be sold. The addition of saccharin in the manufacture of food, further processing, or repacking, after the effective date of the final regulation will cause such products to be adulterated within the meaning of the act and subject to regulatory action.

B. SACCHARIN USED IN HUMAN DRUGS

When a final regulation is issued, holders of approved new drug applications for a drug product containing saccharin as an inactive ingredient and sponsors of IND notices for a drug product containing saccharin as an ingredient will have 9 months to file a supplemental application (NDA) or amendment (IND notice) to revise the formulation removing saccharin as an ingredient. Similar requirements are proposed for antibiotic drug products.

Petitions may be submitted to FDA requesting that a specific use of saccharin as an inactive ingredient be permitted. Such petitions must include the information specified in section II.B. of this preamble.

Manufacture of any drug products containing saccharin as an inactive ingredient would be prohibited after 18 months from the date of publication of final regulations in the *Federal Register* initial shipment of drug products containing saccharin as an inactive ingredient would be prohibited 18 months after the date of publication of final regulation in the *Federal Register*.

This proposal invites comment on the appropriateness of permitting the marketing of saccharin as a single-ingredient drug for use by persons who must restrict their intake of sugar, available without a physician's prescription. If the final regulation should permit such marketing, any manufacturer wishing to ship in interstate commerce a saccharin-containing tablet sweetener would, within 180 days after the date of publication of the final regulation, have to submit a new drug application for the product and comply with the other requirements set forth in proposed § 310.514.

Tabletop sweeteners currently being marketed would be permitted to continue to be marketed as over-the-counter drugs, pending review and action on the new drug applications. The Commissioner cautions against substantial changes in the packaging format of saccharin as a single-ingredient product during this period. Within 120 days after publication of the final regulation, however, those products would have to be labeled with the statements prescribed in § 310.514(b)(2).

C. SACCHARIN USED IN COSMETICS

A final regulation prohibiting the use of saccharin in cosmetics that are likely to be ingested will be effective 30 days after publication of a final regulation in the *Federal Register*. The addition of saccharin to cosmetics that are likely to be ingested after the effective date of a final regulation would be prohibited. Cosmetics containing saccharin that are already on the market and those prod-

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ucts that are fully processed and packaged for sale to consumers or institutions before the final regulation takes effect will be permitted to be sold. The prohibition on saccharin in cosmetics does not apply to products that are not likely to be ingested (e.g., hair tonics).

D. SACCHARIN USED IN ANIMAL DRUGS AND FEEDS

The final regulation prohibiting the use of saccharin in animal feed will be effective on publication in the FEDERAL REGISTER. The Commissioner intends, in the final regulation, to prohibit the addition of saccharin to any animal feed after the effective date of the regulation. The final regulation prohibiting the use of saccharin in animal drugs will be effective 30 days after publication in the FEDERAL REGISTER. After the effective date, it will be unlawful to manufacture an animal drug containing saccharin. Holders of approved new animal drug applications for products that contain saccharin as an inactive ingredient will be required to file a supplemental application within 9 months of publication of final regulations.

E. RECALL OF SACCHARIN-CONTAINING PRODUCTS

The Commissioner has concluded that the protection of the public health does not require the recall from the market of food, drugs (human and animal), animal feed, and cosmetics that contain saccharin or the destruction of products that are fully processed and packaged for sale to consumers or institutions when a final regulation is issued. Thus, at this time, no recall is contemplated and products that contain saccharin on the market or fully processed and packaged for sale to consumers or institutions when a final regulation is issued would be permitted to be sold.

As discussed earlier in this preamble, the Commissioner believes that prolonged consumption of saccharin in ordinary foods, such as soft drinks, and exposure to saccharin from other products (i.e., drugs, animal drugs and feed, and cosmetics) poses a significant risk of cancer and should not be permitted in the future. However, the potential risk of human cancer from saccharin is cumulative; though significant, it is not immediate in the sense that the exposure of consumers to saccharin must be halted at once. The relatively short period of time in which products containing saccharin already on the market will be sold, does not, in the Commissioner's judgment, significantly threaten the public health.

The Commissioner emphasizes, however, that there is a significant potential risk of cancer from prolonged consumption of saccharin. His judgment is that a recall—with all the attendant costs to the industry and consumers—is not required to protect the public health; but this judgment should not be construed as reflecting a lack of concern about the cumulative risk associated with the routine consumption of saccharin by the general population.

All FDA regulations concerning human food were reorganized under Subchapter B—Food for Human Consumption, published in the FEDERAL REGISTER of March 15, 1977 (42 FR 14302). For the convenience of the reader, the following table lists the former designation of the sections in reclassified Subchapter B which would be amended by this proposal.

New section:	Old section:
145.115	27.14
145.120	27.84
145.121	27.78
145.125	27.45
145.171	27.8
145.170	27.34
145.181	27.87
145.111	27.138
145.121	27.109
150.141	29.4
150.161	29.5
173.185	121.1056
173.230	121.1145
145.181	121.1063
190.182	121.1063(d)

The Commissioner has carefully considered the environmental effects of the proposed regulation and, because the proposed action will not significantly affect the quality of the human environment, has concluded that an environmental impact statement is not required. A copy of the environmental impact assessment is on file with the Hearing Clerk, Food and Drug Administration.

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Therefore, under the Federal Food, Drug, and Cosmetic Act (secs. 201(e), 301, 401, 402, 409, 502, 505, 512, 601(a), 701 (a) and (c), 82 Stat. 1042-1043 as amended, 1046-1047 as amended, 1050-1055 as amended, 70 Stat. 919, 72 Stat. 1784-1788 as amended, 82 Stat. 343-351 (21 U.S.C. 321(a), 351, 341, 342, 345, 352, 353, 350b, 361(a), 371 (a) and (c))) and under authority delegated to the Commissioner (21 CFR 3.1), it is proposed that Chapter I of Title 21 of the Code of Federal Regulations be amended as follows:

PART 145—CANNED FRUITS

1. In Part 145:
 - a. By revising § 145.116(a) to read as follows:

§ 145.116 Artificially sweetened canned apricots.

(a) Artificially sweetened canned apricots is the food which conforms to the definition and standard of identity prescribed for canned apricote by § 145.115(a), except that in lieu of a packing medium specified in § 145.116(a)(3), the packing medium

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used is water sweetened with one or more of the artificial sweeteners listed in and complying with Parts 170 through 189 of this chapter. Such packing medium may be thickened with pectin and may contain any mixture of any edible organic salt or salts and any edible organic acid or acids as a flavor-enhancing agent, in a quantity not more than is reasonably required for that purpose.

b. By revising § 145.126(a) to read as follows:

§ 145.126 Artificially sweetened canned cherries.

(a) Artificially sweetened canned cherries is the food which conforms to the definition and standard of identity prescribed for canned cherries by § 145.125(a), except that in lieu of a packing medium specified in § 145.125(a)(3), the packing medium used is water sweetened with one or more of the artificial sweeteners listed in and complying with Parts 170 through 189 of this chapter. Such packing medium may be thickened with pectin and may contain any mixture of any edible organic salt or salts and any edible organic acid or acids as a flavor-enhancing agent, in a quantity not more than is reasonably required for that purpose.

c. By revising § 145.131(a) to read as follows:

§ 145.131 Artificially sweetened canned figs.

(a) Artificially sweetened canned figs is the food which conforms to the definition and standard of identity prescribed for canned figs by § 145.130, except that in lieu of a packing medium specified in § 145.130(c), the packing medium used is water sweetened with one or more of the artificial sweeteners listed in and complying with Parts 170 through 189 of this chapter. Such packing medium may be thickened with pectin and may contain any mixture of any edible organic salt or salts and any edible organic acid or acids as a flavor-enhancing agent, in a quantity not more than is reasonably required for that purpose.

d. By revising § 145.136(a) to read as follows:

§ 145.136 Artificially sweetened canned fruit cocktail.

(a) Artificially sweetened canned fruit cocktail is the food which conforms to the definition and standard of identity prescribed for canned fruit cocktail by § 145.135(a), except that in lieu of a packing medium specified in § 145.135(a)(3), the packing medium used is water sweetened with one or more of the artificial sweeteners listed in and complying with Parts 170 through 189 of this chapter. Such packing medium may be thickened with pectin and may contain any mixture of any edible organic salt or salts and any edible organic acid or acids as a flavor-enhancing agent, in a quantity not more than is reasonably required for that purpose.

e. By revising § 145.171(a) to read as follows:

§ 145.171 Artificially sweetened canned peaches.

(a) Artificially sweetened canned peaches is the food which conforms to the definition and standard of identity prescribed for canned peaches by § 145.170(a), except that in lieu of a packing medium specified in § 145.170(a)(3), the packing medium used is water sweetened with one or more of the artificial sweeteners listed in and complying with Parts 170 through 189 of this chapter. Such packing medium may be thickened with pectin and may contain any mixture of any edible organic salt or salts and any edible organic acid or acids as a flavor-enhancing agent, in a quantity not more than is reasonably required for that purpose.

f. By revising § 145.176(a) to read as follows:

§ 145.176 Artificially sweetened canned pears.

(a) Artificially sweetened canned pears is the food which conforms to the definition and standard of identity prescribed for canned pears by § 145.175(a) except that in lieu of a packing medium specified in § 145.175(a)(3), the packing medium used is water sweetened with one or more of the artificial sweeteners listed in and complying with Parts 170 through 189 of this chapter. Such packing medium may be thickened with pectin and may contain any mixture of any edible organic salt or salts and any edible organic acid or acids as a flavor-enhancing agent, in a quantity not more than is reasonably required for that purpose.

g. By revising § 145.181(a) to read as follows:

§ 145.181 Artificially sweetened canned pineapple.

(a) Artificially sweetened canned pineapple is the food that conforms to the definition and standard of identity prescribed for canned pineapple by § 145.180(a), except that in lieu of a packing medium specified in § 145.180(a)(3), the packing medium used is water sweetened with one or more of the artificial sweeteners listed in and complying with Parts 170 through 189 of this chapter. Such packing medium may be thickened with pectin.

PART 180—FRUIT BUTTERS, JELLIES, PRESERVES, AND RELATED PRODUCTS

2. In Part 180:

a. By revising § 150.141(c) to read as follows:

§ 150.141 Artificially sweetened fruit jelly.

(c) The artificial sweetening ingredients referred to in paragraph (a) of this section are one or more of the artificial sweeteners listed in and complying with Parts 170 through 189 of this chapter.

b. By revising § 180.161(c) to read as follows:

§ 180.161 Artificially sweetened fruit preserves and jams.

(c) The artificial sweetening ingredients referred to in paragraph (a) of this section are one or more of the artificial sweeteners listed in and complying with Parts 170 through 189 of this chapter.

PART 172—FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION

3. In Part 172:

§ 172.135 [Amended]
a. By amending § 172.134 Disodium EDTA by deleting paragraph (b)(3).

b. By amending § 172.812 by revising paragraphs (b) and (c) to read as follows:

§ 172.812 Glycine.

(b) The additive is used or intended for use as a stabilizer in mono- and diglycerides prepared by the glycerolysis of edible fats or oils in an amount not to exceed 0.02 percent of the mono- and diglycerides.

(c) To assure safe use of the additive, in addition to the other information required by the act, the labeling of the additive shall bear adequate directions for the use of the additive in compliance with the provisions of this section.

§ 172.820 [Amended]

c. By amending § 172.820 Polyethylene glycol (mean molecular weight 300-9,500), by deleting and reserving paragraph (c)(2).

PART 180—FOOD ADDITIVES PERMITTED IN FOOD ON AN INTERIM BASIS

§ 180.37 [Revoked]

4. In Part 180, by revoking § 180.37 Saccharin, ammonium saccharin, calcium saccharin, and sodium saccharin, which had permitted saccharin and its salts in food on an interim basis pending additional study.

PART 180—SUBSTANCES PROHIBITED FROM USE IN HUMAN FOOD

5. In Part 180, by adding new § 180.185 to read as follows:

§ 180.185 Saccharin and its salts.

(a) The food additive saccharin is the chemical, 1,2-benzothiazolin-3-one-1,1-dioxide (C₇H₇N₂O₂S). Ammonium saccharin, calcium saccharin, and sodium saccharin are produced by the additional neutralization of saccharin with the proper base to yield the desired salt. Saccharin and the named salts have been used as sweetening agents in food.

(b) Food containing any added saccharin or saccharin salt is deemed to be adulterated in violation of the act.

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PART 310—NEW DRUGS

6. In Part 310, by adding new § 310.514 to read as follows:

§ 310.514 Saccharin; use as an ingredient in drug products.

(a) Saccharin has been used for many years as a flavoring agent in drug products, such as pediatric liquid oral preparations, cough syrups, chewable tablets, and toothpaste with medical claims. Saccharin has also been used in the management or mitigation of diabetes and other conditions in which the available carbohydrate and/or calories of a patient must be controlled. Information now available demonstrates that saccharin causes malignant bladder tumors in test animals and therefore has a potential for causing cancer in humans. The potential risk in humans outweighs the benefits of nontherapeutic use of saccharin. On the basis of this new evidence, saccharin has not been shown to be safe for use as an inactive drug ingredient, with certain exceptions as provided for in paragraph (f) of this section.

(b) (1) Any drug product that contains saccharin, or one of its salts, as a single-active-ingredient product in liquid, tablet or powder form for use as a tabletop sweetener is a new drug within the meaning of section 201(p) of the act and requires an approved new drug application for marketing.

(2) Such products currently being marketed may remain on the market as over-the-counter products: *Provided*, (i) A new drug application complying with the requirements of § 314.1 of this chapter is submitted within 180 days of the date of publication of a final regulation; (ii) All products labeled after 120 days after date of publication of a final regulation shall have the following statements displayed prominently on the principal display panel and on any other labeling, unless revised upon approval of the new drug application:

(A) "For use as a noncaloric sweetener when a sugar-restricted diet is medically indicated, as in patients with diabetes."

(B) "Warning: Saccharin causes bladder cancer in animals. Use of saccharin may increase your risk of cancer."

(C) Any drug product that contains saccharin as an inactive ingredient is a new drug within the meaning of section 201(p) of the act and is misbranded and subject to regulatory action under sections 301, 502, and 505 of the act.

(D) Any holder of an approved new drug application for a drug product containing saccharin as an inactive ingredient shall submit to the Food and Drug Administration on or before 9 months after date of publication of final regulation a supplemental application providing for a revised formulation removing saccharin as an ingredient.

(1) The supplemental application shall contain:

(i) A full list of articles used as components and a full statement of the composition of the drug product.

(ii) Data showing that the change in composition does not interfere with any

assay or other control procedures used in manufacturing the drug product, or that the assay and other control procedures are revised to make them adequate.

(iii) Data to establish that the stability of the product is not adversely affected by the revised formulation. If the data are too limited to support a conclusion that the drug will retain its declared potency for a reasonable marketing period, a commitment from the applicant:

(A) To test the stability of marketed batches at reasonable intervals;

(B) To submit the data as they become available; and

(C) To recall from the market any batch found to fall outside the approved specifications for the drug.

(2) The revised formulation shall not be marketed before the receipt of written notice of approval of the supplement by the Food and Drug Administration.

(e) Any sponsor of a "Notice of Claimed Investigational Exemption for a New Drug" (IND) for a drug product containing saccharin as an inactive ingredient shall amend the IND notice before 9 months after date of publication of final regulation to provide for a revised formulation removing saccharin as an ingredient.

(f) If the holder of an approved new drug application or sponsor of an IND fails to comply with the provisions of paragraph (d) or (e) of this section, the Commissioner will initiate action to withdraw approval of the application or terminate the IND notice in accordance with the applicable provisions of section 506 of the act and Parts 312 and 314 of this chapter.

(g) Any person may file a petition in accordance with Part 10 of this chapter to amend paragraph (c) of this section to specify a use of saccharin in a drug product as not being subject to the misbranding provisions of that paragraph. The petition must be supported by the following information:

(1) The amount of saccharin contained in each dose of the drug;

(2) An adequate showing that there are not technically feasible alternatives to the use of saccharin in the drug product, or an adequate showing that the drug product provides a substantial health benefit or other public benefit that would not be available without the use of saccharin; and

(3) A copy of the proposed labeling clearly specifying the saccharin content and its intended use.

PART 430—ANTIBIOTIC DRUGS; GENERAL

7. In Part 430, by adding new Subpart F—Ingredients No Longer Shown To Be Safe, consisting at this time of § 430.300, to read as follows:

Subpart F—Ingredients No Longer Shown To Be Safe

§ 430.300 Saccharin; use as an ingredient in antibiotic drug products.

(a) Saccharin has been used for many years as a flavoring agent in drug prod-

ucts, such as pediatric liquid oral preparations, cough syrups, chewable tablets, and toothpaste with medical claims. Saccharin has also been used in the management or mitigation of diabetes and other conditions in which the available carbohydrate and/or calories of a patient must be controlled. Information now available demonstrates that saccharin causes malignant bladder tumors in test animals and has a potential for causing cancer in humans. The potential risk in humans outweighs the benefits of nontherapeutic use of saccharin. On the basis of this new evidence, saccharin has not been shown to be safe for use as an inactive drug ingredient, with certain exceptions as provided for in paragraph (e) of this section.

(b) (1) Any manufacturer or other person who holds an approved antibiotic drug file providing for a product that contains saccharin shall submit an amendment on or before 9 months after date of publication of final regulation providing for a revised formulation removing saccharin as an ingredient.

(2) The amendment shall contain:

(i) A full list of articles used as components and a full statement of the composition of the drug product.

(ii) Data showing that the change in composition does not interfere with any assay or other control procedures used in manufacturing the drug product, or that the assay and other control procedures are revised to make them adequate.

(iii) Data to establish that the stability of the product is not adversely affected by the revised formulation. If the data are too limited to support a conclusion that the drug will retain its declared potency for the period allowed by the expiration date, a commitment from the applicant:

(A) To test the stability of marketed batches at reasonable intervals;

(B) To submit the data as they become available; and

(C) To recall from the market any batch found to fall outside the approved specifications for the drug.

(c) No batch of antibiotic drug product containing saccharin as an ingredient will be certified or released after 18 months after date of publication of final regulation).

(d) (1) Any sponsor of a "Notice of Claimed Investigational Exemption for a New Drug" (IND) for a drug product containing saccharin as an ingredient shall amend the IND notice before 9 months after date of publication of final regulation to provide for a revised formulation removing saccharin as an ingredient.

(2) If the sponsor of an IND notice fails to comply with the provisions of paragraph (d) (1) of this section, the Commissioner will initiate action to terminate the IND notice in accordance with the applicable provisions of section 507 of the act and Parts 312 and 433 of this chapter.

(e) Any person may file a petition in accordance with Part 10 of this chapter to amend paragraph (c) of this section to specify a use of saccharin in a

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drug product which justifies certification or release of the product. This petition must be supported by the following information:

(1) The amount of saccharin contained in each dose of the drug;

(2) An adequate showing that there are no technically feasible alternatives to the use of saccharin in the drug product, or an adequate showing that the drug product provides a substantial health benefit or other public benefit that would not be available without the use of saccharin; and

(3) A copy of the proposed labeling clearly specifying the saccharin content and its intended use.

PART 510—NEW ANIMAL DRUGS

8. In Part 510, by adding new § 510.414, to read as follows:

§ 510.414 Saccharin.

(a) There are no approved or documented uses of saccharin as an ingredient in animal drugs intended for use in food-producing animals. Information now available demonstrates that saccharin causes malignant bladder tumors in test animals and therefore has a potential for causing cancer in humans. In food-producing animals, the use of saccharin as an ingredient in animal drugs or animal feed requires a demonstration that no residue will be found in food from the edible products derived from those animals, either by an assay designated in accordance with the provisions of the anticancer clauses of the act if it is a carcinogen, or by an assay designated in accordance with the general safety provisions of the act. No such assay has been submitted, nor, to the knowledge of the Commissioner, does such an assay exist. On the basis of this evidence, saccharin has not been shown to be safe for use as an inactive ingredient in animal drugs intended for use in food-producing animals.

(b) Saccharin has been used as an ingredient in some animal drugs intended for use in non-food-producing animals. Saccharin provides no therapeutic benefit to animals and has not been shown to provide any overriding benefit to a measurable animal treatment population. For these reasons, the Commissioner concludes that any risks to animals from the use of saccharin in such drugs outweigh any theoretical benefit alleged from its continued use. Accordingly, on the basis of the new evidence, saccharin has not been shown to be safe for use as an active or inactive ingredient in animal drugs intended for use in non-food-producing animals.

(c) Any drug product that contains saccharin as an inactive ingredient is a new animal drug within the meaning of section 301(w) of the act, and is unlawful and subject to regulatory action under sections 301 and 312 of the act.

(d) Any holder of an approved new animal drug application for a drug product containing saccharin as an inactive ingredient shall submit to the Food and Drug Administration on or before 9 months after date of publication of final

regulation) a supplemental application providing for a revised formulation removing saccharin as an ingredient.

(e) If the holder of an approved, new animal drug application fails to comply with the provisions of paragraph (d) of this section, the Commissioner will initiate action to withdraw approval of the application in accordance with the applicable provisions of section 312 of the act.

PART 589—SUBSTANCES PROHIBITED FROM USE IN FOOD OR FEED FOR ANIMALS OTHER THAN MAN

9. By adding a new Part 589, consisting at this time of § 589.185, to read as follows:

§ 589.185 Saccharin and its salts.

(a) The food additive saccharin is the chemical, 1,3-benzothiazolone-2-one-1,1-dioxide (C₇H₅N₂O₅S). Ammonium saccharin, calcium saccharin, and sodium saccharin are produced by the additional neutralization of saccharin with the proper base to yield the desired salt. Saccharin and the named salts have been used as sweetening agents in human food and may have been used as a sweetening agent in food or feed for animals other than man.

(b) Information now available demonstrates that saccharin causes malignant bladder tumors in test animals and therefore has a potential for causing cancer in humans. For this reason it has not been shown to be safe for use in food or feed for animals other than man. In food-producing animals, the use of saccharin as an ingredient in animal feed requires a demonstration that no residue will be found in food from the edible products derived from those animals, either by an assay designated in accordance with the provisions of the anticancer clauses of the act if it is a carcinogen, or by an assay designated in accordance with the general safety provisions of the act. No such assay has been submitted, nor, to the knowledge of the Commissioner, does such an assay exist.

(c) Food or feed for animals other than man containing any added saccharin or saccharin salt is deemed to be adulterated in violation of the act.

PART 700—GENERAL

10. In Part 700, by adding a new § 700.22, to read as follows:

§ 700.22 Use of saccharin as an ingredient in cosmetic products.

(a) Saccharin and its salts have been used as an ingredient in cosmetic products. The ingestion of saccharin has been shown to induce cancer of the bladder in rats. The Commissioner concludes that, on the basis of these findings, saccharin is a deleterious substance that may render injurious to users any cosmetic product that contains saccharin or a saccharin salt as an ingredient and is likely to be ingested under normal conditions of use.

(b) Any cosmetic product containing saccharin or a saccharin salt as an in-

redient that is likely to be ingested is deemed to be adulterated and is subject to regulatory action under sections 301 and 301(a) of the Federal Food, Drug, and Cosmetic Act.

Interested persons may, on or before June 14, 1977, submit to the Hearing Clerk, Food and Drug Administration, Rm. 4-85, 8600 Fishers Lane, Rockville, MD 20857, written comments (preferably in quadruplicate and identified with the Hearing Clerk docket number found in brackets in the heading of this document) regarding this proposal. The envelope containing the comment(s) should be prominently marked "SACCHARIN." Received comments may be seen in the above office between the hours of 9 a.m. and 4 p.m., Monday through Friday.

Note.—The Food and Drug Administration has determined that this document contains a major proposal requiring preparation of an inflation impact statement under Executive Order 11811 and OMB Circular A-107 and certifies that an inflation impact statement has been prepared. A copy of the inflation impact statement is on file with the Hearing Clerk, Food and Drug Administration.

Dated: April 12, 1977.

DONALD KERRY, Commissioner of Food and Drugs.

[FR Doc. 77-11189 Filed 4-14-77; 8:45 am]

[21 CFR Parts 145, 150, 172, 180, 189, 310, 430, 510, 589 and 700]

[Doc. No. 77-0081]

SACCHARIN AND ITS SALTS

Hearing

AGENCY: Food and Drug Administration.

ACTION: Notice of Public Hearing.

SUMMARY: The Commissioner of Food and Drugs announces that a public hearing will be held on May 18 and 19, 1977 to receive information and views from interested persons on the proposed regulations regarding saccharin published elsewhere in this issue of the FEDERAL REGISTER.

DATES: The public hearing will be held on May 18 and 19, 1977 at 9 a.m. A written notice of participation must be filed by May 9, 1977.

ADDRESSES: Written notices of participation should be sent to the Hearing Clerk (HFC-30), Food and Drug Administration, Rm. 4-85, 8600 Fishers Lane, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

Mr. Ted Herman, Compliance Regulations Policy Staff (HFC-10), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, MD 20857 (301-443-3480).

SUPPLEMENTARY INFORMATION: Elsewhere in this issue of the FEDERAL REGISTER, the Commissioner is proposing to revoke the interim food additive regu-

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lation, 21 CFR 180.37 (formerly 21 CFR 131.469) prior to reclassification published in the *Federal Register* of March 18, 1977 (42 FR 14931) under which saccharin and its salts (saccharin) are currently permitted as ingredients in pre-packaged food such as soft drinks, and as tabletop nonnutritive sweeteners. The Commissioner is also proposing to accept and promptly review new drug applications for the marketing of saccharin as a single-ingredient drug, available without a physician's prescription. The Commissioner is also proposing to prohibit the use of saccharin in cosmetics that are likely to be ingested, to amend the standards of identity that provide for the use of saccharin, and to prohibit the use of saccharin in animal drugs and animal feed. Comments on the proposal may be submitted until June 14, 1977. Because of the broad public interest in and concern about saccharin, the Commissioner has determined that, in addition to the normal 90-day comment period for receipt of written comments, an informal public hearing should be held regarding the proposal. The purpose of the informal hearing is to provide an open forum for the presentation of information and views concerning all aspects of the proposal by interested persons, be they con-

sumers, scientists, or representatives of manufacturers of regulated products.

In preparing a final regulation, the Commissioner will consider the administrative record of this hearing along with all other written comments received during the comment period specified in the proposal.

The hearing will be held on May 19 and 18, 1977 in the auditorium located on the first floor in the HEW North Building, 330 Independence Ave. SW., Washington, DC 20261. The hearing will begin at 9 a.m. each day. The presiding officer will be Dr. Donald Kennedy, Commissioner of Food and Drugs.

A written notice of participation must be filed pursuant to 21 CFR 12.45 (formerly 21 CFR 2.131) prior to reclassification published in the *Federal Register* of March 23, 1977 (42 FR 15453) with the Hearing Clerk (HFC-30), Food and Drug Administration, Rm. 4-25, 4950 Fishers Lane, Rockville, MD 20857 not later than May 9, 1977. The envelope containing the notice of participation should be prominently marked "Saccharin Hearing." The notice of participation itself must contain the Hearing Clerk Docket No. 77N-0085, the name, address, and telephone number of the person desiring to make a statement, along with any business affiliation, a summary of the scope

of the presentation, and the approximate amount of time being requested for the presentation. A schedule for the hearing will be mailed to each person who files a notice of participation; the schedule will also be available from the FTA Hearing Clerk. Individuals and organizations with common interests are urged to consolidate or coordinate their presentations.

In the event that the responses to this notice of hearing are so numerous that insufficient time is available to accommodate the full amount of time requested in the notices of participation received, the Commissioner will allocate the available time among the persons making the oral presentation to be used as they wish. Formal written statements (preferably in quadruplicate) may be presented to the presiding officer on May 18 and 19 for inclusion in the administrative record.

The hearing will be open to the public. Any interested person who files a written notice of participation may be heard with respect to matters relevant to the issues under consideration.

Dated: April 12, 1977.

DONALD KENNEDY,
Commissioner of Food and Drugs.

(FR Doc 77-11198 Filed 4-14-77; 8:46 am)

INTERIM REGULATED SUBSTANCES

TAB K

Interim Regulated Substances

§ 121.40001 Saccharin; ammonium, calcium, and sodium.

Use: Sweetening agent only in special dietary foods

1970 Production: pounds per year for food use	Saccharin	Calcium saccharin	Ammonium saccharin	Sodium saccharin
	4,400	94,880	33	1,473,730
Ave. Consumption: milligrams per day	78.1	353.8	1.6	335.7

Previous
Regulatory status: GRAS

§ 121.4004 Brominated vegetable oil

Use: Stabilizer for flavoring oils used in fruit flavored
beverages.

1970 Production: No record
pounds per year
for food use

Ave. Consumption: milligrams per day 0.0225

Previous
Regulatory status: GRAS

§ 121.4005 Mannitol

Use: anticaking agent and free-flow agent, formulation
aid, firming agent, flavoring agent and adjuvant, lubricant
and release agent, nutritive sweetener, processing aid,
stabilizer and thickener, finishing agent, and texturizer.

1970 Production: 2.89 million
pounds per year
for food use

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- 2 -

Ave. Consumption: 4,216
milligrams per day

Previous
Regulatory status: GRAS



DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
ROCKVILLE, MARYLAND 20857

Ms. Judy Robinson
Professional Staff Member
Subcommittee on Migratory Labor
Committee on Labor and Public Welfare
United States Senate
Washington, D.C. 20510

JAN 31 1977

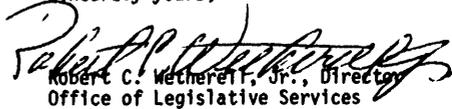
Dear Ms. Robinson:

During the hearing before the Committee on Small Business on January 13, 1977, Senator Nelson and Acting Commissioner Sherwin Gardner discussed the status of the color additive FD&C Yellow No. 5.

I am enclosing two documents, which will be published in the Federal Register on February 4, 1977, pertaining to labeling and restrictions on the use of the above color.

If I can be of any further assistance, please let me know.

Sincerely yours,


Robert C. Wechert, Jr., Director
Office of Legislative Services

2 Enclosures

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Yellow No. 5. The Commissioner, in evaluating the listing of FD&C Yellow No. 5 for external cosmetic use, concludes that such action is inappropriate pending receipt of the new data from chronic studies with FD&C Yellow No. 5.

Accordingly, the Commissioner is announcing in accordance with section 701 (e)(2) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 371 (e)(2)), that the effectiveness has been stayed for the order listing FD&C Yellow No. 5 for use in externally applied cosmetics.

Therefore, under the Federal Food, Drug, and Cosmetic Act (sec. 701(e), 706 (b), (c), and (d)), 79 Stat. 819, 78 Stat. 599-608 (21 U.S.C. 371(e), 376 (b), (c), and (d))) and under authority delegated to the Commissioner (21 CFR 5.1) (recodification published in the FEDERAL REGISTER of June 18, 1976 (41 FR 24282)), notice is given that the effective date of March 22, 1974 for the order amending Part 5 by adding new Subpart G consisting of § 8.7255 is stayed by the filing of timely and valid objections.

Until further notice, FD&C Yellow No. 5 will continue to be provisionally listed for use in cosmetics, generally, and in externally applied drugs.

Dated: January 28, 1977.

STRAWIN GARDNER,
Acting Commissioner of
Food and Drugs.

[FR Doc 77-3337 Filed 2-8-77; 8:46 am]

[Docet No. 77N-0008]

PART 5—COLOR ADDITIVES

Listing of FD&C Yellow No. 5 for Cosmetic Use Subject to Certification; Stay of Effectiveness

The Food and Drug Administration (FDA) is announcing a stay of the effectiveness of the order listing FD&C Yellow No. 5 for use in externally applied cosmetics.

In the FEDERAL REGISTER of January 21, 1974 (39 FR 2358), the Commissioner of Food and Drugs issued an order listing FD&C Yellow No. 5 for use in externally applied cosmetics other than hair straighteners, permanent wave preparations, and depilatories by adding new § 8.7255 (21 CFR 8.7255). The continued use of these three types of products has been permitted under the provisional listing of FD&C Yellow No. 5.

Timely objections to the order were received from a manufacturer of colors, a manufacturer of cosmetics, and a trade association. Two of the letters objected to the order's exclusion of the use of FD&C Yellow No. 5 in ingested cosmetics. Both letters claimed that such use should be included in the order and cited findings from teratological and multireproduction studies as supporting evidence for their safe use. It was also cited that the color was already listed for use in food and ingested drugs. Two of the letters objected to the exclusion of the use of the color in hair straighteners, permanent wave preparations, and depilatories. One letter objected to the omission of a final listing of lakes made from FD&C Yellow No. 5. One letter objected to the omission of the use of FD&C Yellow No. 5 in externally applied drugs. The filing of these objections automatically served to stay the effectiveness of the order because they involved its primary aspects.

A proposal was published in the FEDERAL REGISTER of September 23, 1976 (41 FR 41860) to postpone the closing dates for the provisional listing of certain color additives beyond December 31, 1976. One of the requirements that the proposal would impose is the submission of new data from chronic studies with certain color additives, including FD&C

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[21 CFR Parts 1, & 200, 201]

[Docket No. 77N-0009]

FD&C YELLOW NO. 5

Labeling in Food and Drugs for Human Use and Restriction on Use in Certain Human Drugs

The Food and Drug Administration (FDA) is proposing to require the label declaration of FD&C Yellow No. 5 when used to color foods and ingested drugs and to prohibit its use in certain drugs for human use. These restrictions are considered necessary because of mounting evidence of allergic-type reactions to FD&C Yellow No. 5. Interested persons have until April 8, 1977 to submit comments.

In the FEDERAL REGISTER of May 8, 1968 (34 FR 7447), the Commissioner of Food and Drugs issued an order listing FD&C Yellow No. 5 (also known as tartrazine when not certified by FDA for use in food, drugs, and cosmetics) for use in food under § 8.278 (21 CFR 8.278) and for use in ingested drugs under § 8.4175 (21 CFR 8.4175). This action was supported by safety data in a color additive petition (CAP 23) and other relevant data. The petition was submitted by the Certified Color Industry Committee, c/o Hazleton Laboratories, Falls Church, VA (now the Certified Color Manufacturers Association, 900 17th St. NW, Washington, D.C. 20004); notice of filing was published in the FEDERAL REGISTER of March 27, 1965 (30 FR 4083).

No specific restrictions were placed on the use of FD&C Yellow No. 5 other than the requirements of batch certification by FDA. The color is provisionally listed for use in externally applied drugs and in cosmetics under § 8.501(a). The closing date for this provisional listing is January 31, 1977. A proposal was published in the FEDERAL REGISTER of September 23, 1976 (41 FR 41860) to postpone this closing date to December 31, 1980, conditioned upon the timely submission of reports from new chronic toxicity studies. Regulations finalizing this proposal are expected to be published in the near future.

An order listing FD&C Yellow No. 5 for use in externally applied cosmetics under § 8.7255 (21 CFR 8.7255) was published in the FEDERAL REGISTER of January 21, 1974 (39 FR 2358). However, the effective date of that order was stayed by the submission of objections to, among other things, certain restrictions that were to be placed on use of the color. Published elsewhere in this issue of the FEDERAL REGISTER is a notice announcing the stay of the effective date of that order.

DISCUSSION OF PROBLEM

Since FD&C Yellow No. 5 was listed for use in food and ingested drugs, evidence of allergic-type responses caused by ingestion of substances containing the color has accumulated. These responses to FD&C Yellow No. 5 occur primarily in patients who also have aspirin intolerance, although an absolute association has not been established. The phenomenon of aspirin intolerance in certain persons with underlying allergic

disorders, including bronchial asthma, nasal polyposis, vasomotor rhinitis, and skin allergies to various substances, has been known for over 50 years. The aspirin reaction is manifested by asthmatic symptoms, urticaria, angioedema, or nasal symptoms. The overall incidence of aspirin intolerance in the United States is unknown. Samler and Beers (Ref. 1) cited a report by Pearson on a large asthmatic population in which 2.3 percent were said to be aspirin intolerant. Chafee and Settignano (Ref. 2), on the other hand, reported an incidence of 4.3 percent in their large population of asthmatics. These figures were obtained solely on the basis of clinical history. Chafee and Settignano found that among their patients with rhinitis, 0.7 percent were aspirin intolerant.

It has also long been known that some persons are sensitive to organic chemicals. However, the first person to report an association between FD&C Yellow No. 5 and allergic-type reactions was Lockety (Ref. 3). In 1959, Lockety reported generalized urticaria in three patients after ingestion of one or more tablets of a corticosteroid containing FD&C Yellow No. 5. The patients were an asthmatic, a patient known to be very sensitive to drugs of coal tar origin who was taking a steroid for a skin rash due to a topical mercurial, and a patient with a collagen disease who was known to be aspirin intolerant.

Since the 1960's, there has been increasing numbers of reports establishing that there is a strong association between aspirin intolerance and FD&C Yellow No. 5 intolerance. Chafee and Settignano (Ref. 4) described an asthmatic patient with aspirin sensitivity (angioedema) whose chronic asthma and acute attacks were exacerbated after taking certain antiasthmatic drugs, vitamins, premarin, and certain foods. In double-blind studies, she was reported to be allergic to FD&C Yellow No. 5 and, mildly, to FD&C Red No. 4. Drugs containing FD&C Yellow No. 5 could provoke symptoms with a single dose. On the basis of these findings, the authors recommended that FD&C dyes be required to be listed on food and drug packages.

The precise incidence of intolerance of FD&C Yellow No. 5 in the total population or even in aspirin-intolerant patients is not known. Over an 11-year period, Samler and Beers (Ref. 1) followed over 1,000 aspirin-intolerant patients diagnosed on the basis of history. They hospitalized for study 182 of these aspirin-intolerant patients. All were asthmatic, but they had had vasomotor rhinitis and nasal polyps for years before developing asthma. Of the 182 aspirin-intolerant patients, nine (5 percent) were intolerant of tartrazine, FD&C Yellow No. 5. In a double-blind study using some of these patients, 3 of 40 aspirin-intolerant patients (7.5 percent) receiving 25 milligrams of tartrazine developed symptoms.

Juhlin et al. (Ref. 6) found that seven of seven aspirin-intolerant patients developed asthma or urticaria to only 1 to 2 milligrams of tartrazine. One of the seven reacted only slightly to 1 milli-

gram but reacted strongly to 5 milligrams of tartrazine. Thus, these authors found a 100-percent incidence of tartrazine intolerance in their limited studies. Although the test was single blind, there were no reactions to a placebo. One of the patients had been taking an antihistamine containing only 30 micrograms of tartrazine per tablet for a month in an attempt to relieve urticaria which began after taking an aspirin tablet. There was no improvement in the urticaria until 3 days after the patient stopped taking the antihistamine.

Michaelsson and Juhlin (Ref. 6), in a study involving provocation tests with aspirin, aso dyes, and two commonly used food preservatives in patients with recurrent urticaria or angioedema, found that 39 of 52 patients developed a reaction to something—e.g., 36 of these had urticaria to aspirin; 19 to tartrazine (12 cases after 1 to 3 milligrams, the rest after 5 to 18 milligrams); and 23 to sodium benzoate (42 percent). There were also 10 cases of urticaria due to Sunset Yellow (FD&C Yellow No. 6) and some of these were not sensitive to tartrazine. It is not possible from this paper to ascertain the precise percentage of aspirin-intolerant patients who were also intolerant of FD&C Yellow No. 5, but it would appear to be about 50 percent.

Settipane and Pridemant (Ref. 7) recently performed a tartrazine-placebo-controlled double-blind crossover study in 40 patients who had a history of aspirin intolerance and in 40 normal controls. Most of the aspirin-intolerant patients had asthma, the remainder had rhinitis and rhinorrhea. Many of these also had urticaria. The patients were challenged with 0.44 milligram of tartrazine or placebo (except for two who received 0.22 milligram). Six (15 percent) of the 40 aspirin-intolerant patients given tartrazine developed urticaria or bronchospasm, together with at least a 20-percent reduction in three pulmonary function tests. There were no reactions to the placebo, and none of the normal controls developed any reactions.

It is not possible to state precisely the incidence of intolerance to FD&C Yellow No. 5 in the United States. Further, there is a broad range of degree of intolerance, some patients reacting to a fraction of a milligram and others requiring 5 milligrams or more (the dosages found in foods). Using the incidence of 4.3 percent aspirin intolerance in a population of asthmatics and 0.7 percent in a population with rhinitis, as reported by Chafee and Settipane (Ref. 4) in their large practice involving over 3,781 patients with these diseases, calculations of the incidence can be estimated. In Chafee and Settipane's report, about half the patients had allergic rhinitis, only. The other patients had asthma alone or asthma plus rhinitis. The incidence of asthma in the United States is estimated to be 1 to 2 percent. Thus, if there are 2 to 4 million asthmatics in the United States and about a 4-percent incidence of aspirin intolerance in asthmatics, there could be 80,000 to 160,000 cases of aspirin intolerance among asthmatics. If Chafee and Settipane's prac-

tice is indicative of the relative incidence of asthma vs. allergic rhinitis, there are 2 to 4 million patients with allergic rhinitis, of whom 14,000 to 28,000 could be aspirin intolerant. Thus, a total of 94,000 to 388,000 know aspirin-intolerant patients could be assumed. If it is further assumed that 50 percent of aspirin-intolerant patients are intolerant to FD&C Yellow No. 5, using Michaelsson and Juhlin's urticaria and angioedema population (Ref. 6), then there would be 47,000 to 94,000 FD&C Yellow No. 5 intolerant patients.

The amount of FD&C Yellow No. 5 ingested is undoubtedly important in the potential provocation of a reaction. In many cases, however, it is not possible to ascertain the amount of FD&C Yellow No. 5 ingested by the people who reported the allergic-type symptoms to their physicians and, accordingly, the threshold value for provocation of a reaction to FD&C Yellow No. 5 has not been defined in the literature. The determination of the threshold amount may be particularly difficult in those persons who show allergic-type reactions to FD&C Yellow No. 5 only after having ingested the color additive in foods or drugs over a prolonged period. Samter and Beers (Ref. 1) tested one dose of 25 milligrams of FD&C Yellow No. 5 in 40 aspirin-intolerant patients, three of whom reacted positively. Juhlin et al. (Ref. 5), on the other hand, reported that some patients promptly showed allergic-type reactions after a single ingestion of as little as 1 milligram of tartrazine (FD&C Yellow No. 5).

The Food and Drug Administration has long been concerned about allergies involving food, drugs, and cosmetics. The Commissioner recognizes that many substances to which man is exposed, including those occurring in nature, may elicit allergic-type reactions in some unusually susceptible or idiosyncratic individuals. A great variety of materials has been implicated in allergic-type reactions, e.g., dusts of various kinds, pollens, feathers, insect fragments, bee stings, seeds, dandruff, and a number of foods. In general, hypersensitive persons may react by exhibiting a number of responses, which may include angioedema, urticaria, bronchial asthma, pruritis, and vascular purpura.

In evaluating the reports described above, the Commissioner concludes that there is no evidence in the available information on FD&C Yellow No. 5 that demonstrates a significant hazard to the general population when the color is used at current levels and in the manner now practiced. However, because of the evidence of a causal relationship between FD&C Yellow No. 5 and serious allergic-type responses in certain susceptible individuals, the Commissioner concludes that action must be taken to limit the potential for exposure of such persons to the color through ingestion of food or drugs.

There are no reports of reactions to FD&C Yellow No. 5 from external application and, accordingly, the use of the color additive in externally applied drugs

and cosmetics is not considered to present a likelihood of allergic-type responses. Cosmetic articles such as mouthwashes, dentifrices, and lipsticks are also unlikely to induce allergic-type responses because of the very small amount of the cosmetic that may actually be ingested. Furthermore, as of May 31, 1976, all newly ordered labels for cosmetics have been required to declare the specific colors present. Under these circumstances, persons who are hypersensitive to FD&C Yellow No. 5 will, by careful review of the product labeling, be able to avoid cosmetic products containing the color. The Commissioner concludes, therefore, that no further action is required as to cosmetics in general or for externally applied drugs.

PROPOSAL FOR FOODS

Persons who know they are intolerant of FD&C Yellow No. 5 are likely to be selective in the types of foods that they use and, with appropriate label declaration would be able to avoid the potential hazard from allergic-type reactions to the color in food by reading the label. Accordingly, a label declaration of the presence of FD&C Yellow No. 5 in food for humans, whether added as the straight color, a mixture, or a lake, would enable persons intolerant to FD&C Yellow No. 5 to minimize exposure to the color.

The basis for the proposed action is the provision of section 706(b)(3) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 376(b)(3)), which provides that regulations for the listing of a color additive shall "prescribe the conditions under which such additive may be safely employed for such use or uses (including, but not limited to * * * and directions or other labeling and packaging requirements for such additive)." FD&C Yellow No. 5 has clearly been shown to produce allergic-type responses in humans and thus a requirement for label declaration of the color is justified. The evidence that other color additives may elicit similar responses is limited and, accordingly, the Commissioner concludes that similar labeling requirements should not be extended to other color additives at this time. Under the proposed amendment, foods containing colors other than FD&C Yellow No. 5 can continue to be labeled in accordance with the requirements concerning the label declaration of color additives prescribed by section 403 (i) and (k) of the act (21 U.S.C. 343 (i) and (k)), which permit declaration collectively as artificial color.

There is no evidence that any color, including FD&C Yellow No. 5, elicits allergic-type reactions in animals. Accordingly, label declaration of FD&C Yellow No. 5 in animal feeds and pet food would not be required.

The Commissioner concludes that labeling for food products should be revised as soon as possible to include the declaration of FD&C Yellow No. 5 among the list of ingredients. Therefore, he proposes that the effective date for this portion of the final regulation be 1 year after the date of publication in the *Federal Register*. The Commissioner believes this

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will provide sufficient time to permit use of current stocks of labeling and revision of labeling to include a declaration of the presence of FD&C Yellow No. 5. Manufacturers could, of course, revise their labeling before the effective date of the regulation, and the Commissioner encourages them to do so.

PROPOSAL FOR DRUGS FOR HUMAN USE

The use of color additives in ingested drugs for human use is an old, accepted practice in the pharmaceutical industry. The use of color additives in drugs serves a necessary public health function because it permits drugs of identical size and shape to be distinguished. The distinction provided by the use of colors provides an important quality control tool in the dispensing of drugs to prevent mixups between otherwise similarly appearing drugs. The ability to distinguish among different products is also very important to persons taking many drugs, especially to the patient who may think in terms of taking a drug of a particular color rather than by the name of the drug. Color additives in drugs also assist in the identification of drugs in cases of accidental overdose.

Because yellow is a primary color, it is widely used as a color additive in drugs. Of the three yellow color additives available for use in ingested drugs, FD&C Yellow No. 5 is the most widely used. It is used to produce not only typically yellow shades but also variations of green, brown, orange, and other related colors. It is estimated that approximately 60 percent of all colored drug tablets for human use sold in the United States contain FD&C Yellow No. 5.

Thus, in view of the extent of use of FD&C Yellow No. 5, a substantial number of drugs would have to be reformulated if the color additive were prohibited in drugs for human use. Further, while reformulating their products to eliminate FD&C Yellow No. 5, some firms might decide to eliminate all color additives. The considerable time and effort necessary to reformulate drug products and the loss of product identification would be unimportant if considered necessary for the protection of public health and if there were no suitable alternative course of action. However, on the basis of the current information available concerning the nature and extent of the problem of intolerance of FD&C Yellow No. 5, the Commissioner believes that prohibiting all drug uses of FD&C Yellow No. 5 is not necessary for the protection of patients who are intolerant of FD&C Yellow No. 5, and that a labeling requirement similar to that for foods will be satisfactory.

The Commissioner concludes, however, that for drugs a simple listing of the color as FD&C Yellow No. 5 among the list of ingredients would not provide a sufficient safeguard for the person intolerant of FD&C Yellow No. 5. Generally, there is no uniform procedure for the declaration of ingredients on drug labeling; therefore, susceptible individuals might overlook such a listing. The listing of ingredients for ingested drug products has traditionally been used to designate active ingredients; conse-

quently, listing of FD&C Yellow No. 5 may give an incorrect impression that it is an active ingredient. Finally, there may be physicians who are unaware that FD&C Yellow No. 5 may elicit allergic-type responses in certain susceptible individuals and for whom a simple listing would be inadequate.

For these reasons, the Commissioner concludes that the use of FD&C Yellow No. 5 in drugs should be declared in the form of a precautionary statement, i.e., "This product contains FD&C Yellow No. 5 which may cause allergic-type reactions in certain susceptible individuals".

This above decision would, of course, be subject to modification if new information becomes available indicating that the only way to protect sensitive persons would be to prohibit the use of FD&C Yellow No. 5.

Although a total prohibition against the use of FD&C Yellow No. 5 is not warranted, the Commissioner concludes that some action must be taken to limit the potential for exposure of these sensitive individuals to drugs containing FD&C Yellow No. 5. To achieve this objective, the Commissioner is proposing two alternative approaches for both over-the-counter (OTC) and prescription human drugs. In addition to comments on the proposals themselves, the Commissioner requests views concerning the advantages and disadvantages of the two alternative approaches.

OTC DRUG PROPOSAL I

The first proposal applicable to OTC drugs would amend the color additive regulations (21 CFR Part 8) to require that the presence of FD&C Yellow No. 5 be declared on the labels of all OTC drugs that are ingested as well as those that may be administered rectally or vaginally. A declaration of the presence of FD&C Yellow No. 5 on the label of these OTC drugs would enable persons who know they are intolerant of FD&C Yellow No. 5 to avoid drugs containing this color additive. Further, by having the presence declared on the label, physicians would more easily be able to identify persons intolerant of FD&C Yellow No. 5.

Under this proposal, the principal display panel of OTC drugs containing FD&C Yellow No. 5 that are ingested, as well as those that may be administered rectally or vaginally, would be required to contain the statement "This product contains FD&C Yellow No. 5 which is capable of producing allergic-type reactions in certain susceptible persons". The quantity of FD&C Yellow No. 5 would not have to be given.

OTC DRUG PROPOSAL II

Persons intolerant of FD&C Yellow No. 5, like many other persons, may take a variety of OTC drugs at one time or another to relieve or treat conditions or symptoms of a disease. Some of the drugs that may be taken are used to treat allergic or allergic-type conditions, including those allergic-type conditions that may arise as a result of ingestion of FD&C Yellow No. 5. As previously discussed, most persons reacting to FD&C

Yellow No. 5 have other basic allergic problems including, in many cases, a sensitivity to aspirin. Thus, drugs used to treat allergic problems may be used widely by persons intolerant of FD&C Yellow No. 5. However, if a person intolerant of FD&C Yellow No. 5 is administered a drug containing FD&C Yellow No. 5 to treat an existing allergic problem, severe aggravation of the basic allergic condition may result. Further, in the haste of treating a serious allergic problem, a drug containing FD&C Yellow No. 5 could be taken by a person who knows he is intolerant even though the drug is labeled as containing the color additive. Likewise, a drug containing FD&C Yellow No. 5 could also be taken by a sensitive person to treat a serious allergic problem before the person's intolerance of FD&C Yellow No. 5 had been ascertained.

Another possibility which would not be resolved by the OTC Drug Proposal I is that all available drugs of a particular class that are used to treat a sensitive person's allergic condition might contain FD&C Yellow No. 5. Alternatively, the only drugs in a class which are effective for a person might all contain FD&C Yellow No. 5; thus, it could be impossible to select a drug free of FD&C Yellow No. 5.

In view of these considerations, the Commissioner is offering, as an alternative to OTC Drug Proposal I, a second proposal applicable to OTC drug products. This second proposal would include the labeling requirements of the first proposal plus a requirement that would prohibit the use of FD&C Yellow No. 5 in certain classes of drugs that are ingested, as well as those that may be administered rectally or vaginally. The classes of OTC drugs that would not be permitted under this proposal to contain FD&C Yellow No. 5 are analgesic, antihistaminic, cough and cold, oral nasal decongestant, and antispasmodic drugs. These are the classes of OTC drugs that are most likely to be taken by persons intolerant of FD&C Yellow No. 5 to treat an allergic problem or as a substitute for aspirin.

PRESCRIPTION DRUG PROPOSAL I

The first proposal applicable to prescription drugs is a labeling requirement similar to that proposed for OTC drugs. In addition to a declaration of the presence of FD&C Yellow No. 5 on the label of all ingested prescription drugs (as well as those that may be administered rectally or vaginally) containing this color additive, the labeling required by § 201.100(d) (21 CFR 201.100(d)) would be required by proposed § 4175 (21 CFR 4175) to contain the statement "This product contains FD&C Yellow No. 5 which may cause allergic-type reactions in certain susceptible persons". This statement would be required to appear on the label and in the "How Supplied" section of the package insert, if present.

Although persons intolerant of FD&C Yellow No. 5 may not see the labeling on prescription drugs, they could remind their physicians of their intolerance. The physician could then avoid prescribing a drug containing FD&C Yellow No. 5 for

sensitive patients. In addition, as with OTC drugs, having the presence of FD&C Yellow No. 5 declared on the label would enable a physician to identify more easily persons intolerant of FD&C Yellow No. 5.

PRESCRIPTION DRUG PROPOSAL II

The second proposal applicable to prescription drugs would include the labeling requirements of the first prescription drug proposal and a prohibition against the use of FD&C Yellow No. 5 in seven classes of drugs. The following classes of ingested prescription drugs, as well as those that may be administered rectally or vaginally, would not be permitted to contain FD&C Yellow No. 5: analgesic drugs, antihistaminic drugs, cough and cold drugs, oral nasal decongestant drugs, anti-inflammatory drugs, non-steroidal anti-inflammatory drugs, and glucocorticoid drugs.

The reasons for this proposal are the same as those set forth under OTC Drug Proposal II.

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In the proposed drug regulations set forth below, the Commissioner has decided to propose only the second approach for both OTC and prescription drugs for human use because it provides an optimal degree of safe conditions of use for the color. The second approach, while including the provisions of the first, would be more restrictive. Therefore, the Commissioner believes that the proposed changes to Parts 6 and 201 that would be made if the first proposed approach (i.e., a labeling requirement for all drugs containing FD&C Yellow No. 5) were finalized are readily apparent and do not require presentation. Even though only the second approaches are set forth in the proposed regulations, the Commissioner requests comments on both the OTC and prescription drug proposals. The Commissioner is also interested in receiving comments on the availability of drugs that do not contain FD&C Yellow No. 5 within the five OTC drug classes and the seven prescription drug classes included in the proposal set forth below.

EFFECTIVE DATES

As with the food labeling proposal, the Commissioner believes that the effective date of the final regulations as it pertains to labeling drugs for human use should also be 1 year after the date of their publication in the *Federal Register*. He believes this will provide sufficient time for manufacturers to obtain new labels. Each drug for human use containing FD&C Yellow No. 5 labeled after 1 year after the date of publication of the final regulations in the *Federal Register*, should bear a label indicating the presence of FD&C Yellow No. 5.

If the second proposal were adopted, the effective date of the labeling portion of the final regulation would be 1 year as stated above. With respect to the classes of drugs that would have to be reformulated to remove FD&C Yellow No. 5, the Commissioner proposes to make this portion of the final regulations

effective 6 months after their date of publication in the *Federal Register*. After the effective date of this portion of the final regulations, the use of FD&C Yellow No. 5 in the manufacture of any drug among the classes of drugs prohibited from containing FD&C Yellow No. 5, would render the drug adulterated within the meaning of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 et seq.) and subject to regulatory action. Further, the Commissioner proposes that the distribution by a manufacturer of any drug prohibited from containing FD&C Yellow No. 5 eighteen months after the date of publication of the final regulations will cause the product to be adulterated and subject to regulatory action. The prohibition of FD&C Yellow No. 5 would apply to its use as a straight color, a lake, or mixtures of straight colors. The Commissioner is not proposing to recall from the market any drugs containing FD&C Yellow No. 5 if they were manufactured or in process within 6 months of the date of publication of the final regulations or were distributed for sale within 18 months of the date of publication of the final regulations.

Manufacturers of new drugs containing FD&C Yellow No. 5 may revise their labeling to conform to this proposal at the earliest possible time after the effective date of the final regulations and should not wait until their supplemental application submitted under § 314.8 (21 CFR 314.8) has been approved. If the second proposal were adopted, a manufacturer of a new drug containing FD&C Yellow No. 5 in one of the classes of drugs that would be prohibited from containing the color additive would be allowed to either delete the use of any color additive or substitute other color additives in accordance with § 314.8(d)(2) and (e) pertaining to supplemental new drug applications.

To be in compliance with § 314.8, the holder of a new drug application would be required to submit data providing the new composition and showing that the change in composition does not interfere with any assay or control procedure used in manufacturing the drug, or that the assay and any other control procedure have been revised to make them adequate. The supplement would be required to include data available to establish the stability of the revised formulation. If the data are limited to support a conclusion that the drug will retain its declared potency for a reasonable marketing period, a commitment to test the stability of marketed batches at reasonable intervals and to submit the data as they become available is required. Additionally, there must be a commitment to recall from the market any batch found to fall outside the approved specifications for the drug.

The articles and publications cited in this preamble are listed below. In addition, other articles and publications used in support of this proposal are listed. Copies of the journal articles and other information forming the basis for the proposed actions are on public display in the office of the Hearing Clerk, Food and Drug Administration, Rm. 4-85,

5600 Fishers Lane, Rockville, MD 20857, between 9 a.m. and 4 p.m., Monday through Friday.

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- (1) Samter, M. and R. F. Beers, "Concerning the Nature of Intolerance to Aspirin," *Journal of Allergy*, 40:281-285, 1967.
- (2) Chafco, F. H. and G. A. Settignano, "Aspirin Intolerance, I. Frequency in an Allergic Population," *Journal of Allergy and Clinical Immunology*, 58:199-199, 1974.
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- (6) Michelsson, G. and L. Juhlin, "Urticaria Induced by Preservatives and Dye Additives in Foods and Drugs," *British Journal of Dermatology*, 88:128, 1973.
- (7) Settignano, G. A. and R. E. Podupakam, "Aspirin Intolerance, III. Subtypes, Familial Occurrence of Cross Reactivity with Tartrazine," *Journal of Allergy and Clinical Immunology*, 56:215, 1976.

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- (1) Speer, R., Management of Childhood Asthma, Springfield: Charles C. Thomas, 1958.
- (2) Crisp, L. M., "Allergic Vascular Purpura," *Journal of Allergy and Clinical Immunology*, 48:1, 1971.
- (3) Yurchak, E. J., "Immunologic Studies with Aspirin, Clinical Studies with Aspirin-Protic Conjugates," *Journal of Allergy*, 46:245, 1970.
- (4) Johnson, H. M. et al., "Tartrazine: Solid-phase Radioimmunoassay Studies of an Azo Dye Implicated in Allergic Reactions (Azo dye and Allergy)," (unpublished paper).
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- (6) Cobos, M. S., "Tartrazine Revisited," *Drug Intelligence and Clinical Pharmacy*, 9:196, 1975.
- (7) Smith, L. V. and R. J. Starik, "Drugs Containing Tartrazine Dye," *Journal of Allergy and Clinical Immunology*, 58:486, 1976.

The Commissioner has carefully considered the environmental effects of the proposed regulation and, because the proposed action will not significantly affect the quality of the human environment, has concluded that an environmental impact statement is not required. A copy of the environmental impact assessment is on file with the Hearing Clerk, Food and Drug Administration.

Therefore, under the Federal Food, Drug, and Cosmetic Act (secs. 501, 502, 701, 706 (b), (c), and (d), 52 Stat. 1048-1051 as amended, 1055-1056 as amended, 74 Stat. 392-403 (21 U.S.C. 251, 352, 371, 378 (b), (c), and (d))) and under authority delegated to the Commissioner (21 CFR 6.1), (recodification published in the *Federal Register* of June 19, 1976 (41 FR 24262)), it is proposed that Chapter I of Title 21 of the Code of Federal Regulations be amended as follows:

PROPOSED RULES

6839

PART 1—REGULATIONS FOR THE ENFORCEMENT OF THE FEDERAL FOOD, DRUG AND COSMETIC ACT AND THE FAIR PACKAGING AND LABELING ACT

1. In § 1.12 by revising paragraph (c) to read as follows:

§ 1.12 Food labeling: spices, flavorings, colorings, and chemical preservatives.

(c) A statement of artificial flavoring, artificial coloring, or chemical preservative shall be placed on the food, or on its container or wrapper, or on any two or all three of these, as may be necessary to render such statement likely to be read by the ordinary person under customary conditions of purchase and use of such food. The specific artificial color used in food shall be identified on the labeling when so required by its listing in Part 8 to assure safe conditions of use for the color additive.

PART 2—COLOR ADDITIVES

2. In § 2.275(d) by redesignating the text that follows the *italicized* heading as paragraph (d) (1) and by adding new paragraph (d) (2) to read as follows:

§ 2.275 FD&C Yellow No. 5.

(d) Labeling requirements. (1) . . .
(2) Foods for human use that contain FD&C Yellow No. 5, including butter, cheese, and ice cream, shall specifically declare its presence by listing the color additive in the list of ingredients.

3. In § 2.4175 by revising paragraphs (b) and (c) to read as follows:

§ 2.4175 FD&C Yellow No. 5.

(b) *Uses and restrictions.* (1) Except for the categories of drugs for human use in paragraph (b) (2) of this section, FD&C Yellow No. 5 may be used for coloring ingested drugs in amounts consistent with good manufacturing practice.

(1) FD&C Yellow No. 5 may not be used in the following categories of ingested prescription drugs for human use as well as those that may be administered rectally or vaginally:

Analgesic drugs
Antihistaminic drugs
Cough and cold preparations
Oral nasal decongestants
Antihistamines
Nonsteroidal anti-inflammatory drugs
Glucocorticoid drugs

(2) FD&C Yellow No. 5 may not be used in the following categories of ingested OTC drugs for human use as well as those that may be administered rectally or vaginally:

Analgesic drugs
Antihistaminic drugs
Cough and cold preparations
Oral nasal decongestants
Antihistamines

(c) *Labeling requirements.* (1) The label of the color additive and any mixtures prepared therefrom intended solely

or in part for coloring purposes shall conform to the requirements of § 8.32.

(2) Ingested drugs for human use (as well as those that may be administered rectally or vaginally) containing FD&C Yellow No. 5 shall bear the statement "This product contains FD&C Yellow No. 5 which may cause allergic-type reactions in certain susceptible individuals" on their label and in the labeling on or within the package, if any. For prescription drugs containing FD&C Yellow No. 5, the labeling required by § 201.100(d) of this chapter shall bear the statement "This product contains FD&C Yellow No. 5 which may cause allergic-type reactions in certain susceptible individuals". This statement shall be set forth in the "How Supplied" section of the labeling.

PART 200—GENERAL

4. In Subpart B by adding new § 200.55 to read as follows:

§ 200.55 Drugs for human use not permitted to contain FD&C Yellow No. 5.

Although § 2.4175 of this chapter provides for the use of FD&C Yellow No. 5 in most drugs, it prohibits FD&C Yellow No. 5 from being used in certain categories of systematically administered drugs for human use that appear within one of the categories of drugs for human use listed in § 2.4175 of this chapter contains any quantity of FD&C Yellow No. 5, the drug is deemed adulterated and subject to regulatory action.

PART 201—LABELING

5. In subpart C by adding new § 201.64 to read as follows:

§ 201.64 Declaration of presence of FD&C Yellow No. 5.

The labeling for each ingested over-the-counter drug for human use containing FD&C Yellow No. 5 (as well as those that may be administered rectally or vaginally) shall, as required by § 2.4175 of this chapter, bear the statement "This product contains FD&C Yellow No. 5 which may cause allergic-type reactions in certain susceptible individuals". The labeling statement shall appear on the principal display panel of the OTC drug product. A statement indicating the presence of FD&C Yellow No. 5 shall also appear on any labeling on or within the package.

6. In § 201.100 by revising paragraph (b) (6) and by adding new paragraph (b) (7) and (8) to read as follows:

§ 201.100 Prescription drugs for human use.

(b) . . .
(8) An identifying lot or control number from which it is possible to determine the complete manufacturing history of the package of the drug.

(7) For all ingested drugs containing FD&C Yellow No. 5, the statement "This product contains FD&C Yellow No. 5

which may cause allergic-type reactions in certain susceptible individuals" as required by § 2.4175 of this chapter.

(8) If a container is too small or otherwise unable to accommodate a label with sufficient space to bear all the required information but is packaged within an outer container from which it is removed for dispensing or use, the information required by paragraph (b) (2), (3), and (5) of this section may be contained in other labeling on or within the package from which it is to be dispensed, the information referred to in paragraph (b) (1) and (7) of this section may be placed on such outer container only, and the information required by paragraph (b) (6) of this section may be the crimp of the dispensing tube.

Interested persons may, on or before April 5, 1977, submit to the Hearing Clerk, Food and Drug Administration, Rm. 4-85, 5600 Fishers Lane, Rockville, MD 20857, written comments (preferably in quintuplicate and identified with the Hearing Clerk docket number found in brackets in the heading of this document) regarding this proposal. Received comments may be seen in the above office between the hours of 9 a.m. and 4 p.m., Monday through Friday.

Note.—The Food and Drug Administration has determined that this document does not contain a major proposal requiring preparation of an inflation impact statement under Executive Order 11821 and OMB Circular A-107. A copy of the inflation impact statement is on file with the Hearing Clerk, Food and Drug Administration.

Dated: January 28, 1977.

SHERWIN GARDNER,
Acting Commissioner of
Food and Drugs.

[FR Doc 77-3358 Filed 2-3-77; 8:45 am]

MARSHFIELD CLINIC
MARSHFIELD, WIS. 54449

DEPARTMENT OF ALLERGY
Pattaya Chotiwutvinit, M.D.
Raymond L. Hansen, M.D.
Pediatric Allergy
Robert M. Heywood, M.D.
Medical Allergy

21 February 1977

The Honorable Gaylord Nelson
U. S. Senate
Senate Office Building
Washington, D. C. 20510

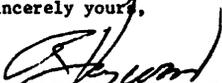
Attn: Miss Judy Robinson

Dear Senator Nelson:

I have received a letter from Robert C. Wetherell, Jr., Director, Office of Legislative Services, Department of Health, Education and Welfare dated February 14th, along with a copy of the Federal Register of February 4, 1977 concerning the proposed regulations regarding FD7C yellow #5 food color.

May I give you the thanks of the many patients who have allergic-like reactions to this dye. We thank you for your help and attention in the development of these new regulations which will help these patients avoid this dye when necessary.

Sincerely yours,



ROBERT M. HEYWOOD, M. D.
Allergy Unit
Department of Internal Medicine
Marshfield Clinic

RMH:mk/31

CC: Dr. Ben Lawton
Dr. Ray Hansen
Dr. P. Chotiwutvinit



NATIONAL JEWISH HOSPITAL AND RESEARCH CENTER
3800 EAST COLFAX AVENUE • DENVER, COLORADO 80206
PUBLIC RELATIONS DEPARTMENT

January 17, 1977

The Honorable Gaylord Nelson
United States Senate
Senate Office Building
Washington, D.C. 20510

Dear Senator Nelson:

We understand consideration is being given to banning Yellow Dye No. 5. You may be interested in the attached article from one of our recent hospital newsletters, which indicates the dangers associated with the dye.

Sincerely,

A handwritten signature in cursive script that reads "Del Harding".

Del Harding
Director of Public Relations

DH:pb

Enc.

Yellow dye can trigger asthma

A dye which may trigger asthma and other adverse reactions in some individuals is widely used in the manufacture of anti-asthma medication, two National Jewish Hospital investigators have reported.

Dr. Richard S. Buswell and Pharmacist Martin S. Leskowitz identified the dye as tartrazine, designated FD and C (foods, drugs and cosmetics) Yellow No. 5 by the Food and Drug Administration.

Writing in *The Journal of the American Medical Association* the investigators reported tartrazine, used as a coloring in a variety of food, drugs, and cosmetics, has been implicated in adverse reactions including bronchospasm and urticaria (hives) in tartrazine-sensitive individuals.

Among drugs frequently colored with tartrazine, the authors said, are oral bronchodilators containing theophylline or theophylline-related compounds, drugs commonly used to control asthma. A survey of 149 such drugs on the market disclosed 29 (nearly 20 percent) containing the dye, they said.

Sensitivity to tartrazine has been particularly noted in patients with aspirin sensitivity. The authors cited a 1967 study which reported tartrazine sensitivity in 14 (8 percent) of 182 aspirin-sensitive patients. Later investigators found the incidence far higher, with a 1972 study reporting tartrazine sensitivity in seven of eight patients sensitive to aspirin.

"It is well documented that many patients with asthma have adverse reactions to aspirin," the NJH researchers said. "A number of these same patients will have similar reactions to tartrazine.

"It seems reprehensible that a substantial percentage of theophylline-containing or -related bronchodilators contain a dye capable of causing bronchospasm and other adverse reactions," they concluded. "It would seem that, at the very least, all tartrazine-containing oral bronchodilators should be clearly labeled as such. Perhaps it would be more appropriate to remove tartrazine from bronchodilators altogether."

FDA regulations do not now re-

quire manufacturers to list additives used for coloring on the labels of their products.

The authors noted that some companies have voluntarily removed the dye from bronchodilating drugs, and a few others indicate they plan to discontinue use or make it known that their product contains tartrazine.

"It actually has been known since about 1959 that tartrazine can cause adverse reactions in some asthmatics," Dr. Buswell said. "Numerous cases and studies have been reported since then. In view of this, it is hard to understand why some drug companies still use it."

From Spring-Summer 1976 issue of NJH Report, the quarterly newsletter published by the National Jewish Hospital and Research Center, 3800 East Colfax Ave., Denver, CO 80206.

NJH is the only accredited hospital in the nation specializing in treatment, education and research in chronic respiratory diseases and immunological disorders.

SUMMARIES OF GAO STUDIES

**REPORT OF THE
COMPTROLLER GENERAL
OF THE UNITED STATES****Need To Establish The Safety Of
Color Additive FD&C Red No. 2**

Food and Drug Administration
Department of Health, Education, and Welfare

The Food and Drug Administration has permitted the use of FD&C Red No. 2, a color additive, in food, drugs, and cosmetics for 15 years without making a final determination of its safety although the Federal Food, Drug, and Cosmetic Act requires that color additives used in such products be determined to be safe.

During this period, scientific studies have raised questions about the safety of FD&C Red No. 2. Permitting continued use of the additive before resolving the safety questions exposes the public to unnecessary risk.

MWD-76-40

OCT. 20. 1975



COMPTROLLER GENERAL OF THE UNITED STATES
WASHINGTON, D.C. 20548

B-164031(2)

The Honorable Gaylord Nelson
United States Senate

Dear Senator Nelson:

In response to your January 30, 1975, request, this is our report on the need for the Food and Drug Administration to establish the safety of color additive Food, Drug, and Cosmetic Red No. 2. As requested, reports on saccharin and aspartame will be forwarded separately as our reviews concerning them are completed.

The Administration is part of the Department of Health, Education, and Welfare. As requested by your office, we have not obtained the Department's written comments on the matters in the report. However, we have discussed these matters with Administration officials and have considered their comments in the report.

We invite your attention to the fact that this report contains a recommendation to the Secretary of Health, Education, and Welfare. As you know, section 236 of the Legislative Reorganization Act of 1970 requires the head of a Federal agency to submit a written statement on actions he has taken on recommendations to the House and Senate Committees on Government Operations not later than 60 days after the date of the report, and the House and Senate Committees on Appropriations with the agency's first request for appropriations made more than 60 days after the date of the report.

We will be in touch with your office in the near future to arrange for copies of this report to be sent to the Secretary of Health, Education, and Welfare and the four Committees to set in motion the requirements of section 236.

Sincerely yours,

A handwritten signature in black ink, appearing to read "Thomas B. Stearns".

Comptroller General
of the United States

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ABBREVIATIONS

FDA	Food and Drug Administration
FD&C	Food, Drug, and Cosmetic
FD&C Act	Federal Food, Drug, and Cosmetic Act, as amended
GAO	General Accounting Office
HEW	Department of Health, Education, and Welfare
NCTR	National Center for Toxicological Research

CHAPTER 5CONCLUSIONS AND RECOMMENDATIONSCONCLUSIONS

Since July 12, 1960, the FD&C Act has required FDA to review the safety of color additives used in food, drugs, and cosmetics and to issue regulations prescribing their safe use. Color additives, such as Red No. 2, that were commercially established at that time could continue to be used in these products on an interim basis for a reasonable period, pending completion of scientific investigations needed to determine their safety.

FDA, however, has permitted the use of Red No. 2 in food, drugs, and cosmetics for 15 years without making a final determination of its safety. FDA has repeatedly extended the interim period for using Red No. 2 in food, drugs, and cosmetics on the basis of requests from manufacturer or industry associations to allow time to complete scientific investigations concerning its safety. In some cases, however, the requests did not identify investigations underway or indicate when they were to be completed.

Moreover, since 1970 several scientific studies involving animals, including some performed or sponsored by FDA, raised questions concerning the safety of Red No. 2 in food. In some of these animal studies Red No. 2 or amaranth was shown to be either toxic to reproductive systems or carcinogenic. Because of its concern about the safety of Red No. 2, FDA in July 1972 issued a proposal to limit human exposure to the color additive. As of September 1, 1975, FDA had not made a final determination on the safety of Red No. 2 or restricted its use in food, drugs, and cosmetics. Permitting its continued use for an extended period while questions concerning its safety remain unresolved results in unnecessary risks to the public health. To minimize such risk, FDA should act promptly to establish the safety of Red No. 2 or take appropriate regulatory action.

RECOMMENDATIONS

We recommend that the Secretary, HEW, direct the Commissioner, FDA, to act promptly to establish the safety of Red No. 2 or take appropriate regulatory action to prevent its use in food, drugs, and cosmetics.



*REPORT OF THE
COMPTROLLER GENERAL
OF THE UNITED STATES*

Regulation Of The
Food Additive Aspartame

Food and Drug Administration
Department of Health, Education, and Welfare

On July 26, 1974, the Food and Drug Administration published a regulation approving the use of aspartame, an artificial sweetener. Later, questions were raised regarding adverse effects of the additive on health.

Before these questions were answered, preliminary results of an agency investigation indicated discrepancies existed in the data submitted in support of aspartame's safety.

On December 5, 1975, the regulation approving the use of aspartame was suspended. Aspartame has not been, nor will it be, marketed until all questions about its safety have been answered.

MWD-76-111

APRIL 8, 1976



COMPTROLLER GENERAL OF THE UNITED STATES
WASHINGTON, D.C. 20548

B-164031(2)

The Honorable Gaylord Nelson
United States Senate

Dear Senator Nelson:

In your letter dated January 30, 1975, you requested that we review the Food and Drug Administration's (FDA's) methods for determining the safety of three additives-- Food, Drug, and Cosmetic Red No. 2; saccharin; and aspartame--for use in food. You asked that we furnish separate reports on the three additives and that the reports focus on

- the history of FDA's regulation of the additives, including in-house and outside tests leading to a change in their regulated status;
- the current status of testing the additives and FDA activities affecting their status;
- the extent to which FDA has examined alternatives to the additives if their safety is questioned; and
- whether the regulatory action taken by FDA on these three additives, based on the scientific evidence available, complies with the Federal Food, Drug, and Cosmetic Act, as amended (21 U.S.C. 301).

We were also requested to determine FDA's legal authority for allowing a food additive regulation to remain in effect when scientific evidence has raised questions about the additive's safety.

This report on aspartame is the second of three reports to be issued. Our report entitled "Need To Establish The Safety of Color Additive FD&C Red No. 2" (MWD-76-40) was issued October 20, 1975.

In our review of aspartame, we concentrated on the period since February 1973, when a petition for its use was submitted to FDA for approval. We reviewed pertinent legislation, regulations, and practices relating to FDA's regulation

B-164031(2)

of food additives; examined FDA records relating to the regulatory status of aspartame; and reviewed documents submitted by its petitioner in support of the additive's safety. We interviewed officials of FDA; Canada's Food and Drug Directorate, Ottawa, Canada; and G. D. Searle and Company, Chicago, Illinois.

REGULATION OF FOOD ADDITIVES

Since enactment of the Food Additives Amendment of 1958 on September 6, 1958 (Public Law 85-929), the Federal Food, Drug, and Cosmetic Act has required FDA to establish regulations prescribing the conditions under which a food additive may be safely used.

The act (21 U.S.C. 348(b)(1)) provides that any person may file a petition with FDA proposing the issuance of a regulation prescribing the conditions under which an additive may be safely used. A petition must contain:

- The name and all pertinent information concerning the food additive, including, where available, its chemical identity and composition.
- A statement of the conditions of the additive's proposed use, including all directions, recommendations and suggestions for its proposed use, and specimens of its proposed labeling.
- All relevant data on the physical or other technical effect the additive is intended to produce and the quantity of the additive required to produce such effect.
- A description of practicable methods for determining the quantity of the additive in or on food and any substance formed in or on food because of its use.
- Full reports of investigations made about the additive's safety, including full information on the methods and controls used in conducting the investigations.

In determining whether a proposed use of a food additive is safe, the act (21 U.S.C. 348(c)(5)) requires FDA to consider



REPORT OF THE COMPTROLLER GENERAL OF THE UNITED STATES

Need To Resolve Safety Questions On Saccharin

Food and Drug Administration

Department of Health, Education, and Welfare

In February 1972, the Food and Drug Administration published an interim regulation to allow the continued use of saccharin in food for a limited time, to resolve the question of its potential to cause cancer. Resolution of the question is not expected before mid-1978.

Allowing an interim food additive regulation to remain in effect for about 6 years while safety questions concerning the additive are being resolved seems contrary to the Food and Drug Administration's intent of permitting use of such additives for a limited time. Extended use of a food additive, such as saccharin, whose safety has not been established and for which a question of carcinogenic (cancer causing) potential has been raised could expose the public to unnecessary risk.

HRD-76-156



COMPTROLLER GENERAL OF THE UNITED STATES
WASHINGTON, D.C. 20548

B-164031(2)

The Honorable Gaylord Nelson
United States Senate

Dear Senator Nelson:

This is the last of the three reports you requested on January 30, 1975. It is on the need for the Food and Drug Administration to resolve safety questions on saccharin. Our reports "Need to Establish the Safety of Color Additive FD&C Red No. 2" (MWD-76-40) and "Regulation of the Food Additive Aspartame" (MWD-76-111) were issued October 20, 1975, and April 8, 1976, respectively.

The Food and Drug Administration is part of the Department of Health, Education, and Welfare. As requested by your office, we have not obtained the Department's written comments on the report. However, we have discussed it with Food and Drug Administration officials and have considered their comments in the report.

We invite your attention to the fact that this report contains a recommendation to the Secretary of Health, Education, and Welfare in chapter 5. As you know, section 236 of the Legislative Reorganization Act of 1970 requires the head of a Federal agency to submit a written statement on actions taken on recommendations to the House and Senate Committees on Government Operations not later than 60 days after the date of the report and to the House and Senate Committees on Appropriations with the agency's first request for appropriations made more than 60 days after the date of the report.

We will be in touch with your office in the near future to arrange for copies of this report to be sent to the Secretary of Health, Education, and Welfare and to the four Committees to set in motion the requirements of section 236.

Sincerely yours,

A handwritten signature in cursive script, appearing to read "Thomas P. Stead".

Comptroller General
of the United States

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ABBREVIATIONS

FDA	Food and Drug Administration
GRAS	generally recognized as safe
OTS	o-toluenesulfonamide
WARF	Wisconsin Alumni Research Foundation Institute, Inc.

CHAPTER 5CONCLUSIONS AND RECOMMENDATIONCONCLUSIONS

In February 1972 the Food and Drug Administration published for saccharin and its three salt forms an interim food additive regulation because certain animal study results raised questions about their potential to cause cancer. Additional animal study data has since raised similar questions concerning saccharin's safety. The question of saccharin's carcinogenicity is not expected to be resolved before mid-1978.

Allowing an interim food additive regulation to remain in effect for several years while safety questions concerning the additive are being resolved seems contrary to FDA's intent of permitting use of such additive for a limited period. Extended use of a food additive, such as saccharin, whose safety has not been established and for which a question of carcinogenic potential has been raised could expose the public to unnecessary risk.

Moreover, permitting, under the interim regulation, the continued use of saccharin at the same level used as a generally-recognized-as-safe substance, with a safety factor of 30 to 1 rather than the conventional 100 to 1, seems questionable. Potential hazards from the use of saccharin could be further minimized by reducing the levels of o-toluenesulfonamide in saccharin to the lowest level practically achievable under present manufacturing technology.

Because saccharin has been used under an interim food additive regulation for about the past 4 years and because safety questions about it are not expected to be resolved for about 2 more years, FDA should reevaluate the justification for saccharin's continued use pending resolution of the safety questions.

RECOMMENDATION

We recommend that the Secretary, Department of Health, Education, and Welfare, direct the Commissioner, FDA, to promptly reassess (1) the justification for continued use of free saccharin and its three salt forms under the interim food additive regulation and (2) the need for issuing a permanent regulation or possibly discontinuing their use in food.

If continued use under the interim regulation is justified, consideration should be given to the need for increasing the safety factor to the conventional level set forth in FDA's regulations and to reducing the permissible levels of OTS in saccharin to the lowest achievable levels.

Senator NELSON. Our next witness is Dr. Sidney Wolfe, and he is accompanied by Ms. Anita Johnson.

I understand that Ms. Anita Johnson is not here today to give her expert advice to you, Dr. Wolfe, so you will have to struggle along without your very able associate.

Dr. Wolfe represents the Public Citizen's Health Research Group.

Dr. Wolfe, we are pleased to have you present today.

I regret that we are going to have to squeeze you a bit more on time than we would like.

First, I would like to ask that, if we have questions, which I am sure we will have, to submit to you in writing, you would be willing to respond for the record, so we would at least have a complete record, because we will not have time to ask the questions, and then your statement will be printed in full in the record, and if you are comfortable with it, I think it would be helpful if you would take the main points of your testimony, and summarize them, and if you wish to comment on any of the testimony the GAO and the FDA gave, on any of their points, you are free to do so. I might say to the GAO, the FDA, and Dr. Wolfe, the committee would be happy to accept for the record any additional commentary each of you may have on the testimony of the other, because we are intending to get as complete and accurate a record as possible.

STATEMENT OF SIDNEY WOLFE, M.D., PUBLIC CITIZEN'S HEALTH RESEARCH GROUP

Dr. WOLFE. Thank you.

While the subject is still fresh in the air, I would like to make a couple of additional comments about Yellow No. 5.

The estimated incidence of allergies in the United States to Yellow No. 5 is figured out in the following way: Approximately 0.2 percent of the population is allergic to aspirin.

Senator NELSON. Point 2?

Dr. WOLFE. Point 2 percent of the population is allergic to aspirin, that makes about 400,000 people.

Senator NELSON. Did you say 0.2 percent?

Dr. WOLFE. Yes. The number you estimate of 400,000 is I believe the correct one.

Of these, about 70 percent, according to the authors of a recent study also have allergies to Yellow No. 5. About 70 percent of aspirin allergic people, are also people allergic to Yellow No. 5. This shows up as hives or urticaria. The authors of the study were concerned enough about the possibility of asthma being induced in these people, when they did their study, they used even smaller amounts of Yellow No. 5 in those people that had the history of asthma.

I think it is important to point out that the amount of Yellow No. 5, that is consumed, by the public on the average is considerably higher than the amount that it takes to provoke these allergic responses, according to a memo from the FDA, which we have looked at as part of our review of all of the FDA data on the so-called safety and food color.

The average intake of Yellow No. 5 was 7 milligrams per day, the average per person for the whole country; the amount it takes to

provoke the allergic response is anywhere from 0.1, or a tenth of a milligram, to 2 milligrams. So we are talking about an ordinary person consuming as much as 70 times more per day than it takes to provoke an allergic response.

The idea of everyone having to shuffle through the office of an allergist to identify the cause of their symptoms is ridiculous. The idea of solving this problem by putting on the label of those foods that they contain Yellow No. 5—as the FDA is about to propose—is irresponsible.

There are some circumstances in which the consumer does not see the label, so that will not be an adequate solution even for those who have already identified their allergies, such as eating in a restaurant, eating at a friend's house, eating any of the FDA formula foods that do not require any listing on the label. So when a food color provokes an allergy in about 300,000 people, 70 percent of the 400,000 that are allergic to aspirin, it should have been banned a long time ago.

Senator NELSON. What is the function of Yellow No. 5?

Dr. WOLFE. The function of Yellow No. 5 is to make the consumer think that foods contain a quantity of naturally-yellow ingredients when they do not; for example, Yellow No. 5 is added to a lot of ice cream and beverages that are called "lemon." Lemon soda, lemon ice cream, rarely have lemon in them.

The function of the dye, in short, is to deceive or defraud the public.

In addition, it produces an allergy in over 300,000 people. Under the circumstances, there is no usefulness.

Senator NELSON. No safety, no preservative?

Dr. WOLFE. None.

Senator NELSON. No emulsifying, is that correct?

Dr. WOLFE. That is correct, and that is certainly true of all of these substances in the category of food colors.

We have stated that many of the food additives, not just the colors, would be better called food cosmetics, and not food additives.

The only ingredients many of them add to the food product are ingredients of cost and risk unaccompanied by any nutritional or other health benefit.

The two items we reviewed in the testimony, I will go over these briefly because of the need to conclude the hearing, are the review of generally recognized as safe food additives, the so-called GRAS review, and food colors.

In brief, we looked at all of the studies available on the substances FDA plans to reaffirm as generally recognized as safe, about 50 chemicals.

On page 2 of the testimony, we review the information available on these substances. Now, the information available is merely whether the study was done or not, not whether it was positive or negative, and not whether the study was competently designed and performed. If there is a plus (+) on the chart, it merely means that some study which is arguably of that type, has been performed. A plus (+) does not mean that the study is a reliable one.

Of the over 50 GRAS chemicals we looked at, 72 percent had not had mutagenic testing, 58 percent had not had reproductive studies, 70 percent had not had long-range studies.

The percentage that had not had cancer is even larger than 70 percent, because some of the long-range studies did not look for cancer but looked instead for other long-range effects.

Eighteen percent of the substances proposed for GRAS status had not even had acute toxicity studies.

This review, which has been going on for some time, has been very slow, and if these 50 chemicals are any indication, the studies FDA adjudges to support safety are very inadequate.

Substances not tested for some of the most important toxicities are being proposed to remain as generally recognized as safe.

We include in our testimony the details on which type of studies have not been done on all 50 of these GRAS additives.

Mr. GORDON. Dr. Wolfe, who proposed these studies; is it the FDA?

Dr. WOLFE. Yes.

I will now briefly review some of our findings as far as food coloring is concerned.

We made a freedom-of-information request for all data in FDA possession that had been used to substantiate the finding of safety, for the six permanently listed coal-tar food color additives, the three provisionally listed coal-tar dyes, and a number of nonsynthetic food colors. As amended, the Food and Drug Cosmetic Act requires the FDA to clear every color for safety, and requires consideration of consumption levels in the diet, considering the fact it is taken not alone as in animal studies, but with 10, 20 or 50 other additives, cumulative effects of any such additive, and so forth.

Under this law, the FDA has approved six coal-tar dyes for foods, Citrus Red 2, Blue 1, Orange B, Red 3, Yellow 5, and Red 40.

Our review of all the data in FDA's files on these colors, including review of microscopic slides in the cancer study of Red 3, which was done by a consulting pathologist, shows that in each instance, there is either a very clear finding of carcinogenicity, or suggested evidence of carcinogenicity. The two in which there are positive findings are: (1) Citrus Red 2, which the World Health Organization recommended should not be used as a food additive, but is used in increasing amounts every year to make people think that Florida oranges are California oranges by putting more orange in their skins, and (2) Red 40, which is also on the market, and is the subject of a petition to ban by the Michael Jacobson Center for Science in the Public Interest.

The other four, Blue 1, Red 3, Yellow 5 and Orange B, are at the least suspect carcinogens, to say nothing of the fact that Yellow 5 is a very powerful allergy producing chemical.

To review the other ones, Orange B, has been shown to cause so-called hyperplastic nodules in the livers of dogs fed this dye for a number of years. These nodules according to standards of 1976 or 1977 are thought to be precursors of cancer, and it is difficult to understand how the FDA year after year allows these substances in the food supply.

We are talking about consumption of 5 million pounds of dyes every year by the American public, twice as much as 10 years ago.

According to the FDA's own estimates, there are about 4 million children in this country, 10 percent of the children between ages 1 and 12, who will have eaten over 1 pound of color dye by the time they are 12, some will have eaten as much as 3 pounds.

Many other food colors have been previously banned, because they have long been known to be carcinogens.

How much longer is the American public, or the Congress to tolerate this kind of shoddy, incompetent work that has been done by the FDA, that has allowed these chemicals to stay on the market, chemicals which give no benefit to the public.

A recent survey by Redbook Magazine of housewives found that 59 percent thought all food additives that had only cosmetic value should be banned outright, without even considering the need for doing tests, so the sentiment of the public is clearly in this direction.

Dr. Schmidt, former FDA Commissioner has said repeatedly, if we thought the American public wanted this, we would do it. The American public apparently does want it, but the action is still not forthcoming from the FDA.

Senator NELSON. Do you have a list, or does the FDA have a list of those additives that are purely cosmetic?

Dr. WOLFE. The FDA does. All of the colors, of course, are purely cosmetic, and they have broken other additives down into categories, which would be identified by use.

Let me summarize.

With all its faults, the safety testing of drugs—in which there are benefits in a far greater proportion of cases—is far ahead of the safety testing of food additives which, in many instances, confer no benefit to the consumer.

I do not mean to give the impression that FDA's drug program is very good.

We asked the FDA 7 months ago to renew its warnings because a half million women were given drugs which cause birth defects—progestins—as yet the FDA has done nothing. They have mumbled about doing things, but they have done nothing about it.

With respect to food additives, we propose the following:

1. Remove all coal-tar dyes from the market immediately. The six permanently listed ones and the three provisionally listed colors are a blight on the food supply.

2. Eliminate the present concept of generally recognized as safe (GRAS). All substances should be considered potentially dangerous until complete and adequate tests prove otherwise.

The burden should not be in the direction of giving the benefit of the doubt to safety despite the fact that 70 percent had never been tested for cancer.

3. Disallow the marketing of any new food additive unless it has:
 - a. Been thoroughly tested for all types of toxicity—largely being done at present;

- b. Has evidence of more than a cosmetic benefit to food; and

- c. Is better than an existing food additive of the same category.

There should be no further food additives which offer only a cosmetic effect.

It should be of real benefit, as you, Senator Nelson have proposed over the years for drugs, why should a new food additive with even less value be allowed on the market, unless it performs some function not already performed by existing additives.

4. Disallow further testing of food additives by industry. They should pay the bill, but reliable and closely monitored third parties—such as academic centers—should do the testing.

5. There needs to be a housecleaning at FDA Bureau of Foods. The FDA officials who have allowed the American public to be exposed to these massive amounts of dangerous or untested food additives by their lack of adversarial attitude to the food industry must be replaced by people with more critical scientific faculties.

I thank you. I will be happy to answer any questions.

Let me say that we will submit for the record in addition to our testimony, our recent petition for the FDA to ban the six permanently listed colors, and a statement from Dr. Leo Friedman, now deceased, of the FDA stating quite clearly that this whole variety of studies should be done for food additives, not just the hit and miss approach that they have right now.

Senator NELSON. What is the petition?

Dr. WOLFE. The petition was a petition to ban all six of the permanently listed food colors.

We are submitting that along with the testimony.

Senator NELSON. Does the petition argue the merits of the issue?

Dr. WOLFE. Yes, the petition is based on a review of all documents in the FDA files, supporting the so-called safety.

Senator NELSON. So you are summarizing in your petition the arguments?

Dr. WOLFE. All of the studies in the files.

Senator NELSON. Is that all there?

Dr. WOLFE. Yes, we are submitting this whole petition.

Senator NELSON. Let me say that I would be happy to have your response to the GAO or the FDA, so we see the points at issue, and the arguments on both sides, so the record will be clear.

Dr. WOLFE. I would just like to make one comment. In listening, and reading the rest of the 31 pages of testimony submitted by the FDA this morning, I think that what they are doing is describing a process of doing something without doing anything.

The FDA, in a shorter press release handed out this morning, says that as of this March, they will start doing things like reviewing colors.

In several hundred hours of our time, including that of the consultant working for us, we were able to review the files, and conclude there were serious questions of safety of all of the nine coal-tar colors that are allowed on the market.

The FDA is buying time, and is allowing more and more consumption of these dangerous food colors. Ms. Carter stated very succinctly, a month or two ago, that if there are really any questions about the safety of these kinds of chemicals, they should go off the market during review.

We would agree fully. We will be seeing, unless something other happens, another year or two before these colors go off the market.

The record will attest to the merits of the issues raised by Dr. Michael Jacobson, of the Center for Science and Public Interest, on Red No. 40, and Orange B, and the issues we have raised. Unfortu-

nately, it took 5 years from the time we petitioned to ban Red No. 2, for FDA to enact the ban to take place. Of course, there is also this waste of time going on with this \$18 million reviewing the GRAS list, et cetera.

I submit that instead of protecting the food industry as FDA has done, these food additives should go off the market, and that the public should be protected.

Senator NELSON. Thank you very much, Dr. Wolfe.

Your prepared statement will be made a part of the record.

This record will be open at least for 30 days to receive additional comments.

Tomorrow the hearings will resume in the caucus room, in the old building, the Russell Office Building, room 318, at 9:30 a.m.

The committee stands in recess.

[Whereupon, the committee was recessed at 12:10 p.m.]

[The prepared statement and supplemental information submitted by Dr. Wolfe follows:]

TESTIMONY OF SIDNEY WOLFE AND ANITA JOHNSON
 PUBLIC CITIZEN'S HEALTH RESEARCH GROUP
 BEFORE THE SENATE SELECT COMMITTEE ON SMALL BUSINESS
 HEARINGS ON FOOD ADDITIVES
 JANUARY 13, 1977

The Health Research Group is a consumer research and advocacy organization funded by voluntary donations to Public Citizen, Inc. The group performs oversight on the activities of the Food and Drug Administration in addition to its activities in health care delivery and occupational health.

"GENERALLY RECOGNIZED AS SAFE" FOOD CHEMICALS

The 1958 Food Additives Amendment to the Food, Drug and Cosmetic Act requires all chemicals added to food to be approved by the Food and Drug Administration on the basis of scientific proof of safety, except for food additives which are "generally recognized as safe (GRAS)."¹ "Generally recognized as safe" are additives which are

"generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experiences based on common use in food) to be safe under the conditions of its intended use." 321 U.S. 201(s).

Contrary to what the term "generally recognized as safe" connotes, the GRAS chemicals are those which are exempt from the requirement of petitioning FDA with proof of safety. Shortly after the Food Additives Amendment was passed, FDA compiled in this way a list of additives which were GRAS: three FDA employees compiled a tentative list in 1958 of 189 substances they thought might be GRAS. This list was based at best on educated hunches. No literature searches or tests were conducted, and no explicit estimates of consumer exposure to each substance were made. This list was sent out to 900 scientists, asking for comments on whether they recognized the substances to be safe. Only 324 scientists replied. Of these, only 69 commented on specific substances. FDA records show that the 69 comments which questioned the safety of specific substances on the list were summarily dismissed with written determinations such as "critic not qualified" and "safety established." Again no tests, literature searches or calculations of consumer exposure were performed when views critical of the proposed GRAS substances were rejected by FDA. Even at that stage, the pure hunch approach to GRAS status prevailed. Internal FDA memos in 1959 and 1960 criticized the carelessness of the procedure. Nevertheless, further lists were formed in the same way until over 600 substances were adjudged GRAS. Basically, GRAS meant to FDA that no one had proven the substance unsafe.

Over the years, several substances were removed from the GRAS list as information became available about their lack of safety. Among them were cyclamate, removed after a host of adverse information on gene mutations, birth defects and cancer; saffrole, the flavoring in root beer, because of its carcinogenicity (cancer-causing properties); NDGA because of abdominal cysts and kidney lesions; oil of calamus because of carcinogenicity.

1. Exempt from the Amendment are pesticides and color additives, and chemicals which were sanctioned by FDA or USDA prior to passage of the Amendment.

As use of these GRAS substances soared in the '60s with the boom in use of processed foods, and substances were banned once tests were conducted on them, the GRAS additives became a matter of public concern. The Presidential consumer address to Congress in 1969 called for a review of the GRAS list. FDA is now conducting this review, with the aid of an outside group, an advisory committee connected with the Federation of American Scientists of Experimental Biology (FASEB). This time around, literature searches are being conducted before the GRAS designation of an additive is re-determined. On the following pages is a table of the substances for which GRAS status has been proposed by FDA or the advisory committee, together with the types of toxicological studies available on each. The chart includes all reported studies, many of which are of poor quality.

To repeat, many of the studies represent a "+" on the table are inferior and in some cases ludicrous. Sometimes animals were fed a substance and then examined only for total weight, rather than for effects on individual tissues and body processes. Sometimes only a few animals were used at doses so low that toxicity would not be noted; sometimes the identity and amount of the substance fed was in question. Anything that could conceivably be called a study was marked by "+". A long-range toxicity test was marked "+" if the animals were exposed for a long period of time even if the study lacked examination for cancer-causing properties. Therefore, not all the substances which have had "long-range toxicity studies" conducted on them have been examined for carcinogenicity.

PROPOSED GRAS SUBSTANCES LACKING ANY STUDY
OF TYPE DESCRIBED

Kind of Study	Number Lacking Study	Percent Lacking Study
Acute toxicity	9/50	18
Intermediate toxicity	25/50	50
Metabolism	17/50	34
Long-range-- including cancer*	35/50	70
Reproduction	29/50	58
Mutagenicity	36/50	72

*Long-range studies on only some chemicals included cancer tests, so the percent of chemicals lacking cancer studies is more than 70 percent.

Clearly, the toxicological properties of these substances are not well known, even though FDA is about to re-affirm them as GRAS. Nothing is known about the long-term safety of the majority of these substances; half of the substances lack information on safety within a several month period. Nine substances do not even have acute studies.

Even the advisory committee has expressed concern about the lack of scientific data:

"There is a paucity of information on the biological effects of dill..."

"In view of the prevalence of allergies to gum arabic... additional studies should be undertaken...reported to be toxic to pregnant animals..."

Oil of rue data, "meager."

Paraben, "dearth of closely controlled experimental data." "Few reports of experiments have been found

STUDIES ON PROPOSED GRAS SUBSTANCES

	<u>Acute Toxicity</u>	<u>Intermediate Range Toxicity</u>	<u>Metabolism</u>	<u>Long-range Toxicity</u>	<u>Reproduction</u>	<u>Mutagenicity</u>
<u>Sodium Benzoate and Benzoic Acid</u>	+	+	+	+	+	0
<u>Propyl Gallate</u>	+	+	+	0	+	0
<u>Guar Gum</u>	+	+	0	0	+	+
<u>Gum Arabic</u>	0	+	0	0	+	+
<u>Gum Ghatti</u>	0	0	0	0	+	+
<u>Gum Tragacanth</u>	0	+	0	0	+	0
<u>Sterculia Gum</u>	0	+	0	0	+	+
<u>Dill</u>	+	+	0	0	0	0
<u>Garlic</u>	+	+	0	0	0	0
<u>Oil of Rose</u>	+	0	0	0	0	0
<u>Pulses</u>	No tests. The principle ingredient has been tested to some extent.					
<u>Paraben</u>	+	+	+	+	+	0
<u>Sorbitol</u>	+	+	+	+	+	+

NOTE: Chart includes studies as reported by FASEB; many are poor studies.

	Acute Toxicity	Intermediate Range Toxicity	Metabolism	Long-Range Toxicity	Reproduction	Mutagenicity
Corn Sugar, Invert Sugar, and Corn Syrup	+	0	+	+	0	0
Choline Chloride & Choline Bitartrate	+	+	+	0	0	0
Aluminum Compounds:			+			
a) Aluminum Chloride	+	0		+	+	0
b) Aluminum Sulfate	+	0		0	0	0
c) Aluminum Hydroxide	+	0		0	0	0
d) Sodium Aluminum Phosphate	+	+		0	0	0
e) Aluminum Nitrate	+	0		0	0	0
f) Aluminum Sodium Sulfate	0	0		+	+	+
Sucrose	+	+	+	+	+	0
Carnauba Wax	0	0	0	0	0	+
Hydrogenated Fish Oil	+	+	+	0	0	0
Tallow and Hydrogenated Tallow	+	0	+	0	0	0
Stearic Acid and Calcium Stearate	+	+	+	0	0	0
Sorbic Acid and Salts	+	+	+	+	+	+
Beeswax	+	0	+	0	0	+
Japan Wax	0	0	0	0	0	+
Inositol	+	0	+	0	+	0
Tocopherols	+	+	+	0	+	0
Malic Acid	+	+	+	+	+	+

	Acute Toxicity	Intermediate Range Toxicity	Metabolism	Long-Range Toxicity	Reproduction	Mutagenicity
<u>Dextrin and Corn Dextrin</u>	+	0	+	+	0	0
<u>Carbonates and Bicarbonates:</u>						
a) Potassium Carbonate & Bicarbonate	+	0	0	0	0	+
b) Sodium Carbonate & Bicarbonate	+	+	+	0	0	+
c) Calcium Carbonate	+	0	+	0	+	0
<u>Dextrans</u>	+	0	+	0	0	0
<u>Calcium Salts:</u>						
a) Calcium Chloride	+	+	0	0	0	0
b) Calcium Gluconate	+	+	+	0	0	0
c) Calcium Phytate	+	0	+	0	0	0
d) Calcium Acetate	+	0	+	0	0	0
<u>Calcium Oxide and Calcium Hydroxide</u>	+	0	0	+	0	0
<u>Succinic Acid</u>	+	0	+	0	+	0
<u>Glycerin</u>	+	+	+	+	+	0
<u>Glycerides</u>	+	0	+	+	0	0
<u>Tall Oil</u>	+	+	0	0	+	0
<u>Sodium Hydrosulfite</u>	+	0	0	0	0	+
<u>Zinc Hydrosulfite</u>	0	0	0	0	0	0
<u>Gum Guaiac</u>	+	+	+	+	+	0
<u>Sulfamic Acid</u>	+	+	+	0	0	0
<u>Phosphates (24)</u>	9/24	22/24	13/24	3/24	9/24	4/24

expressly designed to determine the oral toxicity, mutagenicity, teratogenicity or carcinogenicity of the various carbonate compounds. Knowledge of specific toxic levels and effects of long term feeding is lacking."

A paper by the late Dr. Leo Friedman, Director, Division of Toxicology, in FDA By-Lines (January 1974), submitted with this testimony, delineates what toxicological information is deemed adequate for proving the safety of a food additive. Needed are acute studies in two animal species, middle-range studies including physiological measurements and microscopic examination of tissues, long-term studies in two species,¹ including studies for carcinogenicity, reproduction studies to monitor for birth defects or loss of fertility, and studies for adverse effects on genes.²

The gap between what FDA believes it needs to assure the public that an additive is safe, and what it has on each substance is enormous.

These substances should not be called "generally recognized as safe." They should be called "generally recognized as unknown." They are being re-endorsed not because they are in fact safe, but because nothing in the scientific literature proves that they are hazardous, no matter how little actual information either way is available.³ For GRAS substances, the vast bulk of food additives, FDA has assumed the burden of proving harm. FDA's approach to GRAS has effectively undermined the food additives law, which was intended to assure consumers that they were not eating additives of unproven safety.

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1. Unless toxicology tests have been run on a chemical, its effects are largely unknown. While a substance with immediate toxicity may be identified by ordinary use--particularly if the toxicity is bizarre--other effects cannot be identified. A consumer will not ordinarily associate effects of intermediate duration; for example, a headache or a kidney malfunction with a portion of ketchup used several weeks before, even if a ketchup preservative caused it. The possibility of tracing cancer or cataracts to an additive a consumer ingested ten years before is virtually nil. Thus, the fact that an additive has been marketed for many years without anyone noticing any adverse effect is irrelevant to all but the most unusual immediate effects.

Teenage cancer of the vagina was traced to the drug diethylstilbestrol (DES) because the type of cancer was very rare and the chemical was taken in drug form that would be remembered and documented as different from other people's experience.

In contrast, a consumer who contracted breast cancer from DES in beef liver would not associate the cancer with DES exposure years ago. Doctors do not even ask their patients about foodstuffs they consumed yesterday, much less years ago. Most intermediate and long-range effects are identified in animal tests. In the rare case where long-term effects in humans are noticed and documented by scientists, they are confirmed by laboratory animal tests.
 2. It is possible that safety could be assured on some additives without every test being conducted, but exceptions would be made only for specific reasons, such as that closely related compounds had already been thoroughly tested and enough information was available to show that both compounds had the same properties.
 3. Several GRAS substances have, on the basis of very positive information, been taken off the GRAS list. For example, mannitol was removed because female rats fed the substance over a long period of time developed benign thymomas, abnormal growth of thymus gland tissue. However, once off the GRAS list, these substances have not been limited; they are merely given "interim food additive" status by FDA, for an indefinite period of time during which studies are supposed to be conducted while they continue to be sold.

FOOD COLORS

The Color Additives Amendment to the Food, Drug and Cosmetic Act explicitly requires FDA to clear every color for safety and requires consideration of:

- (i) the probable consumption of, or other relevant exposure from, the additive and of any substance formed in or on food, drugs or cosmetics because of the use of the additive;
- (ii) the cumulative effect, if any, of such additive in the diet of man or animals, taking into account the same or any chemically or pharmacologically related substance or substances in the diet (21 U.S.C. 376 (b)(5))

Under this law, FDA has approved six coal-tar dyes for food: Citrus Red 2, Blue 1, Orange B, Red 3, Yellow 5, and Red 40.

Exposure to coal-tar dyes in the food supply is enormous. Approximately 4 million American children (10 percent of children ages 1-12) will have eaten over one pound of coal-tar dyes in food by the time they are 12 years old. Some will have eaten as much as three pounds.¹

Studies in FDA files show that two of the approved coal-tar colors, Citrus Red 2 and Red 40 cause cancer in animals. Citrus Red 2 is used to color oranges orange, and may be ingested by children who chew or such oranges, or in any recipe which calls for orange peel or rind. Red 40 is added to a wide variety of orange, red, purple, and brown foods, including ice cream, candy, and baked goods.

While the other four permanently approved coal-tar colors are not proven carcinogens, the long-term studies on these dyes in FDA files are substandard by any toxicological standard of the last 25 years, and, moreover, provide suggestive evidence of cancer-causation.

The dangerous qualities of these dyes should come as no surprise. A number of the coal-tar colors have been banned by FDA earlier as carcinogens, suspect carcinogens, or in connection with other serious injuries.²

Although these data have been in FDA files for some time, we found little evidence that the studies had been subjected to critical scrutiny by FDA officials. To the contrary, the dyes seem to have been approved in a perfunctory manner; when safety concerns were raised within the agency, they were dismissed, often for spurious reasons. Our report of January 11, 1977, "Hazards of Food Colors," contains more details on these dyes and how they were handled at FDA.

Even if studies were required to be conducted by FDA, the intention of the law can be subverted by allowing shoddy studies and ignoring positive evidence of harm when it does occur. In the case of colors, the law has been systematically destroyed by bad administration over the last 17 years.

1. These figures are based on a July 1976 study by the FDA Division of Consumer Studies.
2. Examples are Red 1, Red 32, Butter Yellow, Green 1, Red 2, Red 4. Some other members of the family, such as Blue 1, Blue 2, and Green 3, are banned in Europe but permitted by FDA (the latter as "provisionally listed" dyes).

In the case of most of the non-coal-tar colors approved by FDA, there is little toxicological information of any kind. The situation is, if anything, worse than with the GRAS additives.

FDA does not appear to believe that toxicological studies are important. Moreover, when, as with the coal-tar colors, studies are conducted, FDA has displayed an absence of critical faculties, approving dyes on the basis of third-rate data. Since most of these studies are sponsored by the manufacturers, a keen sense of scientific skepticism is critical for public protection; yet FDA officials lack the sense of adversariness that a critical scientist must have. Our informal impression is that FDA officials in the Bureau of Foods believe that it is ungentlemanly to question what the manufacturers submit; therefore, they do not.

For the future, mechanisms must be developed to transfer scientific protocol planning and data collection into the hands of competent, disinterested parties, or to foster a healthy scientific adversariness necessary to examine data properly and insist on only the most reliable. We believe that FDA leadership must be replaced by leaders who believe that the burden of proof of safety lies on manufacturers, as the law states.

CONCLUSIONS

Americans are exposed to over 5 million pounds a year of coal-tar dyes all of which are either clear-cut or suspect cancer-causing chemicals aside from having other health risks. In addition, and often in the same foods, are hundreds of million of pounds of other GRAS food additives, generally unlisted.

Most of these chemicals would better be called "food cosmetics"--not food additives. The only ingredients many add to a food product are the ingredients of risk and cost unaccompanied by any nutritional or other health benefit.

With all its faults, the safety testing of drugs--in which there are benefits in a far greater proportion of cases--is far ahead of the safety testing of food additives which, in many instances, confer no benefit to the consumer. The standard for food additives should be stronger, not weaker, than for drugs.

We propose the following:

1. Remove all coal-tar dyes from the market immediately. The six permanently listed ones and the three provisionally listed colors are a blight on the food supply.
2. Eliminate the present concept of generally recognized as safe (GRAS). All substances should be considered potentially dangerous until complete and adequate tests prove otherwise.
3. Disallow the marketing of any new food additive unless it has:
 - a. Been thoroughly tested for all types of toxicity (largely being done at present); and
 - b. Has evidence of more than a cosmetic benefit to food; and
 - c. Is better than an existing food additive of the same category.
4. Disallow further testing of food additives by industry. They should pay the bill but reliable and closely monitored third parties--such as academic centers--should do the testing.
5. There needs to be a housecleaning at FDA Bureau of Foods. The FDA officials who have allowed the American public to be exposed to these massive amounts of dangerous or untested food additives by their lack of adversarial attitude to the food industry must be replaced by people with more critical scientific faculties.

FDA BY-LINES NO. 4
JANUARY 1974

TOXICOLOGICAL METHODS AND TRENDS IN TOXICOLOGICAL RESEARCH

By Leo Friedman, Director, Division of Toxicology

Toxicological methods dealing with problems of chronic exposure have received their greatest development in connection with the evaluation of the safety of food additives and of toxic contaminants and agricultural chemical residues in food. These procedures for safety evaluation are designed to estimate the "maximum no-effect dose." This requires an estimation of the potential of a substance to cause injury and the development of enough data to enable a conclusion that the levels of exposure are so low in relation to the harmful dose that there is a practical certainty no harm can result. Such information can usually be obtained only by studies in animals, and since the emphasis is on the detection of subtle long-range effects deriving from chronic low-level exposures, suitably designed chronic or lifetime studies are the basis for most decisions regarding safety.

The problem of designing animal experiments include two sources of uncertainties: (1) the uncertainty whether the animals chosen for the laboratory studies are appropriate models from which to extrapolate the results to people, and (2) the uncertainty of whether effects that may occur only in very low incidence in the population can be detected with the numbers of experimental subjects that are practical in laboratory investigations. Since the goal of safety evaluation is to insure the least possibility of harm to man, the experimental studies should be designed to detect any and all toxic effects. We do not have available a single ideal animal which has a high susceptibility to every possible adverse effect and in which the induced adverse effects are comparable to those observed in people; thus the inherent limitations of animal studies and the consequent difficulties are evident. These difficulties represent scientific challenges of great practical significance and require that the maximum creative competence be harnessed to solve these problems.

For practical purposes, the Food and Drug Administration over the years has been asked to make and has made specific recommendations as to how safety evaluation data should be obtained. The first of a series of such recommendations appeared in Industrial Medicine, Vol. 12, pp. 55-59, in 1943. Later and more comprehensive articles

Presented at meeting of U.S./U.S.S.R. Environmental Health Delegations, National Institute of Environmental Health Sciences, Federal Research Triangle, N. C., January 22-26, 1973.

by Arnold J. Lehman and his colleagues at FDA were compiled and published in 1949; this publication was revised in 1955 and again in 1959. Since 1959 there have been many developments that would require a new revision of these old guidelines.

In the years since 1959 we have seen many advances in procedures and analytical techniques for metabolic studies, a greater availability of radioactively tagged substances, and a clearer understanding of the microsomal enzymes and their role in metabolic conversion. Advisory committees have made reports emphasizing the importance of reproduction, teratogenesis, and mutagenesis data in safety evaluation, and carcinogenesis testing has been reviewed by several national and international committees.

Essential to the success of a safety evaluation study are proper experimental design and proper interpretation of results. Essential also is the maintenance of good laboratory management and practice so as to prevent or minimize contamination of air, food, water and equipment, to minimize the incidence of intercurrent disease, and to assure adequate records of, and the preservation of, important experimental material. In designing the studies, basic minimum requirements should include observations on growth, food intake, clinical examination, hematology, blood chemistry, urinalysis, gross pathology and histopathology. Additional observations or tests should be included either as a direct result of observations made during interim sacrifices or as a result of prior knowledge based on structural similarities to compounds studied previously or on earlier screening studies, either acute or subacute.

It is clear that safety evaluation studies should be done under the guidance of qualified scientists who, by training and experience, are competent to respond to unforeseen toxicological manifestations noted during the course of the study by initiating reasonable additional experimental procedures or modifications of established protocols. An earlier decision to follow a certain protocol cannot in any way obviate the requirement for data to answer new questions raised by the experimental results of the original protocol when these questions are pertinent at the time of the final evaluation for safe use in food.

The following kinds of specific studies are usually required:

1. Acute toxicity should be determined in several species of experimental animals with emphasis on providing a full and complete description of the effects observed, including observations at necropsy. This information not only gives an indication of relative potency and the types of injury that may be expected, but is also an essential guide to the determination of dosage and the design of protocols of the chronic studies.

2. Cumulative toxicity studies should be made during a period of intermediate duration. This has been the classical subacute 90-day toxicity test. We now think of it as usually extending from time of weaning of an animal to time of sexual maturity and including reproduction according to protocols designed to give valid data on indices of reproductive performance, including teratogenicity and mutagenicity. As has been true in the past, the data should be derived from more than one species of animals including at least one non-rodent species. Full and complete observations should be reported, including gross and microscopic examination of appropriate tissues either at death or at sacrifice. To give valid data on indices of teratogenicity and mutagenicity, it is practically essential that the study be terminated by sacrifice of the pregnant animal before term and examination of the pregnant uterus for viable and dead fetuses and for resorption sites and counting of corpora lutea. It is also important to establish the fertility of the males. It should be noted that, although in most cases the preferred mode of administration is incorporation of the substance into the food of the animal, there are exceptions: in teratogenic studies and in some cases where it may be anticipated that a usage will result in high intakes of a substance in a very short time interval, administration of the dose by gavage, as in acute studies, may be appropriate during certain kinds of subacute experiments. However, in most chronic experiments it is appropriate that the test substance be incorporated in the food.

3. Data are needed on changes that may take place in the additive due to interactions with the food during storage and preparation for consumption.

4. It is always desirable, where possible, to have data from both acute and chronic administration in several species of animal on the substance's absorption, distribution, metabolic transformation, excretion, and accumulation in tissues.

5. When the toxicological data in animals indicate that human beings may be treated safely with tracer amounts of the substance under test, studies to determine the nature of the metabolic conversion patterns should be carried out in humans for comparison with similar studies in animals. Such studies are, of course, always indicated with drugs. They are useful in helping choose the species for the chronic studies. Ideally the test animal should respond similarly to man and the choice should be made so that the test animal should be the one with the metabolic pattern closest to that of man. However desirable this may be, it should be recognized that at the present state of our capability, such a requirement might very well delay the critical chronic toxicity test unduly; in any case, such data may not be as definitive as we think, since the determination of every metabolite, even a major one, is not a simple matter, and the assumption implicit in this recommendation is that the absolute metabolic patterns can be established.

6. The crucial study involves lifetime administration to animals. The classic chronic toxicity test has usually started with weanling animals. It is our present belief that exposure should start at time of conception. In these lifetime studies, animals should undergo at least three cycles of reproduction according to protocols designed to give valid teratogenic and mutagenic data. The design should provide enough animals for interim sacrifice and study. One may combine the lifetime or chronic toxicity test with the 3-generation reproduction test and use the F₁ generation for the lifetime study so that the exposure has started at conception. Our current protocols define lifetime as the point when only 20% of the starting group is still alive. To establish negative findings as valid, more than half the starting rats or mice should have survived at least 18 months.

7. Whenever indicated, more specific tests should be carried out. For example, in the case of organophosphate and carbamate pesticides, cholinesterase inhibition and demyelination studies must be made. In the case of aromatic nitro compounds such as nitrophenol derivatives, cataractogenic studies should be included.

It is important to point out that there are other areas of interest where suitable protocols are not available, or are in early stages of development and should not now be recommended for "routine" testing purposes. These areas of interest include allergy, sensitization, photosensitization, the whole area of food products made from new and unusual raw materials or by new processing techniques, and the area of infant foods where protocols using neonatal animals would be indicated.

Also required are new approaches to the evaluation of experimental data with a view to a more rational estimation of potential hazard under various conditions of use, that is, safety factors should be chosen on a less arbitrary and more rational basis.

In the past -- and we still believe it appropriate -- we have adjusted our suggested requirement for data to different daily levels of exposure. We feel that for any substance, no matter how negligible the amount that enters the food, acute toxicity data are needed to be certain that we are not dealing with a substance of very high or exquisite toxicity, and to provide a basis for dealing with accidental poisonings. For known biologically active substances, we are following an earlier National Academy of Sciences recommendation according to which 1/2000 of the amount found not to produce a deleterious effect in subacute studies in two species of laboratory animals, provided there is not more than 0.1 ppm in the food, is toxicologically insignificant, except when reasons based on chemical structural analogy or other data indicate the need for more extensive study.

For levels of intake greater than what may be considered toxicologically insignificant, lifetime studies have been considered necessary in most cases. When biochemical studies indicate non-absorption from the GI tract or when they show complete conversion

to products normally present in body pools or for some other similar reason, it has not been necessary to do all the studies ordinarily required. In general, acute toxicity studies in several species, a 6 or 12-month study in a non-rodent species, and a lifetime study in two species of rodents, all preceded by suitable range-finding tests, have been required. Our current thinking about chronic studies is that:

- (1) They should include a study of animals which have been exposed to the test substance from time of conception;
- (2) An essential parameter for study is the ability of these animals to produce normal offspring;
- (3) An important test of normalcy is ability to reproduce;
- (4) A lifetime study should continue until an 80% cumulative mortality has occurred in the test group;
- (5) At least 50% of the animals (usually rats and mice) should have survived at least 18 months to establish valid negative findings.

This means that we start with a parent F₀ generation which must be treated for some time before breeding (usually from weaning), so that the F₁ generation is exposed from conception. The F₁ generation must produce a normal F₂ generation, which should be tested for normalcy by breeding an F₃ generation. Exposure to the test substance should ordinarily be continued throughout the course of the entire study. The F₁ generation may also serve for the lifetime test. The F₀ and the F₂ generations should be permitted to breed a second litter, which can be terminated by sacrificing the pregnant female before term. Examination of the pregnant uterus for resorption sites, early and late fetal deaths, and counting of corpora lutea provides valuable data regarding dominant lethal effects, and examination of soft and skeletal tissues of the viable fetuses and newborn gives useful information on teratogenic potential. It is understood that data on reproduction, fertility, fecundity, litter, viability, lactation, etc., are recorded. In all subacute and chronic studies, it is expected that at least 3 dose levels and 1 untreated control would be included, and that one of the test levels should produce a toxic effect. This has been recommended many times in the past. We have suggested that the reproduction aspects be incorporated as an integral part of the chronic toxicity test.

When the comparative metabolic studies indicate that a non-rodent mammalian species is closer to man than the rodent species, this non-rodent should be studied for at least 2 years, during which time there are at least two estrus cycles and one mating. The numbers of animals at the start of the study should be large enough to assure an adequate number of survivors at termination.

In our view, a chronic study in rodents is not valid to demonstrate a "no-effect" level, if most animals fail to survive for 18 months. On the other hand, in the case of unusually good survival or of a long-lived strain, the study should not be terminated until the mortality rate rises and a total mortality of 80% has been reached in a test group, so as to make possible useful longevity comparisons. Survival may vary for different strains of rodents, so that the numbers used are contingent on the judgment of the investigator. He should also consider that statistical "confidence intervals" are determined in part on the number of animals as well as the uniformity of the data. There is always an advantage to having a larger number of animals, especially if it should be necessary to extrapolate to a no-effect level.

Toxicologic data on man should be furnished to the extent available, regardless of how small the anticipated daily intake will be. Human data may come from industrial exposure or accidental poisonings, epidemiological studies, and controlled experiments including metabolism studies. Availability of human data or experience, based on common use in food, may reduce the animal safety data required or may reduce the safety factor. Likewise, where strong or alarming biological effects are observed in test animals, it may be necessary to have additional animal studies beyond those ordinarily indicated before an acceptable intake can be set.

Additional information which may be required includes the following:

1. The background exposure may be needed from all sources, not only that in food. In the case of unavoidable contaminants present in the environment this would require analytical methods of great sensitivity, established accuracy, and specificity.
2. It is important to know the chemical form or forms to which man is exposed, since the chemical form is very important in determining biological activity particularly in the case of metals.
3. The bioavailability and/or biotoxicity of each form and the biological half-life of each form, including the dynamics of absorption, tissue distribution, and excretion, should be established. The need for this kind of information is illustrated by problems with heavy metals such as mercury and methylmercury compounds, the polychlorinated biphenyls, and the like.

The approaches discussed indicate that there are certain assumptions on which the toxicological studies are based, as follows:

1. That there is usually a dose-response relationship;
2. That there is a no-adverse-effect or threshold level for each biological effect;

3. That the need for a sensitive bioassay system can be supplied with the appropriate animal model;

4. That in order to demonstrate a hazard, it is necessary to have empirical evidence that a biological effect has been produced, i.e., that logical inference alone is insufficient for a conclusion that a biological effect exists. This last assumption is important to repeat again and again as we get into the area of biochemical toxicology. There we have the possibility of determining very marked changes in biochemical parameters, but unless they are correlated with physiological changes, their significance in toxicology is not demonstrated. The fact that an effect can be demonstrated does not mean that a hazard exists.

(FDA Advisory Committee)

Internal Analgesic Report page 327
Draft No. 6 (10/22/76)

October 1976

(a) Incidence of adverse effects. The Panel concludes that these adverse effects occur in a significant proportion of the population. They can be serious and even life-threatening in some instances (Refs. 4 through 6). Although very rare, death has occurred within minutes following ingestion of only one or two aspirin tablets in individuals who were known to be hypersensitive to aspirin (Refs. 5 and 6). The incidence of hypersensitivity reactions (dermal and pulmonary) has been estimated to be about 0.2 percent of the general population (Refs. 8 and 9). A much higher incidence of hypersensitivity is found in some subgroups. Six to twenty percent of asthmatics are sensitive to aspirin (Refs. 10 through 13). About twenty percent of patients with chronic urticaria will experience exacerbation when given aspirin (Refs. 14 through 16).

Public Citizen

January 11, 1977

Acting Commissioner
Food and Drug Administration
200 C St., S.W.
Washington, D.C.

Dear Sir:

Enclosed is our report on the color additives permanently approved by FDA as safe for use in food.

After examining the scientific data in FDA files which are supposed to prove the safety of the six coal-tar colors, we have concluded that proof of safety is absent and, in addition, that there is positive evidence of hazard. A summary appears on page 13.

We are also dismayed to learn that FDA has granted permanent approvals for a number of non coal-tar colors on the basis of little or no scientific information.

Therefore, we are petitioning you for an immediate revocation of regulations 21 C.F.R. 8.201 to 8.275, the effect of which would be a ban on these six dyes until they are proven safe by credible evidence. We are also asking you to institute full and competent toxicological studies on each of the non-coal-tar colors.

Yours truly,

Anita Johnson
Anita Johnson, esq.

Sidney Wolfe
Sidney Wolfe, M.D.



HAZARDS OF FOOD COLORS

The Health Research Group has examined the scientific data on the 29 food dyes permanently approved by the Food and Drug Administration. These data have been submitted to FDA by dye manufacturers or, less often, done by FDA itself, and are supposed to demonstrate safety. In fact, the data do not demonstrate safety and in the case of the permanently listed coal-tar dyes--Citrus Red 2, Blue No. 1, Orange B, Red 3, Red 40, and Yellow 5--they showed positive evidence of harm sufficient to warrant banning.¹

Weak FDA officials have undermined a strong consumer protection law, the Color Additives Amendment of 1960 to the Food, Drug and Cosmetic Act. This law requires all food dyes to be proven safe. It provided for a "provisional list" of dyes already on the market which could be marketed for 2-1/2 years or longer pending the completion of scientific studies essential to prove safety. Over the years, FDA has permitted a number of dyes admitted to lack safety proof to be added to food under the "provisional list"; some "provisional" dyes such as Red No. 2 and Red No. 4 have been challenged on safety and banned. Three (Blue 2, Yellow 6, and Green 3) still remain. FDA has granted other dyes--Blue 1, Orange B, Citrus Red 2, Red 3 (erythrosine), Yellow 5, and Red 40-- permanent approval after determining that they have been proven safe. According to our review, there is no evidence that the permanently approved dyes are safe, let alone safer, than dyes still on the "provisional list." Both groups lack proof of safety, and permanently listed dyes, like the dyes still provisionally listed, raise the spectre of mass long-term poisoning that the statute was designed to prevent.

Many food dyes are coal-tar dyes, a suspect family of chemicals, some of whose members have been banned by FDA for their ability to cause cancer and other injuries.²

Approximately 4 million American children, 10 percent of children ages 1-12, will have eaten over one pound of coal tar dyes in food by the time they are 12 years old. Some will have eaten as much as 3 pounds.³

1. New FDA Freedom of Information regulations allow the public to examine files on food additives, including color additives. The Health Research Group made a Freedom of Information request March 19, 1976, and was granted access to the data at convenient times from April to September.
2. Examples of coal-tar food dyes previously banned by FDA are Red No. 1, Red No. 32, Butter Yellow, Green No. 1, Red No. 2, Red No. 4. Some other members of the family, such as Blue No. 1, Blue No. 2, and Green 3, are banned in Europe but permitted by FDA.
3. Based on U.S. Department of Commerce, Bureau of Census population estimated figures for 1976. Amounts include only coal-tar colors--those colors required to be certified for purity of color by FDA as "FD&C colors." The intake of non-coal-tar dye artificial colors is unknown.

These figures are based on a July 1976 study by the FDA Division of Consumer Studies.

ESTIMATED INTAKE OF COAL-TAR FOOD COLORS
IN ONE YEAR BY CHILDREN, IN POUNDS

	<u>Age 1-5</u>	<u>Age 6-12</u>
Average	.047	.060
90th Percentile	.097	.117
Maximum	.250	.254
No. of Children, 1976	15.9 million	25.3 million

According to FDA estimates, some children eat as much as 1/4 pound of coal-tar dye each year. The FDA estimates are based on a 1965 USDA Survey of Food Consumption (the latest available) and 1967 industry figures on dye concentration, and are extremely conservative, since the production of FD & C dyes and of processed foods has increased greatly. The average consumption per person of soft drinks, a major dyed "food," for example, increased from 259 bottles per year in 1965 to 439 in 1975,¹ an increase of 69 percent. In 1965, FDA certified a total of 2,606,499 pounds of FD&C dyes, whereas in 1975, 5,309,750 pounds were certified.²

Artificial food colors pervade our food supply. The FDA study noted that some children had diets comprised entirely of foods containing coal-tar dyes. 95-99 percent of children eat some food containing coal-tar color. Many foods which contain artificial color are required by FDA to label: "artificial color." However, artificial color is difficult for consumers to avoid altogether, not only because it is added to so many products but also because some foods such as cheese, ice cream, butter, red potatoes and oranges, may contain dye which is not labelled at all.

Food dyes offer consumers no real benefit. They are added by manufacturers who believe that the true colors of their products are unappealing to consumers. A Gallup poll commissioned by Redbook magazine and conducted in March 1976 found that 59 percent of women surveyed said they favored banning food additives used only to improve the appearance of food.³

1. National Soft Drink Association, 1975 Sales Survey of the Soft Drink Industry, p. 16.
2. Certification figures from FDA apply to the coal tar dyes approved for food use but also approved for drug and cosmetic use. There are no certification figures for food use alone. The U.S. International Trade Commission estimates that 2,909,000 pounds of FD&C colors were produced in 1964, while 5,725,000 were produced in 1974.
3. Nutrition: The Redbook Nutrition Report, May 1976. The exact question was: "Would you favor or oppose the banning of all food additives used only to improve the appearance of food even if such foods as fruit gelatins, bacon and ham would lack color?" 59 percent favored, 31 percent opposed, 10 percent didn't know.

COAL-TAR COLOR ADDITIVESCitrus Red 2

This is an azo dye which causes bladder cancer in animals and should be banned immediately under the Delaney Clause. It is used to color Florida oranges more orange, to equal the natural orange of the California fruit. The color was approved in 1959, after heavy industry lobbying secured special legislation to permit its use,¹ heading off a ban under the old law which had been used to ban its equally toxic predecessor, Red 32.² After passage of the Color Additives Amendment, FDA gave it permanent approval as safe in 1963. Use of Citrus Red 2 has been increasing: 2,330 pounds were certified in 1965, while 12,172 pounds were certified in 1975.

Citrus Red 2 is permitted in relatively low levels, but it poses a hazard to workers who produce the dye or dye the oranges, to children who chew or suck the outside of oranges, and to anyone who eats grated orange rinds in recipes or orange peel in cocktails.

At the time FDA permanently approved Citrus Red 2 as "safe," on July 21, 1963, it had three long-term "chronic" studies:

1. Hazelton Laboratories, 1957: 2-year rat injection studies.
 - a. Routine microscopic examinations were only done on 20 percent of all animals in the experiment.
 - b. Bladders were not routinely examined.
2. Hazelton Laboratories, 1957: 2-year dog feeding; bladders not examined.
3. Hazelton Laboratories, 1957: 2-year rat feeding study.
 - a. Less than 10 percent of bladders examined microscopically. A subsequent review of this study on November 1, 1972, by FDA pathologist Dr. S. Levin stated: "The insufficient sampling and examination of urinary bladders from the test animals do not allow for a proper evaluation of the carcinogenic potential of Citrus Red 2."

A June 25, 1958, memo from FDA Director of Division of Pharmacology Dr. A. J. Lehman concluded that the color is a toxic substance.

As evident in other FDA memos from the late 1950s, there was concern about possible bladder carcinogenicity of Citrus Red 2 because of its chemical structural similarity to known bladder carcinogens such as betanaphthylamine. Despite this concern, bladder pathology was grossly inadequate in all of the above studies.

In 1962 a study published by Dr. S. L. Radomski,³ University of Miami School of Medicine, clearly demonstrated that Citrus Red 2 was metabolized by rats to a compound very similar to a metabolite of the known animal and human bladder carcinogen beta naphthylamine. This compound itself was demonstrated to be a bladder carcinogen in 1956.⁴ The author concluded in 1962 that this "suggests the possibility that this [Citrus Red 2] dye may produce bladder cancer after prolonged ingestion."

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1. Public Law 86-2.
 2. Radomski, Ann. Rev. of Pharmacol., 14 (1974) 127.
 3. J. Pharm. and Exp. Therapeutics 136, 378-385, 1962.
 4. Brit. J. Cancer 10, 531, 1956.

THE FDA PERMANENTLY LISTED CITRUS RED 2 IN 1963 DESPITE THE KNOWLEDGE THAT ONE OF ITS METABOLIC PRODUCTS WAS LIKELY TO BE A BLADDER CARCINOGEN AND DESPITE THE KNOWLEDGE THAT NONE OF THE CHRONIC ANIMAL STUDIES SUBMITTED BY ITS MANUFACTURER--AMERICAN CYANAMID--HAD ADEQUATELY TESTED FOR BLADDER CANCER.

In the years immediately following the permanent listing of Citrus Red 2, further experimentation--a series of experiments all done not by U.S. industry or FDA but by foreign scientists--established the bladder cancer causing properties of Citrus Red 2:

1. 1965 - Mouse and Rat Feeding Studies.¹ Hyperplasia and benign tumors of the bladder were found in Citrus Red 2 treated mice and rats and bladder cancer in mice.
2. 1966 - Mouse Injection Studies.² A statistically significant increase in malignant tumors (including one case of bladder cancer) was seen in female mice given Citrus Red 2.
3. 1968 - Bladder Implantation.³ A statistically significant increase in bladder cancer was found in mice with Citrus Red 2 treatment.

World Health Organization Opposes Use of Citrus Red 2

In 1969, because of the aforementioned studies, the WHO⁴ recommended that Citrus Red 2 "should not be used as a food additive."

FDA Whitewashes the Danger

Despite the WHO position and all of the studies cited above, a "review" of the studies of Citrus Red 2 by FDA produced a memo (dated January 11, 1971) which can but be described as a total whitewash of dangers of Citrus Red 2 concluding that: "We feel at present that there is no carcinogenic risk to the consuming public from currently regulated and restricted use of Citrus Red 2."

This cover-up of FDA's previous mistakes of permanently listing the color in 1963 and failing to ban it in 1969 after the WHO recommendation is further compounded by the most recent international declaration on Citrus Red 2.

In 1975, the International Agency for Research on Cancer (IARC), in a monograph on the carcinogenic risk of dyes,⁵ concluded that "Citrus Red 2 is carcinogenic in mice and rats" and stated that:

"Following its oral administration it produced hyperplasia and tumors of the bladder. Given subcutaneously, it produced adenocarcinomas of the lung and lymphosarcomas in female mice. Its administration in mice by bladder implantation produced carcinomas of that organ."

1. Proc. Univ. Otago Med. School 43, 31-33, 1965.
2. Food Cosmet. Tox. 4, 493, 1966.
3. Brit. J. Cancer 22, 825-832, 1968.
4. FAO/WHO Nutrition Meeting Report Series No. 46A.
5. IARC Monograph on the Evaluation of Carcinogenic Risk of Chemicals to Man. Monographs are funded by National Cancer Institute (U.S.A.). Volume 8.

Thus, a group of 11 internationally known cancer experts--whose views are represented in the IARC monograph--conclude that Citrus Red 2 is a carcinogen while FDA allows it to stay on the market.

Although limited to use in coloring orange skins, its human consumption will likely fall heaviest on the young children who are often seen chewing on oranges with other consumption as a result of grinding oranges up and using them for marmalade, cakes, other baked goods, and other edibles which call for orange rind.

By allowing Citrus Red 2 to be used, FDA is clearly violating the Delaney Amendment of the Food, Drug and Cosmetic Act which disallows the use of known carcinogens as additives to the food supply.

Blue No. 1 (Brilliant Blue)

Blue 1 is banned in the United Kingdom and in EEC countries.¹ The FDA-blessed ingestion of 150,000 pounds a year in this country is yet another needless exposure to a probably dangerous chemical. It should be banned.

Blue No. 1 is used mainly in beverages, candy, and baked goods. Its use has risen from 85,485 pounds in 1965 to 158,539 pounds in 1975.

Blue 1 was permanently listed as a food color in 1969. A January 10, 1968, memo from Dr. Charles Kokoski, Division of Toxicological Evaluation, FDA Bureau of Foods, had reviewed all toxicological studies and concluded that:

"The results of toxicological studies pertaining to ingestion of FD&C Blue No. 1 demonstrate the safety of this dye for general use in food, vitamin supplements, and ingested drugs."

We shall examine some of the studies cited in Dr. Kokoski's review as supporting "safety" of Blue 1.

1. 1966 - 2-year Rat Feeding Study. Done at FDA and published in Tox. and Appl. Pharm 5(1), 29-36, 1966. Dr. Kokoski concluded that the dye had "no effect" on tumor formation or any other disease process. Examination of the data upon which Dr. Kokoski's conclusions are based shows the following:

Malignant tumors were seen in	
3 out of 24 control animals	(12.5 percent)
6 out of 24 lowest dose Blue 1 animals	(25 percent)
17 out of 96 of all Blue 1 animals	(17.7 percent)

(An additional part of the above study involved injecting other rats with Blue 1. 89 percent of such rats developed tumors--but none of controls--at the injection site.)

This FDA feeding study is the only one with adequate numbers of animals, done for a long enough period of time, and not plagued by serious problems in the animal colony.

2. 1958 - Mouse Feeding Study. This study was done during World War II, during the occupation in the Netherlands and, according to the authors, many mice died during the experiment from infectious diseases or had to be killed before the end of their span of life.²

1. Chemistry in Britain 6, 12-22, 1970.

2. Acta Physiol. Pharmacol. Neerlandica 7, 35-55, 1958.

Animals were fed low doses--1 milligram per day--of Blue No. 1. Looking only at animals surviving more than 400 days after start of feeding, as the authors did, the following incidences of lung tumors were found:

Control males	0 out of 92	0 percent
Blue 1 males	6 out of 27	22.2 percent

The tumors were "solid adenomata" or papillary tumors.

3. Other Subcutaneous Injection Studies (original data not available; summaries reviewed).
 - a. 1953 FDA (Fed. Proc. 12, 397-98). 76 percent of Blue 1 rats and no control animals got injection site fibrosarcomas of "low to moderate malignancy."
 - b. 1961 Germany (Zeitschrift für Krebsforschung 64, 287-304). No injection site tumors in control animals but unspecified number of injection site fibrosarcomas and "metastasis to other organs occurred in several instances."

Only one properly conducted feeding study was done at the time this food dye was permanently listed and it was suggestive of carcinogenicity not entirely negative as Dr. Kokoski stated.

The Netherlands study--with all its problems--is valid or not. If valid, the statistically significant increase in lung tumors cannot be dismissed as Dr. Kokoski has done. If not valid, it cannot be used to support the safety of Blue 1. Nor can dismissing, out of hand, several positive injection studies be used to support the safety of Blue 1.¹

Since 1969, when Blue 1 was permanently listed, no further oral feeding studies have been done. This dye has not been adequately tested but those tests which have been done certainly are far from negative.

Orange B

This color--used exclusively for casings of hot dogs and sausages--was permanently approved by FDA in 1966. 56,493 pounds were certified by FDA in 1966; certification decreased to 31,161 pounds in 1975.

Earlier this year, Dr. Michael Jacobsen, Center for Science in the Public Interest, asked FDA to reexamine the safety of Orange B.² Two reasons were given for his request:

1. A metabolite of Orange B is identical to one of the metabolites of Red 2, now banned; and
2. The long-term feeding studies of Orange B to mice and rats were inadequate because few animals survived to two years and only a fraction of animals were examined

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1. Hueper, Chemical Carcinogenesis and Cancer, Charles Thomas, 1964, defends injection studies noting that arguments against their validity were first raised "rather recently after environmental chemical carcinogenesis had become a serious practical and economically important issue."
 2. Letter to FDA Commissioner Schmidt, February 25, 1976.

microscopically.

These reasons were dismissed by FDA on the basis that this was the way people did studies in the 1950s,¹ and the FDA rejected Dr. Jacobsen's request for action on Orange B.

Our examination of the FDA Toxicological Evaluation Report on Orange B,² shows that in studies on a third species of animal, conducted at the same time, all slides were examined. The results raise serious questions about the safety of Orange B. The following excerpt from the FDA report describes findings in the liver of dogs sacrificed after seven years, having ingested Orange B since age three months.

"In the livers, there were numerous scattered grayish-white nodules ranging in size from 2 mm to 1 cm diameter on the surface and within the livers of both controls and Orange B dogs. The number of these nodules was greater in test dogs and appeared to be roughly related to dietary level of Orange B...One female dog fed 5 percent dose showed distorted architecture; the pathology report stating this as 'suggestive of neoplasia' (tumor)."

Although the significance of hyperplastic nodules of the liver was a subject of debate in the 1960s, it is now generally thought that hyperplastic nodules should be called neoplastic nodules, indicative of the fact that they represent tumors. It is further thought that hyperplastic nodules are a stage in the development of carcinoma of the liver and that, with time, they will likely develop into cancer.³

The Health Research Group requested these slides for examination, but was informed that FDA does not have the slides, even though they were a basis of the approval decision, and does not know where the slides are. Efforts by the Health Research Group to locate the slides in private hands were unsuccessful.

By today's thinking, Orange B is strongly suspect as a carcinogen and should go off the market until the doubts are resolved.

Yellow No. 5 (Tartrazine)

Yellow No. 5 is the most extensively used color for food use in the U.S. as of 1975. 1,391,000 pounds were certified for use in 1975, up from 773,000 pounds in 1965. Its largest uses are in baked goods, candy, dessert powders, ice cream, and pet foods, but it is used widely in virtually every other category of processed foods.

First used for food in 1916, it became permanently listed in 1966. The FDA Division of Toxicology reviewed all of the studies submitted to support the "safety" of Yellow 5 in 1965, and recommended its permanent listing as a food additive.⁴

1. Memo of Dr. Kokoski of meeting with Dr. Jacobson, January 19, 1971.
2. Dr. Kokoski, October 5, 1965.
3. J. National Cancer Inst. 34, 697-723; Gann, Monograph on Cancer Research 17, 301-342, 1975; Cancer Research 35, 3214-3233, 1975.
4. Memo of Dr. Kokoski, November 1, 1965.

Carcinogenicity Studies. As with Blue 1, only 2 chronic feeding studies exist in which animals were exposed to the dye for two years--the minimum time for adequate exposure to test for carcinogenicity.

A brief summary of the findings is as follows:

1. 2-year Rat Feeding Study Done by FDA.¹ Only a fraction of the animals were completely examined microscopically, the author defending this--in Catch 22 fashion--by stating that "the number of rats examined in detail is small since this was part of a study on three other FD&C colors, run concurrently, with similar survival and relative lack of effect." (emphasis added)

Despite the fact that only few animals were thoroughly examined microscopically and that the total number of tumors was the same in control and experimental groups, there were some disturbing findings in Yellow 5 animals not found in control animals:

If we exclude benign mammary fibroadenoma tumors--commonly found in rats--the total number of breast tumors is as follows:

Control	0	in 18 rats "examined"
Yellow 5	8	in 90 rats "examined" (all doses combined)

It is of interest that 4 of the 8 tumors were adenocarcinoma or duct papilloma--the other 4 were not examined microscopically. A similar failure to examine breast tumors from experimental (dye-fed) animals was seen in the case of an early FDA Red 2 study. Why do the control breast tumors get examined microscopically but not the experimental or dye-fed tumors?

Because of the inadequacy of examination of all tissues and the increased breast tumors found in those which were examined, this study cannot be used to show lack of carcinogenicity of Yellow 5.

2. 2-year Mouse Feeding Study.² This study and its problems have already been referred to in the section on Blue 1. There was, however, an increased incidence of lung tumors with Yellow 5 based on the following results in animals surviving more than 400 days after feeding of Yellow 5 was started:

Control (male and female)	1 tumor in 143 animals (0.7%)
Yellow 5	8 tumors in 59 animals (13%)

As with the FDA rat study cited above, this study cannot be used to support the lack of carcinogenicity.

Allergic Problems With Yellow 5. At the time FDA permanently approved Yellow 5, in 1966, there was already published evidence of human allergic reactions to Yellow 5.³ Since then, several more studies have shown the same result: that extremely small amounts of Yellow 5 (well within the amount which could be ingested) can provoke a rash, swelling or other allergic problems such as runny nose, wheezing, or reddening of the eyes.

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1. Tox. and Appl. Pharmacol. 6, 621-626, 1964.
 2. Acta Physiol. Neerlandica 7, 35-55, 1958.
 3. Annals of Allergy 17, 719, 1959.

In the most recent study,¹ 39 known allergic patients with urticaria and 39 nonallergic patients were given small oral doses (0.1 to 2 milligrams) of Yellow 5 with the following results:

Control patients	0/39	with rash after Yellow 5
Allergic patients	19/39	with rash after Yellow 5

According to Dr. Kokoski's memo affirming the safety of Yellow 5, the average daily food intake of Yellow 5 is 7.4 milligrams per day, considerably more than the .1 to 2 milligrams which provoked allergies in the above study. The maximum daily intake, including drug and cosmetic ingestion--according to FDA--may be 69.6 milligrams per day. The same authors report that 70 percent people with allergies to aspirin are also allergic to Yellow 5.

Although there have been plans to require labeling of all Yellow 5 containing products with specific information about this dye--as opposed to the current "artificial color"--such plans have not been made a requirement. Even if they were, the common circumstance (restaurants, parties, dinner with a friend) in which the allergic person does not see the label argue strongly against its continued use.

The allergic problems alone should be grounds for banning this disease-causing food dye but the additional unresolved question of its carcinogenicity further emphasizes the need for this regulatory action.

Red No. 3 (Erythrosine)

Red No. 3 is widely used as a coloring in drugs, cosmetics, and foods, including candy, puddings, cake frostings and cookies. Approximately 337,144 pounds were certified for use this past year, compared with 96,141 pounds in 1965. It was first listed for food use in 1907, and was permanently listed in 1969. The FDA Division of Toxicology evaluated the dye in 1968. "The results of the various toxicological studies carried out with FD&C Red 3 (Erythrosine) demonstrate the safety of this dye for general use in food, vitamin supplements and ingested drugs."³ However, this conclusion was made before the completion of one important study, and despite serious flaws in the studies completed at that time.

Carcinogenicity Studies. Since this dye has been tested more extensively for carcinogenicity than other currently marketed food colors, since FDA unfortunately approved it (permanently) before the last study was complete, and since microscopic slides of these latter studies were available at FDA, we had a pathologist, working as a consultant to Health Research Group, evaluate the studies.

The complete report of our consultant, Dr. Melvin Reuber--also a consultant to the Environmental Protection Agency and now working at the National Cancer Institute--funded Fort Detrick Cancer Research Center--is included as an appendix. His review of two of the studies included review of microscopic slides.

1. Brit. J. of Dermatology 88, 525, 1973.
2. Those allergic patients with a history of asthma were given only .1 milligram to avoid provoking an asthmatic attack.
3. Dr. Kokoski, memo of October 9, 1968.

The results of the studies suggest that Red 3, like other coal-tar colors, is carcinogenic, but they do not definitively prove that it is carcinogenic. Certainly, the available studies cannot be used to demonstrate that Red 3 is not carcinogenic, as FDA has done.

As noted in Dr. Reuber's review, there are increased numbers of malignant tumors in several groups of rats when these groups are compared to animals not ingesting Red 3.

Thus, the available evidence on the carcinogenicity of Red 3 suggests enough of a hazard so that its continued approval as a food dye is not a safe idea.

Mutagenicity. Two studies on mutagenicity are cited by WHO in a monograph on food additives.¹ They show a slight but statistically significant mutagenic effect on bacteria.

Thyroid Effects. A recently published FDA study² (in gerbils) showed that Red 3 "produced a dose-related change in the thyroid reminiscent of human nodular goitre" (1, 2, or 4 percent Red 3 concentration in the diet was used).

These latter two effects of Red 3, when combined with the findings suggestive of carcinogenicity, demand a ban of this dye.

Red No. 40

This dye was permanently approved in 1971. Its use has increased rapidly from 892,282 pounds certified in 1972 to 1,500,760 pounds last year (replacing many uses of Red. No. 2, banned February 10, 1976). Manufactured by Allied Chemical Corporation, it is used in red, brown, purple and orange foods, including soda, ice cream, candy, and baked goods. The dye was approved by FDA on the basis of one grossly inadequate long-term rodent study--rather than the minimum of two recommended by scientific bodies--in which more than half of the animals died from a respiratory disease, and the study had to be terminated early. After the dye was approved and spread throughout the food supply, a second long-term study (on mice) was instituted, which showed early-appearing lymphomas (cancer of the lymph glands). These results have been duplicated in a third study. This dye should be banned immediately.

Red No. 40 has been the subject of a petition to ban from the Center for Science in the Public Interest, Washington, D.C., since February 25, 1976, and details of the studies can be obtained from that group.

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1. Tox. Eval. of Some Food Additives, No. 55A, FAO Nutrition Meeting Report Series, 1975. Studies cited are: Z. Lebensmitt. Untersuch. 122, 157, 1960; Path. et Microbiol. 26, 206, 1963.
 2. Food Cosmet. Toxicol. 14, 233-248, 1976.
 3. See also 41 Fed. Register 236, 53546 (December 7, 1976).

NON-COAL TAR COLOR ADDITIVES

FDA has permanently approved most of the 23 non-coal tar color additives on the basis of little or no information. Some of these colors, such as carrot oil, are of natural origin; others, such as titanium dioxide, used in candy, chewing gum, desserts, etc., are synthetic. Tests should be conducted on additives of natural origin as well as synthetic additives, since even colors of natural origin may pose a hazard, particularly if they are extracted with synthetic organic solvents, contain heavy metals, are stored in harmful preservatives, etc., facts which would be identified in properly conducted toxicological tests.

The amount and quality of scientific evidence underlying FDA approval of these colors varies widely, not with the source, structure or use of the color, but apparently at random.

These colors are not certified by FDA. FDA has little or no information on the amount produced, the amount used in the food supply as a whole or the amount used in different types of products. Knowledge of level of exposure would seem to be a rudimentary prerequisite to any competent toxicology assessment.

Unlike the coal-tar colors, there is little positive evidence of harm contained in FDA files on these colors. The files are objectionable because they contain little evidence of any kind. They make a mockery of the Color Additives Amendment.

FDA appears to have concluded that the majority of these colors are "safe" not because they have been demonstrated to be safe but because there is an absence of evidence of either safety or harmfulness. These color additives, in the view of the FDA officials in charge, should be deemed safe unless they have been proven harmful. For example, FDA approved corn endosperm oil, added to chicken feed to color egg yolks and chicken skins, without normal toxicological data. The only available test was an 8-week study where chickens were fed only low levels of the color. Toxicological tests normally involve feeding relatively high levels of a substance to laboratory animals, in addition to low levels, so that adverse effects, if any, are sure to be detected. The only toxicological measurements used in the corn endosperm oil study were weight of chickens and level of corn endosperm oil in the blood. Tissue examinations by microscope, study of offspring, etc., considered important by most scientists, did not occur. FDA concluded that corn endosperm oil was safe because "this preparation has been in general use as a component of animal feed for over 20 years with no evidence of adverse effect. This extensive 'in use' experience serves in large part as a demonstration of safety." (Memo of Dr. Kokoski, May 24, 1965)

In fact, middle term and long-term safety--and much of short-term safety--cannot be demonstrated by 'in use' experience because consumers cannot detect subtle or gradual adverse effects and, if they could, would be unlikely to associate them with any one environmental chemical. No evidence of adverse effect would be available unless someone had looked for such effects. The only value of 'in use' experience is in detecting effects which are obvious, unusual and immediate.*

*Indeed, FDA put a limitation on amount usable on a similar color, apo-carotenol used in French dressing, because studies on this color were conducted and showed an ability to produce testicular atrophy at higher levels.

Only four of the 23 colors have studies for long-term safety, canthaxanthin, iron oxide, apo-carotenal, and annatto. For apo-carotenal and annatto, the quality and true results of the chronic studies were difficult to assess from the sparseness of the data in FDA files.

Five colors had middle-range or "subacute" studies but no chronic studies, tagetes, caramel, azanth, cottonseed flour, carmine* The fact that subacute studies were conducted on these colors does not mean that they were good studies or that they were critically examined by FDA. For example, in the subacute study on Azanth, rats were fed Azanth for 90 days at levels four times the amount added to food, but at the end of the study microscopic evaluation of the organs was not performed.

For the color cottonseed flour, twenty seven humans were fed the substance for eight weeks. The report of the study concluded that the color was safe. However, there is so little information available on this study, that the effects, if any, could not be assessed. Subacute animal studies for toxicology, with full physiological test and microscopic examination of organs would have shown far more about the safety of the color than superficial observation of humans. Cottonseed flour is widely added to baked goods to make them reddish-brown.

Two colors were approved on the basis of acute studies alone, titanium dioxide and ultramarine blue. These acute studies are themselves shoddy. The only measurement performed in the small short-term rat study conducted on titanium dioxide was weighing the animals. For ultramarine blue, rats were observed for appearance and behavior, and organs were examined by eye, but no physiological or microscopic examination occurred.

The remaining thirteen colors were permanently approved by FDA without toxicological testing of any kind. Turmeric, for example, was approved without data merely because it had previously received "Generally recognized as safe" status from FDA as a flavor additive (memo of Dr. Kokoski, April 8, 1970), a status in itself conferred without studies. The file on grape skin extract, used in beverages, contains no scientific information.

All these colors lack metabolism information or stability information. Without such information, it is not known whether the colors breakdown in the body or in the food itself, into other chemicals which may be harmful. On several occasions, FDA raised questions about the toxicity of contaminants caused by processing but the questions were dropped, such as with Annatto.

Two examples of FDA indifference on these colors: (1) The American Spice Trade Association submitted information to FDA that cayenne, a spice used also for color, was stored in "well-closed containers, adding a few drops of chloroform or carbon

*FDA knew that carmine was an extensively used dye when permanent approval was granted. Carmine is added at concentrations of 1-2% in cough medicine and mouthwash, in addition to its use in soup, liquor, candy and baked goods.

tetrachloride as preservative." Both of these chemicals are highly toxic, but there is no evidence in the file that anyone ever questioned their contribution to the safety of cayenne.

(2) In a Nov. 20, 1963 memorandum on cottonseed flour, an additive used to make baked goods brown, Dr. O.J. Fitzhugh, an FDA scientist of senior status, noted that there had been two studies in which cottonseed flour caused cancer when fed to fish, and also conceded that the second study "reasonably substantiates the earlier conclusion that the cottonseed flour is implicated in the production of hepatoma in the trout." However, knowing this, Fitzhugh went on to approve the color, dismissing the two studies because he had no information on the purity of the cottonseed flour used and because "The cottonseed meal is not the usual diet for fish...The extensive use in human food in this country alone has indicated that this is a good food." Since FDA approved cottonseed flour, the toxicity of the substance has been credited by other scientists to aflatoxin, a contaminant in the substance produced by mold. What is alarming for the consumer, however, is the fact that FDA approved the color in full knowledge of the fish results, by reputable scientists, and ignored them for spurious reasons.

Several colors such as canthaxanthin, have reasonably complete toxicology data. However, most of these non-coal tar colors, have been permanently approved in almost complete ignorance of their toxicological properties. These colors may in fact be safe; but they have not been demonstrated to be so.

CONCLUSION

The Health Research Group has reviewed all data in FDA files with particular emphasis on chronic feeding studies or injection studies to assess carcinogenicity. For each of the six permanently approved coal-tar dyes, there is at least one of the following findings:

- (1) Definitive finding of carcinogenicity: Citrus Red 2, Red 40.
- (2) Suggestive evidence of carcinogenicity: Orange B, Red 3, Yellow 5, Blue 1.
- (3) Other pathology: allergic reactions: Yellow 5 (humans)
goitre formations: Red 3 (gerbils).

All six coal-tar colors had inadequate carcinogenicity studies, making it impossible to make a determination that any are safe.

In addition to these six colors, three coal-tar colors are provisionally listed:

	<u>Lbs. certified in 1975</u>
Blue 2	84,840
Green 3	9,157
Yellow 6	1,084,284

By definition, FDA has been unable to resolve safety questions on these three. Some of the problems associated with these colors are:

- Blue 2: Increased numbers of tumors in Blue 2-fed animals.
- Green 3: Injection study positive for cancer (Banned in United Kingdom and EEC countries).
- Yellow 6: Allergic problems similar to Yellow 5
 - Mutagenic Effect (Dominant Lethal test in rats)
 - A long-term rat study was conducted on this dye, but it was inadequately reported (IARC Monograph #8, Evaluation of Carcinogenic Risk to Man, p. 263).

In summary, not one coal-tar color added to American food has been proven safe, seventeen years after the law requiring proof of safety was passed. All appear to be hazards. FDA administration of the Color Additives Amendment is a disgrace, and the Health Research Group does not believe that consumers will receive proper health protection if the Amendment continues to be administered by the same officials.

As HEW spends close to \$1 billion per year -- through the National Institutes of Health alone -- on cancer research, FDA has allowed the public to eat millions of pounds per year of cosmetic dyes which are carcinogens or suspect carcinogens.

January 11, 1977

APPENDIXPRELIMINARY REPORT ON THE EXPERIMENTAL CARCINOGENICITY
STUDIES FOR ERYTHROSINE (RED NO. 3)

The purpose of this study has been a review of more recent studies on the oncogenicity of erythrosine, and not a review of all studies or of the published reports. The data from two 1969 FDA rat and two FDS gerbil studies on erythrosine have been reviewed in detail. Histopathologic sections from the rat studies were also examined. The results from these studies are given here. The data from two other recent FDA rat studies and a dog study were also briefly reviewed.

1. FDA Rat Intubation Study^{1,2}

Osborne-Mendel rats, 100 days of age, were housed individually and given food and water ad libitum. The basal diet was Purina Laboratory Chow. Five groups of rats were intubated twice weekly with erythrosine (certified as FD & C Red No. 3) at dose levels of 0, 235, 750, or 1,500 mg/kg for 86 weeks. Solutions were prepared in distilled water at concentrations to give a uniform dose volume of 10 ml/kg body weight.

Each group consisted of 25 males and 25 females. Animals were continued on the experiment for two years. At autopsy, organs appeared normal and weights were not recorded. All rats were autopsied and some without gross lesions were discarded. In some cases, pathological examination was limited to the trunk and limbs, but in the majority of rats the entire body,

¹Hansen, W.H., Davis, K.J., Graham, S.L., Perry, C.H., and Jacobson, K.H.: Long-term toxicity studies of erythrosine. II. Effects on hematology and thyroxine and protein-bound iodine in rats. *Fd. Cosmet. Toxicol.* 11: 535-545, 1973.

²FDA memorandum entitled "Pathology report on rats receiving erythrosine," from K.J. Davis to W. Hansen and S. Graham, dated May 18, 1970 (P-54-69), 51 pp.

including the head, was examined. In addition, at the end of the 2-year study a fairly complete series of tissues from some rats in the 1,500 mg/kg and control groups were examined microscopically for any minor morphological changes which could be attributed to long-term toxicity. In other autopsies, histology was limited to liver, testes, tumors (if present), and other organs with abnormalities.

The numbers of female rats receiving erythrosine with carcinomas and sarcomas is given in Table 1, and total number of all rats receiving erythrosine with malignant tumors in Table 2.

TABLE 1. NUMBER OF FEMALE RATS GIVEN ERYTHROSINE BY INTUBATION WITH CARCINOMAS AND SARCOMAS

Dose (mg/kg)	No. Rats with Carcinomas	No. Rats with Sarcomas	Total No. Rats with Tumors
0	1/24 (4%)	1/24 (4%)	2/24 (8%)
100	2/25 (8%)	4/25 (16%)	6/25 (24%)
235	2/25 (8%)	3/25 (12%)	5/25 (20%)
750	6/25 (24%)	3/25 (12%)	9/25 (36%)
1,500	1/21 (5%)	1/21 (5%)	2/21 (10%)

TABLE 2. TOTAL NUMBER OF FEMALE RATS GIVEN ERYTHROSINE BY INTUBATION WITH CARCINOMAS AND SARCOMAS

Dose (mg/kg)	No. Rats with Carcinomas	No. Rats with Sarcomas	Total No. Rats with Tumors
0	1/24 (4%)	1/24 (4%)	2/24 (8%)
100-1500	11/96 (12%)	11/96 (12%)	22/96 (24%)

The incidence of female rats with malignant tumors was increased in rats given 100, 235, or 750 mg/kg erythrosine by intubation (24%, 20%, and 36% respectively), when compared with the 8% incidence in control rats.

There is a three-fold increase in malignant tumors (from 8% to 24%) when all treated rats were grouped together in one group.

The carcinomas and sarcomas were found most often in the reproductive system. Some sarcomas were subcutaneous.

The number of male rats receiving erythrosine with carcinomas and sarcomas is given in Table 3 and total number of all rats receiving erythrosine with malignant tumors in Table 4.

TABLE 3. NUMBERS OF MALE RATS GIVEN ERYTHROSINE BY INTUBATION WITH CARCINOMAS AND SARCOMAS

Dose (mg/kg)	No. Rats with Carcinomas	No. Rats with Sarcomas	Total No. Rats with Tumors
0	0/21 (0%)	3/21 (14%)	3/21 (14%)
100	0/24 (0%)	6/24 (25%)	6/24 (25%)
235	4/24 (16%)	3/24 (13%)	7/24 (29%)
750	1/24 (4%)	2/24 (8%)	3/24 (13%)
1,500	0/22 (0%)	2/22 (9%)	2/22 (9%)

TABLE 4. TOTAL NUMBER OF MALE RATS GIVEN ERYTHROSINE BY INTUBATION WITH CARCINOMAS AND SARCOMAS

Dose (mg/kg)	No. Rats with Carcinomas	No. Rats with Sarcomas	Total No. Rats with Tumors
0	0/21 (0%)	3/21 (14%)	3/21 (14%)
100-1500	5/98 (5%)	13/98 (13%)	18/98 (18%)

There were more sarcomas in untreated male rats than in untreated female rats. Therefore, the differences in incidences for the various groups are less remarkable. The incidence is increased in male rats given 235 mg/kg erythrosine for rats with carcinomas (0% versus 16%), and for total numbers of rats with malignant tumors, i.e., 14% for controls and 29% for treated rats.

Sarcomas in male rats generally were reticulum cell sarcomas in the lung or subcutaneous sarcomas; whereas, carcinomas tended to develop in endocrine organs.

In summary, there were notable increases in the incidences of malignant tumors in adult female rats given three dose levels of erythrosine by intubation. Malignant tumors tended to develop in the reproductive system. There is insufficient data available to determine why the incidence was not increased at the fourth dose level; however, that dose level may well be toxic.

It should be noted that in the results previously reported in *Fd. Cosmet. Toxicol.* rats in this study were not separated by sex; and rats dying during the first few weeks were not excluded from the results.

2. FDA Rat Feeding Study^{1,3}

Osborne-Mendel rats, 100 days of age, were housed individually and given food and water ad libitum. The basal diet was Purina Laboratory Chow. Five groups of rats were fed diets containing erythrosine (certified as FD & C Red No. 3 and containing 95% pure dye) at 0, 0.5, 1.0, or 4.0% for 86 weeks. Each group consisted of 25 males and 25 females except for the control (0%) group, which consisted of 50 males and 50 females. At the end of the treatment period, the animals were fed the control diet until the experiment was terminated at two years.

At autopsy, organs appeared normal and weights were not recorded. All rats were autopsied and some without gross lesions were discarded. In some cases, pathological examination was limited to the trunk and limbs,

³FDA memorandum entitled "Histologic examination of tissues from rats fed erythrosine for two years," from K.J. Davis to W. Hanson and S. Graham, dated March 11, 1970 (P-88-69), 23 pp.

but in the majority of rats the entire body, including the head, was examined. In addition, at the end of the 2-year study a fairly complete series of tissues from some rats in the 4% and control groups were examined microscopically for any minor morphological changes which could be attributed to long-term toxicity. In other autopsies histopathologic examination was limited to liver, testes, tumors (if present), and other organs with abnormalities.

Growth inhibition occurred only in animals given the two highest levels of erythrosine. At the end of one year, male and female rats given the 4% diet and female rats given the 2% diet showed significant growth inhibition.

The numbers of female rats ingesting erythrosine with carcinomas and sarcomas is given in Table 5 and total number of all rats ingesting erythrosine with malignant tumors in Table 6.

TABLE 5. NUMBER OF FEMALE RATS INGESTING ERYTHROSINE WITH CARCINOMAS AND SARCOMAS*

Dose (%)	No. Rats with Carcinomas	No. Rats with Sarcomas	Total No. Rats with Tumors
0	1/45 (2%)	6/45 (13%)	7/45 (15%)
0.5	1/24 (4%)	5/24 (21%)	6/24 (25%)
1.0	2/23 (9%)	4/23 (17%)	6/23 (26%)
2.0	3/24 (13%)	8/24 (33%)	11/24 (46%)
4.0	1/24 (4%)	5/24 (21%)	6/24 (25%)

*Tumors of the mammary gland and pituitary, which occurred in similar numbers of untreated and treated rats, were not included here.

TABLE 6. TOTAL NUMBER FEMALE RATS INGESTING ERYTHROSINE WITH CARCINOMAS AND SARCOMAS

Dose (%)	No. Rats with Carcinomas	No. Rats with Sarcomas	Total No. Rats with Tumors
0	1/45 (2%)	6/45 (13%)	7/45 (15%)
0.5-4.0	7/95 (7%)	22/95 (23%)	29/95 (31%)

The incidence of female rats with malignant tumors was increased in rats ingesting 0.5, 1.0, 2.0, and 4.0% (2%, 26%, 46%, and 25% respectively), when compared with the 15% incidence in untreated rats. The most striking increase was in female rats at the 2% dose level, where the incidence of carcinomas or sarcomas were also increased. The incidence of malignant tumors when all treated rats were grouped together in one group was 31% compared to 15% in the untreated female rats.

Female rats generally developed carcinomas and sarcomas of the ovary and uterus, reticulum cell sarcomas of the lung, as well as a few hemangioendothelial sarcomas.

The number of male rats ingesting erythrosine with carcinomas and sarcomas is given in Table 7 and total number of all rats ingesting erythrosine with malignant tumors in Table 8.

TABLE 7. NUMBER OF MALE RATS INGESTING ERYTHROSINE WITH CARCINOMAS AND SARCOMAS*

Dose (%)	No. Rats with Carcinomas	No. Rats with Sarcomas	Total No. Rats with Tumors
0	1/50 (2%)	10/50 (20%)	11/50 (22%)
0.5	1/24 (4%)	2/24 (8%)	3/24 (12%)
1.0	3/23 (13%)	3/23 (13%)	6/23 (26%)
2.0	3/24 (13%)	4/24 (16%)	7/24 (29%)
4.0	2/25 (8%)	5/25 (20%)	7/25 (28%)

*Tumors of the mammary gland and pituitary, which occurred in similar numbers of untreated and treated rats, were not included here.

TABLE 8. TOTAL NUMBER MALE RATS INGESTING ERYTHROSINE WITH CARCINOMAS AND SARCOMAS

Dose (%)	No. Rats with Carcinomas	No. Rats with Sarcomas	Total No. Rats with Tumors
0	1/50 (2%)	10/50 (20%)	11/50 (22%)
0.5-4.0	9/96 (9%)	14/96 (15%)	23/96 (24%)

The incidence of sarcomas, particularly lymphosarcomas, which was high in the untreated male control rats was not increased in the treated rats. The incidence of carcinomas in both the 1 and 2% dose level male was 13%, compared to 2% in the control rats. Carcinomas tended to develop in endocrine organs (other than pituitary).

In summary, the incidence of malignant tumors was increased in adult female rats ingesting four dose levels of erythrosine. This increase was most striking in the 2% dose level with an incidence of 46% compared to 15% in the untreated controls. Malignant tumors generally involved the uterus, ovary, or lung.

It should be noted that in the results previously reported in Fd. Cosmet. Toxicol. (see ¹), rats in this study were not separated by sex; and rats dying during the first few weeks of the study were not excluded from the results.

3. FDA Rat Study⁴

Charles River male and female rats, of unstated age, given 0 mg, 10 mg, or 20 mg erythrosine two times weekly, were killed after 104 weeks. There were nine rats in each group.

⁴FDA memorandum entitled "Pathology report on Charles River rats given erythrosine. Path. #1637-1960," from K.J. Davis to W. Hansen, dated Dec. 5, 1969 (P-91-69), 10 pp.

Five rats from three groups died at 33 weeks from cardiac puncture, and others from pneumonia before the end of 52 weeks. Most of the high level rats were dead by 78 weeks, and most of the low level and control rats by 104 weeks.

The incidence of female rats with tumors is given in Table 9.

TABLE 9. NUMBERS OF FEMALE RATS GIVEN ERYTHROSINE WITH BENIGN OR MALIGNANT TUMORS

Dose (mg/kg)	No. Rats with Benign Tumors	No. Rats with Malignant Tumors	Total No. Rats with Tumors
0	2/9	0/9	2/9 (22%)
10	3/9	2/9	5/9 (56%)
20	4/9	1/9	5/9 (56%)

The results of this study only suggest that the numbers of tumors in female rats given erythrosine was increased. Tumors were found predominantly in mammary gland and pituitary.

4. FDA Rat Feeding Study⁵

Groups of 12 male and 12 female weanling Osborne-Mendel rats ingested diets containing erythrosine (FD & C Red No. 3) at levels of 0, 0.5, 1.0, 2.0, or 5.0% for 104 weeks.

Tissues were examined histologically by the authors as described under Sections 1 and 2.

Growth depression was observed in male and female rats given the 5% level. Spleen weights were decreased in male rats given the 0.5, 2.0, or 5.0% dose levels and in female rats given the 5% dose level. Slight

⁵Hansen, W.H., Zwickey, R.E., Brouwer, J.B., and Fitzhugh, O.G.: Long-term toxicity studies of erythrosine. I. Effects in rats and dogs. *Fd. Cosmet. Toxicol.* 11:527-534, 1973.

caecal distension occurred in rats given erythrosine at 1% and increased with increasing dose levels. The incidence of chronic nephritis was increased approximately two-fold in rats (not separated by sex) ingesting 0.5% erythrosine, compared with that in the control rats.

The incidence of male rats with malignant tumors is given in Table 10.

TABLE 10. NUMBERS OF MALE RATS INGESTING ERYTHROSINE WITH MALIGNANT TUMORS

Dose (%)	No. Rats with Malignant Tumors
0	1/12 (8%)
1.0	2/12 (17%)
2.0	6/12 (50%)
5.0	3/12 (25%)

The results of this study suggest that the numbers of malignant tumors in male rats ingesting erythrosine was increased. Tumors were predominantly lymphosarcomas. Chronic nephritis developed in the rats ingesting the 0.5% dose level, and may be responsible for the absence of malignant tumors in those animals.

5. FDA Gerbil Intubation Study⁶

Male and female gerbils, 6 months of age, ingested 0, 1.0, 2.0, or 4.0% erythrosine (FD & C Red No. 3) in laboratory chow for approximately 19 months.

All tissues were evaluated histopathologically from animals ingesting the highest dose level and from the untreated controls. A lesser number of tissues (partial screen) was examined from gerbils ingesting the lower

⁶Collins, T.F.X. and Long, E.H. *Fd. Cosmet. Toxicol.* 14:233-248, 1976.

dose levels of erythrosine, Thyroid glands were examined from gerbils at all dose levels.

"Effects induced by erythrosine which may be regarded as somewhat deleterious were: (1) anemia at all 3 levels in the females, and at 4% in the males also, (2) dose-related inhibition of growth, and (3) a two-fold, dose-related change in the thyroid at all levels of treatment, consisting of an increase in size of the large follicles lined by simple squamous epithelium with a concomitant proliferation of small hyperplastic follicles containing sparse colloid and lined by simple cuboidal to simple columnar epithelium."

The histopathology was performed by Long for the FDA. Her conclusions were: "There was no evidence of an effect of erythrosine on tumorigenesis, as there was no significant difference in regard to the incidence of either total tumors or any single type of tumor among the four dosage groups."

There was 17% (4 of 30) malignant tumors in gerbils given 1% erythrosine, compared with 0 of 59 (0%) controls. Three of the carcinomas in treated gerbils were intestinal. The incidence of gerbils with malignant tumors is given in Table 11.

TABLE 11. TOTAL NUMBER OF GERBILS GIVEN ERYTHROSINE BY INTUBATION WITH MALIGNANT TUMORS

Dose (%)	Total	Tumors
0	0/59 (0%)	-
1	4/30 (17%)	1 carcinoma kidney 1 islet cell carcinoma pancreas 1 intestinal carcinoma 1 granuloma cell carcinoma ovary
2	0/28 (0%)	-
4	2/29 (6%)	2 intestinal carcinomas
TOTAL	6/87 (7%)	

Tissue sections from this study have not been reviewed. Conclusions made from this study are for 6-month gerbils and not for younger gerbils. It is strongly recommended that young animals be used for chronic carcinogenicity studies.

6. FDA Gerbil Intubation Study⁷

Male and female gerbils, approximately 6 months of age, were given intubations of erythrosine (FD & C Red No. 3) two times weekly for approximately 19 months. Dose levels were 0, 200, 750, or 900 mg/kg. Those on the 900 mg level actually received 1,200 mg/dose for the first three months.

All tissues were evaluated histopathologically from animals given the highest dose level and from the untreated controls. A lesser number of tissues (partial screen) was examined from gerbils receiving the lower dose levels of erythrosine. Thyroid glands were examined from gerbils of all dose levels.

The histopathology was done by Willigan for the FDA. His conclusions were: "Changes attributable to erythrosine were not observed in any of the tissues evaluated microscopically."

Tissue sections from this study have not been reviewed. The gerbils were 6 months of age at the start of the treatment with erythrosine; therefore, conclusions made from this study are for 6-month old gerbils and not for younger gerbils. It is strongly recommended that young animals be used for chronic carcinogenicity studies.

7. FDA Dog Feeding Study⁵

Young beagle dogs ingested 0, 0.5, 1.0, or 2.0% erythrosine (FD & C Red No. 3) in laboratory chow for 104 weeks. There were 3 males and 3 female dogs at each dose level.

⁷Willigan: Erythrosine Toxicity Study. Summary of Histopathological Observations. Project P-180-70.

Dogs were killed and autopsied. Most tissues were evaluated histopathologically from the 6 dogs ingesting 2% erythrosine and from the 6 control dogs. "Although these 12 dogs received the more thorough microscopic examination, tissues from other dogs were also examined."

The authors concluded that "there were no compound-related histological effects, and only minor incidental abnormalities were seen."

Histologic sections from this study were not reviewed. Two-year carcinogenicity studies in dogs are inadequate, and the numbers of dogs used were small. Referring to age of the dogs as "young" is not satisfactory.

8. Comments

The FDA rat intubation and feeding studies of erythrosine (Sections 1 and 2) are the best designed and carried out studies; therefore, the results can be considered as the most important. The incidence of malignant tumors was increased in adult female rats ingesting erythrosine, particularly in the reproductive system.

The workers analyzing the data failed to separate the results by sexes. They did not separate benign from malignant tumors. Small numbers of histological sections were examined from lower dose treated rats thereby overlooking microscopic tumors. The criteria for distinguishing between benign and malignant tumors are not acceptable by today's standards, i.e., tumors need not have "invaded surrounding tissues or metastasized to more distant locations."⁵

The other FDA rat studies (Sections 3 and 4) are not adequate studies because of the small numbers of animals per group, incomplete histopathologic examinations, and inadequate examinations of the results. These studies do not demonstrate the carcinogenicity of erythrosine. Furthermore, they do

not show that erythrosine is not carcinogenic. They do not show that erythrosine is not toxic as concluded: "most results have been negative."¹

The studies mostly were concerned with the carcinogenic effects of erythrosine in adult rats. The results in the gerbil studies are applicable only for 6-month old gerbils. In view of the enlarged thyroid glands, studies in young gerbils would have been indicated. The duration of the dog studies was not long enough to rule out carcinogenicity. Data concerning carcinogenicity in weanling rats would be desirable; however, only one inadequate study used weanling rats (Section 4).

LETTERS; ADDITIONAL TESTIMONY SUBMITTED FOR THE RECORD; ADDITIONAL INFORMATION

January 31, 1977

FOOD CHEMICAL NEWS

FDA REGULATES HALF OF BACKLOGGED CHEMICALS IN NCI TESTING PROGRAM

Of the nearly 200 chemicals backlogged in the National Cancer Institute's Carcinogen Bioassay Program, 126 are regulated by the Food and Drug Administration as food additives, both direct and indirect, as ingredients of color additives, or as new animal drugs or ingredients of animal drugs.

According to an analysis of the chemicals in the NCI program for which studies have been completed but not yet reported, FDA has some regulatory control over more than half of the substances. Thirty-seven of the chemicals on the list are pesticides regulated by the Environmental Protection Agency.

Other chemicals on the NCI list are used in dyes, cosmetics and other substances which have no direct use in foods, and have the potential to contaminate only through accidental or environmental routes. Few are believed to have the potential to become serious food contaminants.

Only nine on the list are regulated as direct food additives. These are:

- (1) L-Tryptophan regulated under §121.1002; (2) Calcium disodium EDTA under §121.1017; (3) Disodium EDTA under §121.1056; (4) Tetrasodium EDTA under §121.1088; (5) BHT under §121.1034; (6) NTA under §121.1088; (7) Trisodium NTA under §121.1088; (8) 1,2-Dichloroethylene (ethylene dichloride) under §121.1040; and (9) Sodium nitrite under §121.1064 and §121.1230.

In addition to the additives directly added to food there may be residues from the use of other additives which are on the NCI list.

Azodicarbonamide (§121.1085), which is used in flour, reacts to form biurea, which is the dimer of urea. Urea is on the NCI list.

Thiourea intermediates arabinosylcytosine used as a food adduct, and some solvents used in processing are also on the NCI list. Indirect additives include:

- (1) Dioxane -- permitted for use in adhesives; (2) NTA -- permitted as a boiler water additive; (3) Tetrachloroethylene -- permitted as a foaming agent for polystyrene; (4) 1,1,2-Trichloroethane -- permitted for use in adhesives; (5) β -Nitrostyrene -- basic polymer in paper for dry food; (6) Iodoform -- permitted for use in adhesives; (7) Piperonyl butoxide -- permitted for insect control in paper bags for dry food; (8) Phthalic anhydride-- permitted for use as a modifier for rosins, reactant for polyurethane, reaction control reagent, retarder for rubber articles; (9) Toluene diamine -- permitted for use as an antioxidant in rubber articles; (10) BHT -- Prior sanctioned for use in polyolefin and saran coatings; (11) Ethyl tuads (Tetraethylthiuramdisulfide) -- permitted for use as a rubber accelerator; and (12) Dibutyltin diacetate -- permitted for use as a catalyst, which may be used as the food-contact surface of articles intended for contact with bulk quantities of dry food. No food uses.

Drugs for which NADA's have been approved include (1) 2-Amino-5-nitrothiazole; (2) Daraprim; (3) Dichlorvos; (4) Dibutyltin dilaurate; (5) Prednisone; and (6) Niathizide.

January 31, 1977

FOOD CHEMICAL NEWS

Components of animal drugs on the NCI list include: (1) Chloroform; (2) EDTA; (3) Uridifirn; (4) Carbon disulfide; (5) Hexachlorophene; (6) Urea; and (7) BHT.

Among the ingredients of color additives on the NCI list are: (1) Aniline; (2) EDTA; (3) o-Anthranilic acid; (4) Phthalic anhydride; (5) Sodium nitrite; and (6) Urea.

PPI DENIES INTENTIONALLY FATHERING ACIDIFIED FOODS GMP'S

The Pickle Packe.. International has protested that although "the record will reflect that this proposal had its genesis in a petition submitted by PPI," the Food and Drug Administration proposed good manufacturing practice regulations for acidified foods "differs greatly in two major respects."

"First," PPI explained, "it proposes that the GMP's be applicable to all 'Pickled, Fermented, and Acidified Foods' rather than to pickled vegetable products only; Second, it enlarges the concept of GMP's to encompass not merely protection of the public health, but also the 'cosmetic' aspects of food production."

PPI recommended that the "only feasible and necessary GMP is the control of pH," adding: "There is no need to talk of salinity, or specific gravity, or heat, or cold, or any other method of preservation."

As for applying the emergency permit provisions to acidified foods, PPI suggested that FDA not impose these requirements "until the Congress grants the Commissioner the power ..."

"Since 1938, the Congress has seen fit to give to the Commissioner of Food and Drugs varied, and ever-increasing power," PPI admitted, continuing, "The Congress has not, however, seen fit to give the Commissioner the power to require:

"(1) Registration of food processing plants; (2) Establishment and filing of 'scheduled processes'; (3) Maintenance of records of equipment calibration and testing; (4) Specific food manufacturing processes; (5) Product coding; (6) Maintenance of processing and production records; (7) Setting aside and reworking certain batches of product; and (8) Sending supervisory personnel to 'approved' schools."

"Successfully exceeding one's authority once is not a license for doing so again," PPI commented, arguing that while a temporary emergency permit regulation may be issued, "... the Commissioner cannot proceed beyond that and impose requirements that have no relation to the public health."

"No person in this country has ever been made ill because a food manufacturer failed to register, or to file a scheduled process, or to keep detailed records of various plant activities," the group maintained.

"... even if some such requirements could be deemed necessary, that is only one-half the requisite finding," PPI insisted, continuing:

December 16, 1976

Dr. Paul F. Hopper
Acting Executive Director
Food Safety Council
c/o General Goods Corporation
250 North Street
White Plains, New York 10625

Dear Dr. Hopper:

The Senate Small Business Committee has scheduled hearings on January 13 and 14, 1977, on the marketing, regulation and safety of food additives.

The Committee would be grateful if you would appear at 9:30 a.m. on Friday, January 14, in Room 1318 Dirksen Senate Office Building to discuss the objectives of the Food Safety Council as they relate to the following subjects: a) testing of food additives (the state of the art); b) independent testing systems; c) the desirability for new statutory suspension authority for regulatory agencies to utilize when safety questions about additives arise; d) benefit-to-risk considerations in reaching regulatory decisions; e) research into alternatives for existing additives that pose safety questions; and f) any other aspect of the subject that you believe will be helpful to the Committee.

We would appreciate receiving 25 copies of your statement by January 7, 1977, and 75 copies on the date of your appearance before the Committee.

If there are any questions or if our staff can be of assistance, please don't hesitate to contact Benjamin Gordon at the office of the Senate Small Business Committee, 224-8489.

Sincerely,

GAYLORD NELSON
Chairman

GN:bgk

FOOD SAFETY COUNCIL, INC.

Columbia, Maryland

December 29, 1976

Senator Gaylord Nelson, Chairman
Select Committee on Small Business
United States Senate
Washington, D.C. 20510

Dear Senator:

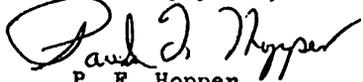
Thank you very much for your kind invitation of 12/16 inviting the Food Safety Council to appear before the Senate Small Business Committee on Friday, 1/14. As you may know, the Food Safety Council is a new organization and has just recently (11/17) elected its Board of Trustees.

While we would very much like the opportunity to appear before the Committee to discuss the goals and objectives of the Food Safety Council, we feel that the organization is still so new that it would be premature for us to participate at this time.

We would, however, appreciate the opportunity to submit a brief statement for the record and perhaps if the hearings are continued at some future date, the Food Safety Council would be in a better position to appear before this committee.

Again, thank you for the invitation to participate on 1/14; we will be following the progress of your hearings and reporting on them to our Trustees.

Very truly yours,



P. F. Hopper

Interim Executive Director

PFH:pms



Robert W. Harkins
Vice President, Scientific Affairs

February 24, 1977

The Honorable Gaylord Nelson
United States Senate
Washington, D. C. 20510

Dear Senator Nelson:

Enclosed is our statement on the use, regulation, and safety evaluation of food ingredients. We respectfully request that this statement be included in the record of the January 13-14, 1977 hearings held by the Senate Select Committee on Small Business.

We appreciate the opportunity to submit this material to the Committee.

Sincerely,

A handwritten signature in dark ink that reads "Robert W. Harkins". The signature is written in a cursive style with a large initial 'R'.

Robert W. Harkins, Ph.D.
Vice President
Scientific Affairs

RWH/ew

Enclosure

cc: Members of the Select Committee
on Small Business
Judy Robinson
Benjamin Gordon

STATEMENT OF ROBERT W. HARKINS, PH.D.
VICE PRESIDENT, SCIENTIFIC AFFAIRS
GROCERY MANUFACTURERS OF AMERICA, INC.

FOR THE

SENATE SELECT COMMITTEE ON SMALL BUSINESS

FEBRUARY 24, 1977

The Grocery Manufacturers of America, Inc. (GMA) is a national trade association representing the interests of approximately 140 manufacturers of food and grocery products sold throughout this country. As such, GMA and its membership are interested in all phases of the marketing, regulation, and safety of food ingredients. We appreciate this opportunity to contribute the viewpoint of the regulated industry on the use of food ingredients.

Overview

It is important, at the outset, to understand the statutory provisions of the Federal Food, Drug, and Cosmetic Act under which the Food and Drug Administration regulates the various components of our food supply. For purposes of regulation under the statute, there are basically two types of food: (1) unprocessed agricultural products and (2) processed food.

An unprocessed agricultural product -- such as raw milk, and fruits and vegetables which are washed but otherwise not processed -- is subject only to the safety provisions in section 402(a)(1) of the Act, under which it may lawfully be marketed unless it contains a "poisonous or deleterious substance which may render it injurious to health." If such a substance is not an added substance, the food is not considered adulterated if the quantity of the substance does not "ordinarily" render it injurious to health. As long as raw agricultural produce remains unprocessed, it is not subject to any

of the statutory provisions relating to food additives. Thus, unprocessed agricultural products are required to meet a relatively low statutory standard for safety.

In contrast, once any agricultural produce is processed in any way or is incorporated in any processed food -- for example, when raw milk is pasteurized or homogenized or dried or made into butter, or when apples are made into apple sauce, or when any fruit or vegetable is canned -- far more complex and stringent statutory provisions apply. The status of each component of the resulting processed food must then be analyzed to determine compliance with sections 402 (adulterated food), 406 (tolerances for poisonous ingredients in food), and 409 (food additives) of the Act. Of major importance, each component of the food must be analyzed to determine compliance with the food additive requirements. The agricultural produce component of the processed food is subject to analysis under the food additive requirements to the same extent as any chemically synthesized component.

In order to be included lawfully in any processed food, every component must meet the statutory requirement of: (1) being generally recognized as safe (GRAS), or (2) being subject to a sanction or approval for use in food granted by FDA or USDA prior to September 6, 1958, or (3) being subject to a food additive regulation promulgated by FDA, or (4) if used for color purposes, being approved by FDA for provisional or permanent use by a color additive regulation. This statutory requirement does not distinguish between natural and synthetic components. And since most of the food that we eat today (except fresh meat, fruit, and vegetables) is processed in one way or another, it means that virtually all components of our food supply, whether produced by nature or synthetically, are subject to the same legal standards for safety.

The present legal requirements are undoubtedly not well understood. Many people believe that components of our food supply that are derived from

agricultural produce are in some way exempt from compliance with the food additive requirements of the law. This simply is not true. Although an apple is not subject to the food additive requirements when sold as fresh fruit, it is fully subject to analysis under the food additive provisions of the law the minute that it is processed in any way, for example, when it is made into applesauce.

In discussing food safety, it is important that the term "food additive" be used properly, in the way that it has been defined by Congress in section 201(s) of the Federal Food, Drug, and Cosmetic Act. A "food additive" is any food ingredient -- including, as we have already noted, any food ingredient derived solely from natural origin as part of agricultural produce -- which is not either GRAS or subject to a prior sanction. A food additive may be either natural or synthetic in origin, just as other food ingredients which are not food additives may be either natural or synthetic in origin. The term simply encompasses all those components of the food supply which have not achieved the status of general recognition of safety or were not approved by FDA or USDA for food use prior to 1958. Many synthetic chemicals used as food ingredients are, of course, not food additives; and a number of natural components of our food supply are regulated as food additives.

Indeed, it is a paradox that we have less knowledge about the safety of food components that are not food additives than we do about the safety of food additives, because the statute requires specific testing of food additives before they may be approved for use in food whereas specific testing of other food components is not required. Every food is composed of hundreds of individual chemical substances. The composition of each complex chemical mixture which we call a "food" is imprecisely known, and the toxicological manifestations of these individual chemicals, let alone the combination, are simply not available for most food components. The common conception that food components

which are not food additives are somehow "better" or "safer" than food additives is therefore demonstrably false.

The popular belief that chemically synthesized food ingredients are inherently less safe than those of agricultural origin is equally false. The list of natural poisons is impressively long, and many synthesized chemicals have been proved to be entirely safe for food use.

Modern chemistry has permitted the food industry to produce by synthesis many chemicals that are also produced in nature. Perhaps the best examples are the vitamins that are so commonly consumed today. Virtually all vitamins added to food or consumed as pills are chemically synthesized, but are equally effective and no less safe than their natural counterparts. It is likely that well over 99 percent of all chemically synthesized food components are identical to chemicals that are also found in food of agricultural origin. Man adds only a relatively few substances directly to food which are not also found in nature.

The "GRAS" List

Particular emphasis has been placed, in recent years, on the so-called "GRAS list" published in the Code of Federal Regulations by FDA in 1959 and 1960. This is an extremely limited list of GRAS food components, as FDA has itself acknowledged. Section 121.101(a) of the FDA regulations states:

It is impracticable to list all substances that are generally recognized as safe for their intended use. However, by way of illustration, the Commissioner regards such common food ingredients as salt, pepper, sugar, vinegar, baking powder, and monosodium glutamate as safe for their intended use.

The regulation then goes on to state that the GRAS list includes some, but obviously not all, GRAS ingredients.

In April 1958, in testimony before the House of Representatives during consideration of the legislation that later became the Food Additive Amendments of 1958, the then Commissioner of Food and Drugs included in a list of "chemical food additives" the following substances which FDA would regard as GRAS for use in food:²⁵

Brandy	Lemon Juice
Butter	Margarine
Coffee	Molasses
Corn Oil	Mustard
Cream	Olive Oil
Dry Skim Milk	Wine
Lard	

None of these illustrative GRAS food ingredients appears on the FDA GRAS list or in any other list of GRAS substances. Similarly, peas, carrots, potatoes, apples, beef, and other common food ingredients of agricultural origin that are also GRAS do not appear on the FDA GRAS list. It is impossible to determine exactly how long a GRAS list would be if it were to contain all of these GRAS food components.

Beginning in 1969, the Food and Drug Administration undertook to review the safety of the food ingredients on its published GRAS list. Since there are so many GRAS food ingredients, this decision reflected the practical conclusion that any review of GRAS substances must begin somewhere, and the published GRAS list was as good a place to begin as anywhere else. As part of its review, FDA contracted with the National Academy of Sciences to survey the industry for use levels of the substances on the published GRAS list.

Continuing Support for the Orderly Review of Food Ingredients

GMA and other major food-based trade associations have given vigorous support to the review of the safety of food ingredients on the published FDA GRAS list. In May 1971, twenty-one trade associations joined together to promote and sponsor a briefing session at which the National Academy of

Sciences launched a user and producer survey of GRAS substances. Mr. William O. Beers, President of Kraftco Corporation, said at that time:¹

We need a comprehensive, orderly review of GRAS substances not only to assure the continued safety of food ingredients, but especially, to forestall conditions which could lead to a loss of public confidence in our food supply.

Therefore, we all have something to gain by assisting in this review. We will not only benefit by an accurate, scientific assessment of the safety of food ingredients, but we will also reassure the public that both industry and government are working toward a common objective -- that of continuing protection of the well being of the American consumer.

Industry remains fully supportive of an orderly, systematic review of the safety of food ingredients. In preparation for the survey currently being undertaken by the National Academy of Sciences on the use of food ingredients (Phase III of the GRAS list survey), GMA participated in a briefing program in December 1975. The text of this presentation was published¹⁹ under the title "Incentives for Further Industry Cooperation and Participation." The first reason invoked for participation in the survey was "a deep sense of corporate responsibility." While there are other significant reasons for participation in the survey, the continued protection of the public is far and away the most important justification for this activity.

Number of Food Ingredients Used in Food Production

For many years, questions have been raised about the number of food ingredients that comprise the food supply. To the best of our knowledge, a single,

comprehensive listing of all of the individual food ingredients does not exist. In the Code of Federal Regulations, the Food and Drug Administration lists the following numbers of GRAS food ingredients, food additives, and color additives for direct use in food production:

GRAS Food Ingredients

251 nonflavor substances³
 223 natural flavorings and spices⁴
 26 synthetic flavorings⁵

500 total GRAS Food Ingredients

Food Additives

187 nonflavor substances⁶
 3 nonflavor substances (interim basis)⁷

190 total Nonflavor Additives

131 natural flavorings⁸
 728 synthetic flavorings⁹

859 total Flavoring Additives

Color Additives

31 permanently approved color additives¹⁰
 3 provisionally listed color additives¹¹
 3 provisionally listed color lakes¹¹

37 total Color Additives

The Code of Federal Regulations also lists some, but not all, ingredients that are indirectly added to food, i.e., those substances permitted in food packaging materials, food contact surfaces, and other applications where they may become a component of food. FDA has referred to an estimated 10,000 indirect additives,¹⁷ but this appears to be largely speculative and the number could actually be much larger.

Thus, the number of substances which comprise the food supply is quite large -- more than 1000 agricultural products, approximately 2000 food components, and 10,000-plus indirect additives. It would be a formidable task indeed to subject each of these 13,000-plus substances to detailed toxicological testing and analysis at this time.

Level of Use of Food Ingredients in Food Production

According to figures from the U. S. Department of Agriculture, the U. S. per capita consumption of food totaled 1297 pounds per year -- or 3.6 pounds or 56.9 ounces per day -- in 1973.³⁰ Recent data permit the following approximate breakdown of this daily food consumption:^{18,29}

	<u>Per Capita Use (ounces/day)</u>	<u>Percentage of Diet (%)</u>
Major food components of natural or agricultural origin, including the following categories:	<u>56.42</u>	<u>99.2</u>
Apples, potatoes, meat, eggs, etc.	50.72	89.2
Sugar	4.47	7.9
Salt	0.66	1.1
Corn syrup and dextrose	0.57	1.0
32 common food ingredients*	<u>0.40</u>	<u>0.7</u>
All other functional ingredients of natural and synthetic origin added at low levels	<u>0.04</u>	<u>0.1</u>
TOTAL	<u>56.86</u>	<u>100.0</u>

*These ingredients and their functions are listed in Appendix A.

Although flavoring agents are the most numerous ingredients used in food production, such substances are used in very small quantities. Many are, of course, of natural agricultural origin, and others are chemically identical to natural flavors. Results from a 1971 survey conducted by the Flavor and Extract

Manufacturers Association on over 1400 flavors indicated that 71 percent of these flavorings were used in food processing at levels less than 1000 pounds annually, or less than 2.7 pounds per day.¹⁶ This national use level corresponds to 0.000000013 pounds or less per capita per day.

Increased Complexity of Toxicological Testing Requirements

Over the past several decades, the requirements for toxicological evaluation of food chemicals have become more complex and elaborate. In 1940 it was not uncommon to call a study of 30 days' duration a chronic toxicity study. Total testing of safety of food chemicals and drugs was commonly conducted in a few rats, a few rabbits, and a few mice, which was considered an adequate toxicological data base at that time.¹²

By the late 1950's, safety testing of food chemicals had become more elaborate and more formalized. A 1958 World Health Organization (WHO) report²¹ distinguished between three types of toxicity studies: acute, short-term, and long-term (chronic). Acute toxicity studies included testing both sexes in three species of animals (one a non-rodent species). Numbers of animals required were relatively small and were based on the statistical precision desired in the estimated LD₅₀ for the substance tested. Short-term toxicity studies required two species of animals (one a non-rodent), 10-20 animals of each sex at each dosage level in the test, and usually a 90-day observation period. Chronic toxicity testing was usually conducted in the rat, with 25 or more animals of each sex at each dosage level in the test. The total period of observation was usually 12 to 18 months.

In 1959 the staff of the FDA Division of Pharmacology published a major review of the then existing requirements for toxicological testing of chemicals.¹⁴ This review incorporated the principles of the WHO report of the prior year and provided additional information on the techniques used in the interpretation of

toxicologic findings in animals. It was a major milestone in toxicologic testing in the United States and served as a guideline for such testing for a number of years.

Throughout the 1960's and 1970's further elaboration of toxicological testing has taken place. Chronic toxicity testing sometimes included both rodent and non-rodent species, and the period of observation in non-rodents frequently extended for half a decade or more. Further attention was directed toward appraisal of teratogenicity, mutagenicity, and embryotoxicity.²² A National Academy of Sciences report gives a good summary of the status of the general requirements for toxicologic testing of food chemicals as of 1970.¹⁵ Because of the complexity of this type of testing, increasing emphasis is being placed on the development of rapid, in vitro screening tests, particularly in the testing for carcinogenesis.^{2,20,23,24}

It is clear that the past 35 years have seen a major change in the accepted requirements for toxicologic testing of food ingredients, from relatively simple testing to a very complex battery of testing procedures. These current procedures are designed to elicit not only the conventional adverse reactions that can occur to a chemical or drug but also the more subtle and complex expressions of toxicity that may only be observed over the entire life span of the animal, for example, in carcinogenicity testing or in the multigeneration reproduction studies. We anticipate that the next 35 years will produce similar improvements in toxicity testing. Toxicology is, of course, a very dynamic field, and we doubt that new types of testing will ever cease to be discovered.

The battery of testing procedures currently utilized in the testing of food ingredients requires both a considerable period of time (minimum of three years) and substantial funding (approximately \$500,000) to complete. It is for these reasons that rapid and less costly in vitro screening procedures are

receiving so much attention at the present time, not just in an effort to reduce the time and cost of testing, but also to utilize the available testing facilities of the country most effectively. The screening tests, however, are not now capable of replacing in vivo studies in animals. Their use in regulatory decision making should be as a supplement to, not replacement for, conventional studies.

National Constraints on Safety Evaluation

There are several limitations on our country's capability to undertake safety studies on all food ingredients. The major limitations are:

- (a) limited number of qualified scientists, particularly pathologists;²⁶
- (b) limited number of qualified laboratories, both inside and outside government;
- (c) competing priorities for testing other substances;
and
- (d) economic burden.

An examination of the limitations on cosmetic safety testing, which are analogous to those affecting food ingredient safety evaluations, is illustrative. Arthur D. Little, Inc., conducted a study²⁶ to evaluate the impact of legislation pending in 1974 that would have required safety testing of all cosmetics and ingredients used in cosmetics -- a much smaller number of ingredients than are used in food. Based upon the estimated 1340 qualified pathologists practicing in the United States in 1974, Arthur D. Little concluded that safety testing of the more than 25,000 cosmetic products and ingredients on the market would take at least 30 years at an estimated cost of \$6.5 billion. This proposed legislation would have resulted in a staggering use of laboratory animals -- a minimum of 60 million mice, 38 million rats, 6 million rabbits,

and 0.5 million dogs. The report concluded that this proposed cosmetic safety testing "would thus almost certainly have a serious adverse impact on other major research activities, such as the cancer and heart programs, new drug and food additive testing, etc., which compete for the same relatively limited number of qualified scientific personnel and facilities."

The severe limitations imposed by shortages of trained scientists and facilities, which can only be slowly corrected, are reasons why the impracticality of testing every food ingredient in every possible toxicological test protocol poses a major societal dilemma. Safety judgments can be based on experience with common food use and on known toxicity information without requiring repeated, periodic studies of food ingredients with the newest toxicological testing procedures.

Cost is also a significant factor. FDA has estimated that the total cost of the GRAS list review program has been approximately \$18 million to date.¹⁷ For this expenditure, FDA has been able to reach, in its judgment, the half-way point in the review of 439 nonflavor GRAS substances, a program initiated in 1969. One needs to compare the \$18 million expenditure, the seven years, and roughly 220 compounds reviewed with the total number of food ingredients already discussed above. If FDA could complete the review process for the remaining compounds in half the time that it took for the review of the first half of the GRAS list, and at the same rate of economic cost, it would require more than our lifetime and hundreds of millions of dollars.

By and large, the work done to date by government and industry in reviewing the safety of existing food ingredients is based on a compilation of available data. The generation of new laboratory data -- for example, chronic feeding studies in rats, dogs or other species -- will add significantly to the costs of the review of the safety of food ingredients. Recently, FDA outlined a

comprehensive program to resolve the status of provisionally listed color additives.¹³ Appropriate scientific investigations must be undertaken on about 30 color additives and data must be submitted to FDA according to a prescribed schedule before final decisions will be made on the status of these colors. It is estimated that it will take four years and \$3.2 million to conduct chronic toxicity feeding studies on just eight of these colors, each of which has already been evaluated in at least two species during earlier tests.

The "Food Lag"

According to the President's Science Advisory Committee report, there has been an overall decline in the number of new chemical entities introduced each year as intentional food additives.²⁹ The reason for this "food lag" is the increased regulatory requirements (i.e., the Food Additive Amendments to the Federal Food, Drug, and Cosmetic Act) that must be met for approval of a new food ingredient.²⁸ Few companies are willing or able to spend upwards of \$500,000 per compound to conduct, over a period of three to ten years, the required series of toxicological tests needed to support a food additive petition.²⁷ Unless a company receives a patent on a particular chemical, once the substance meets FDA approval any company is free to manufacture it. Furthermore, once a company makes such a financial investment on toxicological testing, there is still the possibility that FDA will delay acceptance for months or years or reject the food additive petition.

At a time when the world is deeply concerned about its ability to feed an ever-growing population, we should be very concerned indeed about national policies that discourage innovation in food technology. This is the time when new methods of food production and processing should be advanced as a national priority.

Essentiality of Setting Priorities

When setting priorities for safety evaluation of food ingredients, two broad overlapping areas of concern must be recognized: (1) the total universe of chemicals in man's environment of which food ingredients are a small and relatively well-defined segment, and (2) the relative potential hazard of the individual food ingredients.

Foods, drugs, cosmetics, medical devices, toxic chemicals, pesticides, environmental contaminants, and an enormous number of consumer products all pose a risk of hazard to man. Society must set a priority for food safety evaluation within this broad context, taking into consideration the available resources for toxicological evaluation -- qualified scientists, testing facilities, and funds. Unfortunately, there is today no organized effort within government or society at large to rank these hazards in order to set priorities for toxicological testing and evaluation. In an effort to test first those materials which may pose the greatest risk to man, we need an overall assessment before national commitments are made for food ingredient safety testing.

Achieving a comprehensive, orderly review of the safety of food ingredients and reassuring the public that both industry and government are working together toward a common objective are mutual goals to which the Congress and the food industry need to strive. We appreciate this opportunity to submit comments to the Committee on the use, regulation, and safety evaluation of food ingredients.

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APPENDIX A

FUNCTIONS OF 32 COMMONLY USED FOOD INGREDIENTSFlavoring Agent/Flavor Enhancer

Monosodium glutamate
Mustard
Black pepper
Hydrolyzed vegetable protein

Stabilizer/Thickener - imparts or maintains the desired texture, consistency and thickness in foods

Sodium caseinate
Acacia
Modified starch

Leavening Agent - produces a gas that lightens dough or batter

Yeasts
Monocalcium phosphate
Sodium aluminum phosphate
Sodium acid phosphate

pH Control Agents - controls the acid-alkaline balance in foods

*Sodium carbonate
*Calcium carbonate
*Dicalcium phosphate
*Disodium phosphate
Sodium bicarbonate
Hydrogen chloride
Citric acid
Sulfuric acid
Sodium citrate
Sodium hydroxide
Acetic acid
Phosphoric acid
Calcium oxide

*Also acts as leavening agent

Emulsifier - permits dispersion of tiny particles or globules of one liquid in another liquid

Lecithin
Mono- and diglycerides

Preservative - inhibits bacteriological spoilage of foods

Sulfur dioxide

Firming Agent - produces desirable crispness or texture in foods

Calcium chloride

Processing Aid - assists in filtering or removing unwanted color

Calcium sulfate

Effervescent - causes bubbles when escaping from a liquid

Carbon dioxide

Humectant - retains moisture in foods

Sodium tripolyphosphate

Coloring Agent

Caramel

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February 23, 1977

The Honorable Gaylor Nelson
 Chairman, Subcommittee on Employment, Poverty
 and Migratory Labor
 221 Russell Senate Office Building
 Washington, D.C. 20510

Dear Senator Nelson:

I wish to present some comments on misrepresentations and important omissions in the statement submitted to your committee by the Food and Drug Administration,

The statement that the Wisconsin study "was a carefully designed study which represented the first systematic attempt to test the hypothesis in well controlled settings" is inconsistent with the record, while the statement "the preliminary findings of the researchers show no significant overall effect from the Feingold diet as measured by classroom behavior and by parents" is contrary to the data reported by the Wisconsin investigators.

The first controlled study was that funded by the National Institute of Education under a contract awarded to Dr. C. Keith Conners of the University of Pittsburgh. The Conners study reported in Pediatrics, volume 58, number 2, August 1976, pp 154-166, states the following: "The results of this study strongly suggest that a diet free of most natural salicylates, artificial flavors and artificial colors reduces the perceived hyperactivity of some children suffering from the hyperkinetic impulse disorder."

To label the Wisconsin study as "well controlled" is contrary to the facts. None of the children in this study in the 6 to 12 years of age group were under supervision or control to assure dietary compliance at school.

In support of this fault in the experimental design of the Wisconsin study, I submit the following statement by Mr. John Wacker, a member of the National Board of Directors of the Association for Children with Learning Disabilities (ACLD):

I accidentally discovered that aspirin gave our LD daughter some severe behavioral problems, so I did considerable research on the Feingold theory. Using my company's WATS line, I talked to dozens of parents and professionals over the

USA and was amazed to hear of the sometimes almost miraculous results of the Feingold diet with hyperactive children.

I prepared an article entitled "Eliminating the Additives" based on some of these interviews. However, before it was published, I came upon a report in the Medical World News which described a "primarily negative" report on Feingold's theory by J. Preston Harley. Copy of that report is attached. Since 14 fathers and 13 mothers -- or about 37 percent -- rated their sons as improved, the "negative" terminology seemed strange. Even Dr. Feingold claims the diet will only help from 30 to 50 percent of the children. The article mentioned that the "Food Research Institute" had funded the study so I tried to track down the Institute through Washington and New York sources. Unsuccessful at that, I called Dr. Harley. He said the Institute was part of the University of Wisconsin and was funded primarily by the food industry. I asked him about supervision during the schoolday of the 6 to 12 year old participating children to insure adherence to the diet and make certain they did not drink soft drinks, trade lunches, eat items from vending machines, etc. -- any of which, of course, would have negated the research effort. He said no one supervised them and the research program relied entirely on statements made by the children each night as to what they had eaten during the day. I noted that lying is a frequently noted symptom of learning disabilities. However, Dr. Harley said that all the children were very interested in the study and were considered truthful. Further investigation on my part led me to the attached news item, headlined "public nutritionists' ties to industry told", which mentions the University and the Institute. I also have read Feingold's rebuttal of the Wisconsin "negative" report, summarized in the attached article from Food Chemical News. I went ahead and printed my article, and a copy is enclosed. The first case history noted is that of my daughter.

The FDA report fails to indicate the following very important conclusions reported to the Assistant Secretary for Health:

It is the opinion of the group that these studies have neither proven nor disproven the hypothesis that a diet free of artificial food colors and flavors reduces the symptoms in a significant number of children with the hyperkinetic behavior syndrome. The group feels, however, that the evidence taken as a whole is sufficient to merit further investigation into the relationship of diet and the hyperkinetic syndrome.

A study restricted to children 1 to 6 years of age, focussing on the colors only, has been funded under a contract with the FDA. This program is in progress under the direction of Dr. Hicks Williams, Chief of Pediatrics at the Santa Clara unit of the Kaiser-Permanente Medical Care Program; Dr. Sheldon Margen, professor of human nutrition at the University of California at Berkeley, California; and Dr. Bernard Weiss, professor of behavioral toxicology at the University of Rochester, New York, as coinvestigators.

Dr. Connors is continuing his studies at the University of Pittsburgh under a grant from NIMH.

A pilot study reported in the Medical Journal of Australia for July 17, 1976 is appended.

A letter to the editor of the Medical Journal of Australia by Doctors Cook and Woodhill, supporting the ethics and morality of clinical application of the Kaiser-Permanente Diet is also attached.

I am enclosing a reprint of a recent report in the Journal of Learning Disabilities in which I extend the application of dietary intervention to individuals with seizures, retardation and autism.

Approximately 100 parents associations have sprung up in this country, organized spontaneously by parents whose children have responded favorably and oftentimes dramatically to dietary management. A similar movement is in progress in Canada and in Australia. The parents meet once or twice a month for the exchange of experiences, as well as menus, recipes and lists of permissible foods in the local markets. The parents groups are already exerting considerable influence upon the school luncheon programs as well as the general food supply.

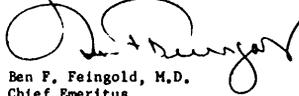
The parents associations are not only instructive and supportive for the parents but also for the children for whom picnics and parties are scheduled. The awareness that other children also have a problem of dietary restrictions is extremely helpful in encouraging compliance.

A program has been funded by the Kaiser-Permanente Medical Care System for the indoctrination of parents and training of resource personnel required for instructing parents in dietary management in various communities of this country.

I would appreciate it if you would incorporate this report into your record.

With best wishes,

Sincerely,



Ben F. Feingold, M.D.
Chief Emeritus
Department of Allergy

BFF/mm
Enclosures

American Cancer Society
Seminar for Science Writers
March 26-30, 1976

CARCINOGENS ARE MUTAGENS:
A SIMPLE SYSTEM FOR DETECTION

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ABSTRACT: About 300 carcinogens and non-carcinogens of a wide variety of chemical types have been tested for mutagenicity in the simple *Salmonella*/microsome test. The test uses bacteria as sensitive indicators of DNA damage, and mammalian liver extracts for metabolic conversion of carcinogens to their active mutagenic forms. There is a high correlation between carcinogenicity and mutagenicity: 90% (157/175) of the carcinogens were mutagenic in the test including almost all of the known human carcinogens that were tested. Despite the severe limitations inherent in defining non-carcinogenicity, few "non-carcinogens" showed any degree of mutagenicity. Damage to DNA by environmental chemicals and radiation is likely to initiate most human cancer and genetic defects. The *Salmonella* test can play a central role in a program of prevention: to identify mutagenic chemicals in the environment (all indications are there are many) and to aid in the development of non-mutagenic products to prevent future human exposure.

We have been involved over the last ten years in the development of a simple test¹⁻¹⁷ for identifying carcinogens (chemicals which can cause cancer). The test detects these chemicals by means of their mutagenicity (ability to damage DNA, the genetic material). *Salmonella* bacteria are used for measuring DNA damage, combined with tissue homogenates from rodents (or humans) to provide mammalian metabolism of chemicals. The

test is very sensitive and inexpensive and the results can be obtained in two days. One of the main results to come out of this work, in addition to the test system itself, is that 90% of the carcinogens tested in the system can be detected as mutagens whereas very few non-carcinogens are mutagenic. As a result of this work we have supported the theory, now enjoying a rebirth, that almost all chemical carcinogens cause cancer by mutation. A mutation damaging a normal regulatory mechanism in a cell could result in uncontrolled growth in that cell and in its descendants, thus giving rise to a tumor.

The test measures back mutations in a special set of histidine-requiring tester strains of *Salmonella* bacteria. These histidine-requiring mutations used were chosen after screening many hundreds of histidine-requiring mutants to obtain those most sensitive to reversion by a wide variety of different mutagens. A number of other genetic modifications were then introduced into the mutants to increase sensitivity. About 1 billion bacteria of the mutant tester strain are treated on a single petri plate and the number of bacteria which are reverted back to ability to grow without added histidine are measured by counting the colonies appearing on the plate after an incubation of 48 hours. Many carcinogens mutate the bacteria because they are directly active, while many other carcinogens are not mutagenic directly. These latter carcinogens are metabolically converted, in animals, but not bacteria, to active forms. We have been able to detect this class of carcinogens as mutagens in the *in vitro* test by adding to it rat (or human) liver homogenates for metabolic conversion of carcinogens to their active forms, thus incorporating in the test an important aspect of mammalian metabolism of chemicals. The compound to be tested, the bacterial tester strain, and the liver homogenates are combined directly on the petri plate, and the plates are then incubated.

We have also suggested another approach to the analysis of mammalian metabolites: to examine mutagenic metabolites in urine^{9,20,21}. We are developing methods for the screening of concentrates of human urine for mutagenic activity.

My colleagues (J. McCann, E. Yamasaki and E. Choi) and I have recently published extensive data on the validation of the test with over 300 chemicals^{14,15}, under standard conditions¹². 90 percent (157) of 175 carcinogens tested for mutagenicity were shown to be mutagens, and these 157 carcinogens cover a wide variety of classes of chemicals known to be carcinogenic. Also, almost all of the tested chemicals known or suspected to be carcinogenic in humans were shown to be mutagenic. These include 4-aminobiphenyl, chlornaphazine, β -naphthylamine, benzidine, cigarette smoke condensates, *bis*-chloromethyl ether, aflatoxin B₁, vinyl chloride, 4-nitrobiphenyl, and cyclophosphamide. We tested 108 "non-carcinogens" (including 46 common biochemicals, none of which were mutagens). Despite the severe statistical limitations in defining "non-carcinogenicity" in the conventional animal tests which use small numbers of animals, few (13%) of the "non-carcinogens" showed any degree of mutagenicity. The test is also highly selective in discriminating between carcinogens and closely related "noncarcinogenic" analogs^{14,15}.

Therefore, there is an extremely high probability that chemicals found to be mutagenic in the *Salmonella* test will turn out to be carcinogens. In fact, many chemicals in the environment which were found to be mutagenic, subsequently were tested in animals and found to be carcinogenic, among these are the major Japanese food additive AF-2 (now banned)²² and the widely used industrial chemical and grain fumigant ethylene dibromide^{2,14,15}. Two hair dye components, which we found to be mutagenic⁶, have been shown to

cause cell transformation and to break mammalian chromosomes^{23,24} (thorough cancer tests are still under way). Fractions of cigarette smoke condensate first shown to be mutagenic¹⁰ have also been shown to cause cell transformation.²⁵

We believe that the test can play a central role in a long-term program of cancer prevention aimed at identifying, and minimizing human exposure to, environmental carcinogens and mutagens. It is a *complement* to traditional animal carcinogenicity tests (which take 2-3 years and cost about \$100,000) as it can be used in a variety of ways not feasible with the animal tests. 1) Chemical and drug companies can now afford to test routinely all new compounds at an early stage of development so that mutagens can be identified and this information taken into consideration before there is a large vested interest in the compound. The *Salmonella* test is now being used by over 60 major chemical and drug companies. 2) If a drug is found to be mutagenic, a variety of derivatives can be synthesized to find a non-mutagenic form^{26,27}. 3) The mutagenicity of a chemical may be due to a trace of impurity and such knowledge could save a useful chemical (R. Gustafson, American Cyanamid, personal communication). 4) Complex mixtures or natural products with carcinogenic activity can be investigated, using the test as a bioassay for identifying the mutagenic ingredients; e.g., cigarette smoke condensate is mutagenic¹⁰ and tobacco companies are trying to identify the chemicals responsible. 5) Human feces (W. R. Bruce, personal communication) and urine^{9,20,21} can be monitored to see if ingested products or drugs are giving rise to mutagens. 6) The variety of substances that humans are exposed to, both pure chemicals and mixtures, are being assayed for mutagenicity by hundreds of laboratories: e.g., water supplies; soot from city air; hair dyes⁶ and cosmetics; drugs; food additives; food; mold toxins; pesticides; industrial

chemicals; fumigants. 7) The active metabolic forms of chemical carcinogens, and their metabolism, can be determined using the test as a bioassay¹³.

8) The test system is useful in clarifying basic mechanisms of mutagenesis by chemical carcinogens, e.g., the demonstration that many aromatic carcinogens are reactive frameshift mutagens with particular base sequence specificity^{1,5,7,8,19}, and the clarification of the role of different repair systems in mutagenesis by various carcinogens^{7,11}. 9) The sensitivity of the *Salmonella* test may make it particularly useful for detecting chemicals which have weak carcinogenic activity and would be difficult to identify in animal tests because of statistical limitations. Weak carcinogens could be of great importance to the human population where millions of individuals could be exposed.

It has been estimated that environmental factors initiate on the order of 80%²⁸ of human cancer and it is becoming increasingly apparent that the causative agents in these environmental factors are likely to act by damaging DNA, e.g., cigarette smoke, asbestos, ultraviolet light, X-rays, known human chemical carcinogens. It seems clear that many more chemicals will be added to the list of human carcinogens, as we are being exposed to an increasing flood of chemicals that have not been tested before use for carcinogenicity or mutagenicity, from flame retardants in our children's pajamas to pesticides accumulating in our body fat. In general, the approach to this problem has been to ignore it and even very large volume chemicals, involving extensive human exposure, have been produced for decades without adequate carcinogenicity or mutagenicity tests, e.g., vinyl chloride (2.5 billion kg/yr, U.S.A.) and ethylene dichloride (3.5 billion kg/yr, U.S.A.)¹³, and a host of pesticides. A small fraction of these chemicals is now being tested in animals, but for the vast bulk of them the only experimental animals are humans, and epidemiological studies on humans are impractical

in most cases because of the 1 to 3 decade delay between exposure and the appearance of human cancer. An explosive increase in the incidence of birth defects and human cancer may be the outcome if too many of the thousands of new chemicals to which humans are exposed turn out to be powerful mutagens and carcinogens.

Damage to DNA by environmental mutagens may be the main cause of death and disability in advanced societies²⁹. We believe that this damage, accumulating during our lifetime, initiates most human cancer and genetic defects and is quite likely a major contributor to aging^{30,31} and heart disease^{32,33} as well. The solution is prevention: identifying environmental mutagens and minimizing human exposures. Rapid, accurate, *in vitro* tests, such as the *Salmonella*/microsome test, should play a crucial role in realizing this goal.

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I would like to acknowledge the invaluable help of J. McCann & E. Yamasaki.

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Hyperkinesis and Learning Disabilities Linked to the Ingestion of Artificial Food Colors and Flavors

Ben F. Feingold, MD

The historical background of hyperkinesis and learning disabilities (H-LD) is reviewed briefly and followed by a discussion of food additives. Focus on artificial food colors and flavors as important etiologic agents is explained and supported by the favorable response of 30 to 50% of various samples of H-LD children managed with the Kaiser-Permanente (K-P) Diet.

The hyperkinetic syndrome, commonly labeled "hyperactivity," has captured public attention in practically every developed country. Whether this public interest represents a greater awareness of the problem or reflects an actual increase in incidence is an often debated question. However, among those who deal with children, particularly educators and teachers, as well as thousands of beleaguered parents, the consensus favors not only a real increase in the incidence of hyperkinesis but a prevalence of epidemic proportions affecting approximately 5,000,000 children in this country.

The increased frequency with which hyperkinesis is encountered has led to a common impression that the condition is a new syndrome, although it is perhaps as old as man. A search of the literature discloses clinical patterns described as early as 400 BC (Benton 1964-65) that were quite similar to those currently labeled as "hyperkinesis" or "minimal

brain dysfunction" (MBD) in which hyperactivity is absent or not prominent. During the 18th, 19th, and early 20th centuries terms such as *tanzruh* (dance mania), fidgety Philip (Hoffman 1864), St. Vitus' dance (Abt 1929), and chorea minor (Gibson 1937) were often applied to clinical descriptions closely similar to the hyperkinetic syndrome or MBD.

The epidemic of encephalitis that occurred during World War I left in its wake a number of cases with true brain damage characterized by signs and symptoms that are now identified with hyperkinesis. Following the collation of the clinical experience of this period by Cohen and Kahn in 1934, it became common practice to label all patients presenting a similar clinical pattern as having brain damage (BD). Abetted by the reports of Strauss and his collaborators (Strauss & Lehtinen 1948, Money 1962, Strauss & Kephart 1955), the practice of labeling individuals as organically brain damaged without adequate substantiating evidence continued into the 1950s.

In the early 1960s, to mitigate the stigma associated with the diagnosis of brain damage, the word "minimal" was introduced. Later the diagnostic term was further tempered by substituting "dysfunction" for "damage"; this led to the commonly encountered term, "minimal brain dysfunction." In 1957 Lauffer and Denhoff suggested the term "hyperkinetic

TABLE I. Etiologies in Neurologic Damage

During Pregnancy
Toxemia of middle later (A, B)
Hemorrhage
Infection
Drugs
Environmental toxicants (air, food, water)
During Delivery
Anesthesia
Trauma
Post Partum
Respiratory distress
Infection
Environmental toxicants

well as hypotheses suggesting the etiology or underlying biological disorder.

A number of etiologies have been proposed for hyperkinesia (see Table I). Causal factors include toxemia, hemorrhage, and drugs during pregnancy; anesthesia causing asphyxia neonatorum and trauma during delivery; psychological and emotional factors; environmental pollutants of the air, water, and food supply.

One of the most widespread and critically important, yet not fully recognized, group of pollutants in the environment is food additives. Food additives may be classified as intentional or nonintentional. Nonintentional food additives are the chemicals and substances that accidentally gain entrance into the food supply — e.g., insect parts; animal hairs and feces; soil, water, and air pollutants; packaging materials, etc. Intentional additives represent the chemicals that are deliberately introduced into the food supply for specific functions or purposes.

impulse disorder" which, through usage, has been abbreviated to "hyperkinesia" and frequently to "hyperactivity."

During the last 50 years, a considerable literature has accumulated which reports attempts to categorize the kaleidoscope of signs and symptoms representing this condition into specific clinical entities (Bax & MacKeith 1963). Depending upon the orientation of the observer and the dominant characteristic presented when the patient was examined, numerous labels were invented for variations of the identical problem (Clements 1966). Many times the descriptive classification carried with it a proposal for treatment and management, as

The classification of intentional food additives in Table II lists 13 categories consisting of 2,764 compounds compiled from data gathered by the National Science Foundation in 1965. This is not a complete list. The precise number of intentional additives may approach 3,800 or even 4,000. The exact number is not known.

Of all additives introduced into foods, synthetic colors and flavors are perhaps the most common. By virtue of this, synthetic

TABLE II. Classification of Intentional Additives.*

Preservatives	33
Coloring agents	28
Flavoring agents	48
Antioxidants	111
Emulsifiers	39
Stabilizers	24
Thickeners	60
Acidulants	34
Chelating agents	4
Enzymes	117
Other	1818
Total	2764

*Compiled by National Science Foundation, 1965.

TABLE III. Adverse reactions induced by flavors and colors.

1. Respiratory
 - Rhinitis
 - Nasal polyps
 - Cough
 - Laryngeal edema
 - Hoarseness (laryngeal nodes)
 - Asthma
2. Skin
 - Pruritus
 - Dermatographia
 - Localized skin lesions
 - Urticaria
 - Angioedema
3. Gastrointestinal
 - Macroglossia
 - Fatulence and pyrosis
 - Constipation
 - Buccal chancres
4. Neurological Symptoms
 - Headaches
 - Behavioral disturbances
5. Skeletal System
 - Arthralgia with edema

colors and flavors are the most common cause of adverse reactions, affecting practically every system of the body (Table III). It is because of this comparatively high frequency of reactions attributed to the added colors and flavors that we have focused our attention upon these two classes of additives. This does not imply that the remaining categories do not cause adverse reactions. No chemical is exempt, since any compound in existence, whether natural or synthetic, may induce an adverse reaction if its consumer has the appropriate genetic profile, i.e., predisposition. This being true, it becomes essential to evaluate each compound or class of compounds for its benefit compared with the risk associated with its use.

In addition to being the most common cause of adverse reactions, the synthetic colors and flavors have no nutritional value. Their function is purely cosmetic, so that deleting them from the food supply would cause no significant loss. Accordingly, on balance, the risk associated with synthetic colors and flavors outweighs their benefits.

Of all observed reactions to such compounds, perhaps the most dramatic and most critical are the behavioral disturbances.

Initially, it may be surprising that food additives can cause behavioral disturbances. Closer analysis allays surprise. Except for terminology, there is no difference between certain compounds when they are used as medicines or when they are introduced into foods as additives. Both are low molecular weight compounds. The availability of behavior-modifying drugs is common knowledge. There are drugs that stimulate, drugs that depress, and others that modify the subject's mood. It is not remarkable that among the thousands of additives in the food supply there may be compounds with similar effects upon behavioral and emotional patterns.

It is surprising indeed to recognize that none of the thousands of chemicals introduced into food as additives has ever been subjected to pharmacological studies such as those that are required for a compound before it can be licensed for use as a drug (Schmidt 1975). Certainly, there is little knowledge of the behavioral toxicology of these additives.

The patient who first attracted my attention to the possibility of a link between behavioral disturbances and the ingestion of artificial food colors and flavors was a 40-year-old woman who reported to the Allergy Department because of angioedema of the face and periorbital region (Feingold 1973). Her food intake was restricted according to the K-P Diet (Table IV) and her angioedema cleared. During the initial interview, the patient had failed to report that she had been in psychotherapy for two years because of a behavioral disturbance characterized by hostility toward her husband, inability to socialize with her peers, and conflict with her coworkers. While she adhered to the K-P Diet, her behavior improved. She also noted that any infraction of the diet induced an immediate recurrence of both the angioedema and the disturbed behavioral pattern.

Having been alerted to a possible link between food additives and behavior, we observed other adults with a similar association, and also children with the apparent same relationship. Since the children were reporting to the Allergy Department, their primary complaints were somatic — e.g., pruritus, urticaria, angioedema, localized skin lesions, nasal

TABLE IV. *The Kaiser-Permanente (K-P) Diet.*

Omit the following, as indicated:

I. Foods containing natural salicylates

Almonds
Apples (cider & cider vinegars)
Apricots
Blackberries
Cherries
Cloves
Cucumbers and pickles
Currants
Gooseberries
Grapes or raisins (wine & wine vinegars)

Mint flavors
Nectarines
Oranges
Peaches
Plums or prunes
Raspberries
Strawberries
All tea
Tomatoes
Oil of wintergreen

The salicylate-containing foods may be restored following 4 to 6 weeks of favorable response provided no history of aspirin sensitivity exists in the family.

II. All foods that contain artificial colors and flavors

III. Miscellaneous items

All aspirin-containing compounds
All medications with artificial colors and flavors
Toothpaste and toothpowder (substitute salt and soda or unscented Neutrogena[®] soap)
All perfumes

Note: Check all labels of food items and drugs for artificial coloring and flavoring. Since permissible foods without artificial colors and flavors vary from region to region, it is not practical to compile a list of permissible foods. Each individual must learn to read the ingredients on the label. When added colors and flavors are specified, the item is prohibited. If in doubt, the food should not be used. Instead, it is advisable to prepare the substitute at home from scratch.

symptoms, and at times gastrointestinal complaints. Early in the course of these observations, none of the parents volunteered information that a child was experiencing behavioral disturbances, often associated with problems at school. After the K-P Diet was ordered for treatment of the physical complaint, the parents would report not only control of the physical problem, but also a marked change in the child's behavioral pattern (Feingold 1975).

To test whether the observations of the parents would be confirmed, we arranged for management by the K-P Diet of children whose primary complaint was a behavioral disturbance, usually labeled MBD or hyperkinesia. Using the Conners Rating Scale (Conners 1969), ratings were made prior to the initial visit and periodically following dietary management, initially at 2-week and then at 4-week intervals. We were soon able to confirm the

parents' earlier reports. Children with a history of signs and symptoms* usually leading to a diagnosis of MBD or hyperkinesia, when managed with the K-P Diet, experienced a marked change in behavioral pattern within 3 to 21 days, depending upon the age of the child. Children who had been receiving various behavior-modifying drugs could discontinue these agents, while the behavioral pattern continued to improve. When rated by teachers on a quarterly or semester basis, children who had had difficulty at school showed a marked adjustment to the classroom environment and

*The history developed at the initial visit covered all developmental periods - prenatal, perinatal, infancy, nursery school, kindergarten, elementary and secondary school. For older patients, performance before and after puberty was stressed.

rapid improvement in scholastic achievement. Any dietary challenge, inadvertent or deliberate, induced a recurrence of the behavioral disturbance which persisted for 24 hours to four days, so that a child experiencing an infraction only twice a week could have a persistence of the clinical pattern.

A double-blind crossover study funded by the National Institute of Education and directed by Dr. C. Keith Conners of the University of Pittsburgh (Conners, Goyette, Southwick, Lees, & Andrulonis 1976) has confirmed that dietary management favorably influences hyperactivity at the .005 level of significance on a teacher-rating scale and at .05 on a parent-rating scale. The subjects of this study initially comprised 57 children who were reviewed in advance of the investigation. Through attrition, chiefly failure to comply with the structure of the study, the group was finally reduced to 15 children who fulfilled all the requirements of the protocol. Five of the 15 children demonstrated unequivocally that dietary management influenced hyperactivity as long as there was full compliance with the diet. Any infraction or challenge was followed within hours by a recurrence of the behavioral pattern.

The Food Research Institute (1976) of the University of Wisconsin conducted a double-blind crossover study on 36 boys of school age (6 to 12 years) and 10 children who were three to five years of age. In the school age group, four children in the sample showed significant improvement as rated by both parents and teachers and/or on several of the objective measures employed. The younger children (age 3 to 5) showed a greater positive response to the experimental diet as indicated by parent rating. All ten mothers in this group rated their child's behavior as improved as did four of the seven fathers in this sample.

The numerous variables of the hyperkinetic syndrome coupled with the many environmental variables do not permit valid statistical conclusions on the basis of the short-term, segmental observations employed in both the Conners and the Wisconsin study. These studies merely confirmed that the K-P Diet influences behavior.

All our clinical observations have been repli-

cated in a pilot clinical study (Cook & Woodhill 1976) in Australia, directed by a psychiatrist with the Sydney Ministry of Health, and the chairperson of Prince Henry Hospital's Department of Nutrition at Little Bay, New South Wales.

Both the Department of Health, Education, and Welfare in this country and the Medical Research Council of Australia are funding further studies of the problem.

RESPONSES TO MANAGEMENT WITH THE K-P DIET

Five separate programs, representing a total of 360 children managed with the K-P Diet, showed favorable responses ranging from 30 to 50% of the sample, depending upon the mean age of the children and the presence or absence of a history suggestive of neurologic damage. Precise determination for the percentage of responders to dietary management will require large samples, perhaps 1,000 subjects or more, studied longitudinally over a period of several years. At this level, it is important to recognize that dietary intervention does influence the behavioral deficits of the hyperkinetic syndrome and, particularly, hyperactivity.

All of the deficits associated with the hyperkinetic or MBD syndrome listed in Table V are not observed in every child. Not only does each child have his own mosaic of deficits, but for any given child, the pattern may vary from day to day, and at times even from hour to hour. Hyperactivity is usually the dominant feature of the pattern, but it is not always present. One child may exhibit features of only a single group listed in Table V; in other children, various combinations of deficits drawn from one or more of the three groups may characterize the behavioral pattern. At times only a single deficit may be observed. However, if this deficit is a critical one — e.g., an auditory perceptual or a visual perceptual disturbance — severe learning disabilities may result.

Although we have observed the response to dietary management for five years, we are still unable to predict from history, physical examination, and neurologic and psychometric tests the ultimate response of the individual. Similarly, assumptions regarding the speed and

TABLE V. Descriptive characteristics of clinical pattern of H-LD.

GROUP I

Marked Hyperactivity and Fidgetiness

Constant motion

Rocks and jiggles legs

Dances, wiggles hands

Runs, does not walk

In infancy, crib rocking, head knocking, fretfulness

Compulsive Aggression

Disruptive at home and at school

Compulsively touches everything and everyone

Disturbs other children

Perseverates — Cannot be diverted from an action even when life threatening

Excitable — Impulsive

Behavior is unpredictable

Panics easily

Frustration leading to temper tantrums

No Patience

Low tolerance for failure and frustration

Demands must be met immediately

Short Attention Span

Unable to concentrate

Poor Sleep Habits

Difficult to get to bed

Hard to fall asleep

Easily awakened

GROUP II

Gross Muscle Incoordination

Exceptionally clumsy

Trips when walking

Collides with objects

Cannot function in sports

Cannot bicycle or swim

Fine Muscle Incoordination

Eyes and hands do not seem to operate together

Difficulty with: buttoning and tying

writing and drawing

speech - stuttering

reading - dyslexia

GROUP III

Cognitive and Perceptive Disturbances

Auditory memory deficits

Visual memory deficits

Deficits in understanding

Difficulty in reasoning, e.g., a math problem

Normal or high IQ but fails at school

Boys Involved 7:1

Merely more than one child in a family affected

degree of response to dietary management cannot be made on the basis of estimates of neurologic damage, previous use of medicines, or age of the child. Children with a history suggesting a possible cause for neurologic damage may experience a complete recovery on the diet, while others with a completely negative history may fail to respond, or may show a partial response, such as improved behavior with deficits in coordination, or cognition, or perception. The history cannot always be precise in disclosing neurotoxic factors. Not infrequently the mother, relying upon memory, cannot accurately reconstruct the events before or during pregnancy. In addition, consideration must be given to less overt factors, such as environmental pollutants of air, soil, and water, which serve as neurobehavioral toxicants during gestation or early childhood.

A determination can be made only through strict application of the diet.

When a favorable response follows dietary management, the initial improvement is in the behavioral pattern (Table V, Group I). Control of aggression, impulsiveness, and the tendency to perseverate results in a calmer child; the improvement in mood enables the child to concentrate, which leads to improved attention. If there are no cognitive or perceptual deficits, there is rapid improvement in learning. The child who was abusive, disobedient, incorrigible, and disdainful of attention moves toward becoming affectionate, lovable, and responsive to guidance.

Correction of the behavioral pattern may be followed rapidly by improved muscular coordination (Table V, Group II). Improvement of the gross muscle involvement corrects awkwardness in gait and permits participation in sports, such as swimming, ball games, and bicycle riding. Improvement of the fine muscle coordination leads to improved writing and drawing skills and, in some children, improved speech.

Cognition and perception (Table V, Group III) are next to respond; improvement in these permits increased scholastic achievement. Learning ability may show a slow improvement over months or even years. An improved behavioral pattern always precedes the correction

in coordination, cognition, and perception. The latter deficits do not improve unless behavior responds to the diet. Cognitive and perceptual deficits are those that persist most commonly, causing learning disabilities even after a marked improvement in behavior and muscle coordination.

Age influences the speed and degree of response to dietary management. Usually, the younger the child, the more rapid and more complete the response. In early infancy improvement or reversal of all signs and symptoms may occur within 24 to 48 hours after elimination of pediatric vitamin drops, a rich source of synthetic colors and flavors. The two- to five-year-old child may improve after five days, while the five- to 12-year-old child may respond in 10 to 14 days. In some children, particularly those who have received long-acting drugs, e.g., dextroamphetamine Spansules® or large doses of behavior-modifying drugs [amphetamine, methylphenidate (Ritalin®), Stelazine®, Mellaril®, Tofranil®, Elavil®, Vistaril®, Cylert®], the response may be delayed for 3 to 5 weeks. Very often, following such delays the improvement may occur abruptly rather than develop gradually.

The older child, postpubescent or adolescent, usually requires a longer period, frequently several months, before improvement is noted; even then, the response is not always complete. The highest incidence of failure to respond to dietary management is observed among patients treated during adolescence.

As the child passes through puberty, a spontaneous improvement in the behavioral pattern may be observed. If the deficits persist, the adolescent cannot perform to his full potential. This makes it difficult for him to cope with his environment, leading to frustration resulting in withdrawal, antisocial behavior, lying, stealing, and ultimately, in many cases, juvenile delinquency.

BEHAVIORAL TOXICOLOGY

The behavioral disturbances with learning disabilities attributed to the ingestion of artificial food colors and flavors represent a small band in the broad spectrum of the newly emerging discipline of behavioral toxicology. Accord-

ingly, the variety of clinical patterns observed can best be interpreted relative to some considerations that are basic to this discipline.

Since molecules of almost any substance can cross the placental barrier, it must be recognized that any environmental compound, whether ingested, inhaled, or injected, has the potential of being toxic to the fetus. The developing organism does not have the same capacity as a fully developed person to metabolize and detoxify potentially toxic substances. Accordingly, the fetus, particularly during the stage of organ differentiation, is highly susceptible to the insults of any substance crossing the placental barrier.

The teratogenic damage which may be manifest as either overt or covert alterations in the organism is governed by the genetic profile of the individual, the nature and doses of the offending compound, and the stage of organ development at the time of the insult. It is conceivable that substances of low toxicity or in small doses can induce covert alterations in the organism which can later manifest as behavioral disturbances without gross functional impairment or structural birth defects. The overt damage to organs and to the nervous system is usually obvious, and its patterns are well known.

It is not generally recognized that the disturbances caused by such alterations are not necessarily obvious at birth, but emerge as the child grows older. Nor is it commonly appreciated that teratogens which have an affinity for developing brain centers may induce subtle alterations which may manifest in later life as behavioral disturbances and learning disabilities. In 1968 Nair and Dubois reported that a morphological or biochemical lesion may remain dormant and not be manifest as a behavioral disorder or functional impairment until later life. This may explain the delayed onset of the behavioral disturbance and learning disabilities so frequently noted when the history suggests possible intrauterine damage.

Relative to the mode of action of artificial food colors and flavors, there are two possibilities to consider. The food additives may play a primary role as the sole etiologic agent. Or, they may serve as irritants superimposed

upon a substratum created by any one of the commonly cited causes of neurologic damage (Table I). In either situation, the toxicants may be ingested by the mother during pregnancy or encountered by the patient during extrauterine life.

Although it is possible that the artificial colors and flavors ingested by the mother may play a primary role in the induction of teratogenic alterations, there is as yet no supporting evidence for this concept. The primary extrauterine role is suggested by the complete reversal of all deficits, both behavioral and learning, following the elimination of the colors and flavors from the diet. The completeness of the favorable response and the ability to induce a rapid recurrence within hours indicate that this is a functional disturbance.

In addition to the functional disturbances induced by the artificial colors and flavors, it is conceivable that irreversible neurologic damage may result, particularly from continued exposure to the chemical over many years. Such damage could explain the persistence of various degrees of muscle incoordination and learning disabilities when a dramatic improvement in the behavioral pattern follows dietary management. Since the higher association centers are the last to differentiate, they are the most susceptible to neurotoxicants. In the human, these centers are not fully developed at birth and are ready targets for damage which can be manifest as the hyperkinetic syndrome. It is conceivable that the high incidence of failure to respond among adolescents may be attributed to irreversible neurologic damage.

As secondary agents acting upon pre-existing neuropathology, the synthetic colors and flavors can produce a variety of clinical patterns — e.g., the hyperkinetic syndrome, seizures labeled as petit mal, mental retardation, and learning disabilities. The secondary role is suggested by a history positive for neurologic damage attributed to other causes, and improvement of various degrees in response to dietary management.

For example, the elimination of colors and flavors may be followed by improved behavior but persistence of muscle incoordination with perceptual and cognitive deficits. Seizures may

be controlled without the use of drugs; but muscle incoordination and learning ability improve only partially or fail completely to respond. In retardation the clinical response may be dramatic, as evidenced by improved behavior, better coordination of both fine and gross muscles, and improved learning ability. All of these gains induce a marked transformation in the patient, whose expression becomes more alert and bright, his social adjustment improves, permitting him to function as a self-sufficient person who does not require one-to-one attention or instruction. In most patients labeled as "retarded," however, the level of learning ability usually remains below the normal estimated for age.

At times, residuals may persist with nothing in the history to suggest a cause. Behavioral toxicology is not yet sufficiently developed to provide guidelines for correlation of behavioral patterns and learning disabilities with all of the potential neurotoxicants in the ecosystem.

CONCLUSION

Artificial food colors and flavors have the capacity to induce adverse reactions affecting every system of the body. Of all these adverse reactions, the nervous system involvement, as evidenced by behavioral disturbances and learning disabilities, is the most frequently encountered and most critical, affecting millions of individuals in this country alone.

The K-P Diet, which eliminates all artificial food colors and flavors as well as foods with a natural salicylate radical, will control the behavioral disturbance in 30 to 50% (depending upon the sample) of both normal and neurologically damaged children. — *Allergy Department, Kaiser-Permanente Medical Center, 2200 O'Farrell Street, San Francisco, Calif. 94115.*

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March 4, 1977

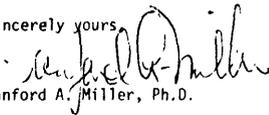
Ms. Judith Robinson
Legislative Assistant to
Senator Gaylord Nelson
United States Senate
Committee on Labor and Public Welfare
Washington, D.C. 20510

Dear Ms. Robinson:

I am enclosing a copy of the manuscript including the comments to which I referred in our telephone discussion. This is the text of the remarks I made at the Bicentennial Conference on Food and Nutrition sponsored by the New York Academy of Sciences in November of last year. This will appear in an Academy monograph to be published this July.

I believe that this statement fairly outlines my feeling on the subject of food additives. This is a difficult and emotional area which has not, in my opinion, been reasonably approached by industry, consumer advocate nor legislator. I hope that your Committee will see fit to break with tradition and make a real contribution to the solution of these problems. Please feel free to call on me for any future services.

Sincerely yours,


Sanford A. Miller, Ph.D.

SAM/lcb

Enc.

ADDITIVES IN OUR FOOD SUPPLY

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For a civilization to flourish, it must, in some way, establish and maintain control over the environment in which it exists. One of the major factors in the environment is food.

Food has always been a determinant of civilization. The first need mankind had to satisfy, even before shelter, protection and defenses, was his need for food. Eight thousand years ago, neolithic man was able to develop the arts of masonry, pottery and weaving only after he had become a food cultivator rather than a hunter and food gatherer.

But mankind had to move a step further before his culture could truly begin the long march to civilization. For farming alone did not remove all of the uncertainties of a food supply. Insecurity of weather, agricultural pests and diseases and, most of all, the lack of a stable distribution system and an inability to store food against the future still kept man locked to the soil, existing from year to year with no sense of future security. More importantly, this sustenance level of food production could not permit any significant number of people to do anything but produce food.

The first step to modify this situation was in the area of crop improvement. Plant varieties such as wheat and barley, foods that were readily stored and transported, were developed not by

by pure chance alone but, conceivably, as a means of protecting against future famines and freeing man's mind from the drudgery of the soil.

The introduction of processing techniques such as drying and baking were also important contributions to the process of increasing storage life, permitting development of reserves and allowing transportation of food over significant distances. The discovery that the addition of several chemical materials such as salt or vinegar could also permit longer storage and better distribution was one of the most significant contributions to this process. The result of these developments in storage and distribution was the rise of the great civilization of the past, for when all man did not have to raise food for individual needs, when only a part of the population was required to maintain a consistent food supply, the remainder were free to use their minds for other purposes, to congregate, interact and found cities.

Thus, early in his existence on this planet, man had learned a fundamental rule of reciprocity still in existence today: as man is able to control the production, storage and distribution of foods, so is he able to enhance the arts and sciences. And as he advances the arts and sciences, so is he able to control better his food supply by improving the production, the storage and the distribution of foods.

The capacity to produce "surplus" food, store and transport it and the need to supply increasing "urban" populations led to..

the development of and increasing dependence on a third party in the food chain, intermediate between producer and consumer. Originating as millers and transporters of grain, the food processing industry really became of significance with the introduction of new preservation methods and the discovery of chemical additives. With these, greater variety and amounts of food could be supplied over a greater distance providing for the consumer potentially more nutritious and more easily handled products. Thus it is not surprising that the first great merchants of the past were millers and later brokers of food. Even more importantly, the control of this aspect of the food system was earlier, and, in the middle ages played a major role in the religious communities.

Thus it seems clear that the introduction of chemical additives was not only an important step in mankind's attempts to control his environment and thus a contribution to the growth of civilization, but also was an action that was almost as old as mankind itself. As shown in Table 1, early man had several options open to him. For example, he could preserve his flesh foods by smoking thus introducing a whole series of organic materials to his food. He could dry the product or add alcohol by fermentation or add salt, or vinegar. In the classical period, the use of spices and herbs to prevent spoilage and improve palatability added new dimensions to his armamentarium of approaches.

In more modern times, the development of thermal processing techniques at the end of the eighteenth century gave new impetus to the drive to control this aspect of the environment. With

the later introduction of economical and reliable refrigeration and freezing techniques, the capacity of the industry to store and distribute food products expanded enormously and led, in part, to the increasing movement of people to the cities as well as increasing dependence of people on the industry to provide them with this most basic of needs. On the part of the industry, these new techniques demanded new means of preserving quality or improving efficiency. With the rise in organic chemistry, new additives became available for this purpose in response to the need.

In the future, techniques already in the lab or pilot plants offer not only the possibility of even greater available food supplies but also the need for even more additives of new capability.

The rise in the capacity of the food industry to do its job is unquestionably one of the most dramatic examples of technologic success. But this success itself has given rise to the set of problems faced by the industry today. In general, these problems are concerned with the questions of safety and wholesomeness of foods. The public is beginning to question its food supply based on an increasing feeling of uncertainty. Are, they ask, the "chemicals" we add to our "pure" food safe? Will they cause cancer, produce monsters or, perhaps, cause my hair to fall out? The result of this uneasiness has been the rise of consumer activism ~~activity~~ and increasing demand for greater control over the industry.

Is there reason for this concern or more importantly perhaps why has the perception of food and its associated industry fallen in the precipitous manner it has? There are perhaps several factors that have contributed to the problem.

First is the question of time. As shown in Table 1, the introduction of new techniques and, more importantly, new additives, took centuries in the past. Thus, society had extensive experience with them and since few were introduced at a time, dislocation in prejudice was relatively slight. Even more importantly, greater experience in terms of hazard was possible based on direct human ~~experience~~ ^{EXPOSURE}. Thus, relatively few people were harmed by an acutely toxic product and ~~thus~~ it could be removed before general introduction.

In more recent times, the time scale of introduction has been contracted even further. Decades or less are all that ^{ARE} ~~is~~ necessary for the exposure to new materials to become widespread. The time for experience has become short, too short to be used as a basis for judging hazard. These perceptions of the problem led, in my opinion, to the establishment and proliferation of the food regulations in this country and to the development of the concept of toxicity testing in animals. It is important to note however that the testing approach was originally designed to deal with acute effects, and, even today, is concerned largely with the elucidation of clearly definable toxic responses.

The problem will become even more acute in the near future when the time of introduction becomes even shorter as a result of

better communications and distribution. Under these conditions, no reliance can be placed on "experience" since before any such "experience" can be obtained it is probable that major portions of the population will be exposed to the new material. Moreover, the nature of these new products, for example fabricated foods, offer increasing problems of combination and amounts of additives that have not been considered in the past.

Second is the problem of the exponential rise in numbers and diversity of such additives. For the first 90% of the time of their use, the number of additives generally in use probably did not exceed 50, including spices and flavorers. Today they represent a number over 2000 (1). Their variety and functional use is legion and of equal importance they represent an enormous volume of use (Table 2). Thus, the number and quantity of such materials has increased so enormously that actual levels of exposure become important criteria in determining safety, based not only on a single use of the substance but on multiple uses in multiple products.

The third problem is concerned with the perception of the concept of "toxicity". In general, the industry and the public tend to view toxicity of additives in the pharmacologic sense, i.e. a substance that demonstrates potential to adversely affect health (2). The problem lies in the definition. For the professional and the industry, this is related to a clearly definable abnormal response capable of being quantitated and compared statistically. For the public, this may mean something considerably less precise, more associated with a feeling of well

being rather than with any specific pathologic lesion. This is largely the result of our increased lifespan and control over some aspects of our health environment. The general availability of food, the decreasing lack of danger from such acute environmental hazards such as infectious disease have led to the decrease in the concern over lifespan and an increase in demand for a better way of life in which health and happiness are equated. When this concern is extended "even unto the future of generation", the problem facing the development of model systems to test these possibilities becomes awe inspiring. It is this difference in the perception of safety that, I believe, lies at the root of the problem dividing professional and public. The professional offers safety from hazards he can measure based upon traditional approaches to relatively acute known pathology; the public demands safety so that their life and that of their descendants can be healthy, happy and complete. For them the question is not whether they will live to 90 but rather how they will live to 90.

The matter is made even more complex by the phenomenally rapid development of knowledge in molecular biology and other areas associated with the exploration of life processes. These have, in part, based upon the incompleteness of our knowledge, given rise to fears and questions concerning the subtle effects of additives in food on these most basic aspects of life without offering reliable methods of studying these effects and predicting accurately their importance to human welfare. Thus it presents another amorphous contribution to the disquiet of the consumer.

In large measure then, the public is asking for measurements of safety that begin to approximate the noise level of the system itself, that is, levels equivalent to and perhaps surpassing the toxicity of naturally occurring materials. That these ^{"NATURAL"} substances themselves can be diverse and widespread is suggested by the list shown in Table 3, itself representative and by no means complete. Nearly every food has in one way or another a toxic substance. It must be pointed out however that these are generally of low activity in their respective classes and that they are often inactivated or removed by processing. What is important however is that, for the public, they represent a basal level of acceptable risk and for the toxicologist should represent a goal towards which technology should strive.

The traditional approaches to the judgment of safety of food additives are shown in Table 4. With few exceptions, it is the list of techniques used to evaluate new substances. It is clear, I believe, that they do not completely approach the question raised by consumers concerning their perception of safety. Accepting this, one may ask if their application ^{OVER} of the past several years has offered any degree or assurance of the safety of food additives at any levels.

For the past five years, the Select Committee on GRAS Substances of the Federation of American Societies for Experimental Biology has, under contract from FDA, been reviewing the safety of GRAS substances. These compounds designated as "Generally Recognized as Safe" occupy a unique position in the list of food

additives and are among the most commonly used of these substances. The result has been the most comprehensive review of the safety of any group of environmental compounds yet performed and have offered to the Committee members a unique experience in this field. One result of this review was the development of a document outlining areas of concern and suggestions for future actions based on this experience (3). Some of these conclusions bear on the questions raised earlier in this report.

First, there is no evidence of acute, major toxicological problem associated with the use of any of the additives reviewed by this Committee. This is not to say that questions were not raised about several of these substances but rather, in each case, the problem was not knowing rather than conviction of hazard. Since, in the general opinion of the Committee, a conservative approach was prudent, suggestions were made for limitation of use of such substances or, in a limited number of cases, elimination from the diets of special groups in the population.

Yet, in spite of this extensive review, many questions remained. In part these were generally associated with the lack of information on several substances. For example, the number of references associated with specific compounds ranged from 20 or less in the case of carnauba wax to over 20,000 in the case of vitamin A and glutamic acid (3). More importantly, important areas were not covered for many compounds particularly the so-called natural products or derivatives. Information was deficient (concerning fetal exposure) for example for more than four-fifths of the substances reviewed. This was a particularly important consideration in view of the

probability that most GRAS substances are consumed by at least some pregnant women. The matter is made more complex by the fact that most traditional multigenerational studies designed to test such compounds do not permit the observation of long-term effects in succeeding generations inasmuch as the test animals are usually killed when they are six months of age.

Information concerning the hazard to the neonate and infant was also lacking for nearly all substances. The neonate stands peculiarly at risk when exposed to substances that may offer only minimal hazard to the adult. For many xenobiotic substances, major shifts in toxicity have been found between neonate and adult (4). In addition to the fact that many such substances pass readily into milk (5), the modern tendency to early weaning and the introduction of the infant to table foods during the first months of life (6) has increased the urgency with which studies of the direct effect of such additives on the neonate must be performed.

The problems of evaluating carcinogenicity hazard are another major area of concern. Not only is there lack of agreement among professionals concerning the adequacy of test protocols but more importantly there is a central conceptual issue concerning the threshold effect for such substances. Is there a "no effect" level for carcinogens as there is for other substances or are these unique in that the presence of any number of such active molecules offer danger to cellular organization? Thus the conclusion of carcinogenesis is a difficult judgmental matter in which the lack of agreement among "experts" has led to increasing public concern.

Although practically all guidelines for toxicological testing for food additives call for teratologic observation (Table 4), the translation of results from experimental animal to human experience is still in a primitive state. For example, in a recent compilation of over six hundred agents producing congenital abnormality in animals, only approximately twenty are known to cause human defects (7). Not only do different species react differently but even different members of the same litter are dissimilar in reaction. When these uncertainties are combined with the newly awakened public concern over the welfare of future generations, the question of teratogenic hazard becomes an important and significant contribution to uneasiness.

Additional questions may be raised about many other issues such as the problem of estimating exactly how much of each additive the public is exposed, what margin of safety is to be used and so on. In each case, however, these all are important in the evaluation of two essential points. First, how much risk is the consumer assuming by the use of the substance and for what benefit? This concept of risk-benefit ratio has become an institutional magic phrase that appears to suggest some quantitative way of automatically determining whether or not a substance should be used in food. While of great value in assessing the use of therapeutic and prophylactic agents, its application to the problems of food additives is much more difficult. The fact that the levels of hazard in the use of such materials is so low, and, for many substances information is lacking, and that there are

are no good techniques for evaluating health as the public probably views it makes the assignment of an order of magnitude for risk difficult. On the other hand, agreement on the relative value of the various benefits resulting from the use of such substances is also difficult to attain. Thus the establishment of adequate quantitation of each of the terms of such an equation is really not favorable at the present time. The acceptance of the concept in general terms does provide a framework, however, vague for judgmental considerations.

The second part concerns the question of how much regulation is required for the industry. In the first place, the unique position of the food industry as a principle maintainer of urban civilization and concomitant dependence of the public on its activity makes unquestionable the need for public control of its actions. In addition, this increasing dependence is coupled with decreasing freedom of choice on the part of the consumer in excluding particular ingredients in their food also supports the argument for governmental control of the industry. In contrast to these arguments is the danger^{that} increasing restriction of options for the industry leading to rigorous control will also lead to reduction in innovation, a commodity much in demand in this world of increasing population and limited food. It should also be pointed out that, for biological systems, the ultimate in rigor is death. The determination of the proper amount of regulation to satisfy both of these needs requires accurate estimation of hazard to man. Without them, prudent bureaucracy will respond properly with greater and more severe restriction.

Even if all of these questions were answered or answerable and even if the public and professional agree on the definitions of risk, benefit and hazard, there would still exist a major problem of credibility of the industry. Beginning from a position of the highest status and one in which public trust was implicit, the industry today occupies a position in which few of its claims are accepted by the public. Moreover, this reputation has so spread that individuals associated with or supported by the industry come under suspicion. Perhaps, the problem began at the turn of the century when it became more important to determine what to say about a food product rather than to become concerned about what was in it or for what it was to be used. The growth of merchandising and marketing influence on food company policy has, in my opinion, led to many of the problems of trust and confidence that the industry faces today.

The current public concern over the use of additives in our food supply is then based upon several considerations. The reduced time for introduction, the increased number and amount of each in use, the growing dependence on the industry and the concurrent reduction in freedom of choice, the change in emphasis from questions of survival to those of a better life not only for ourselves but for our future generations, the lack of information on many materials and the lack of agreement among experts are all contributors to the problem. To argue that many of these are based on public ignorance or "activism" begs the issue. In the end, it is the public that must decide its future and its needs and it is the responsibility of the industry and the professional to

satisfy these requirements. The public must also decide whether it wants to pay directly or indirectly for the development of techniques adequate to fulfill its expectations and desires and, if so, to insist that its legislators honor this request.

The problem of industry credibility is also one of concern. To solve it, the industry must begin to develop a greater sense of its own responsibility and importance to the community. The question must not only be one of satisfaction of legal requirements but more of satisfaction of public responsibility not only in toxicity testing but also in advertising, merchandising and public information.

The fact is that the provision of a safe, constant and highly acceptable food supply requires the conscientious efforts of both public and industry working together as partners, not adversaries. We have perhaps permitted legalistics to interfere too often with this natural alliance of consumer and industry. The establishment of a center, operated by a board of trustees consisting of industry, government, academic and legislative and consumer representatives, whose function would be to establish and perhaps perform new, unique and innovative evaluation of the safety of food additives could go a long way towards solving these problems of mutual trust. Moreover such a center supported by both government and industrial funds, staffed by the best people in the field, whose entire activity was public, could provide a new basis for ensuring the future of our food supply. In the end, it is this last-goal that may prove to be the most important sustainer of our civilization.

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TABLE 1
 CONTROL OF THE FOOD ENVIRONMENT
 PRESERVATION TECHNIQUES

	<u>TIME FOR GENERAL INTRODUCTION</u>
A. <u>PAST</u>	
1. DRYING	CENTURIES
2. SMOKING	" "
3. FERMENTATION	" "
4. SALTING	" "
5. PICKELING	" "
6. SPICES	" "
B. <u>PRESENT</u>	
1. CANNING	DECADES
2. REFRIGERATION AND FREEZING	" "
3. SYNTHETIC ADDITIVES	" "
4. PACKAGING	" "
C. <u>FUTURE</u>	
1. RADIATION	YEARS
2. FABRICATED FOODS	" "
3. NEW ADDITIVES	" "

TABLE 2
 MAJOR CLASSES OF FOOD ADDITIVES (1967)
 AND THEIR USE IN U.S.*

ITEM	QUANTITY LB/MILLIONS	VALUE \$ MILLION
PRESERVATIVES	37.0	14.9
ANTIOXIDANTS	15.5	20.5
SEQUESTRANTS	0.3	0.1
SURFACTANTS	162.5	46.6
STABILIZERS--THICKENERS	306.5	86.9
ACIDULANTS	101.5	25.0
LEAVENING AGENTS	113.0	8.6
FOOD COLORS	81.1	19.4
NUTRIENT SUPPLEMENTS	19.4	10.1
FLAVORING MATERIALS	174.5	140.0
FLAVOR ENHANCERS--POTENTIATORS	43.8	22.5
BASIC TASTE MODIFIERS	2.7	5.0
ENZYMES	N/A	19.7
FUNCTIONAL PROTEIN ADDITIVES	222.0	37.1
MISCELLANEOUS (INCLUDING SORBITAL, ANTICAKING AGENTS, YEAST FOODS, AND DOUGH CONDITIONERS)	<u>68.7</u>	<u>16.0</u>
TOTAL	1,348.5	472.4
AVERAGE/LB		35¢

*SOURCE: ADI ESTIMATES BASED ON FIELD INTERVIEWS

TABLE 3
 NATURALLY OCCURRING TOXIC SUBSTANCES

<u>SUBSTANCE</u>	<u>FOOD</u>
A. GOITROGENS	TURNIP, RUTABATA, CABBAGE
B. "ESTROGENS"	PALM KERNEL
C. TUMORIGENS AND CARCINOGENS	
1. ERGOT (C. PURPUREA)	RYE
2. YELLOW RICE (P. ISLANDICUM)	RICE
3. AFLATOXIN (A. FLAVUS)	PEANUTS
4. SAFROL	SASSAFRAS
D. HEMAGGLUTINIUS	LEGUMES
E. STIMULANTS AND DEPRESSANTS	NUTMEG, COFFEE, TEA, TOBACCO
F. PRESSOR AMINES	CHEESE, PINEAPPLE JUICE, PLANTAINS, BANANAS
G. CHOLINESTERASE INHIBITOR	EGGPLANT, VALENCIA ORANGE
H. SEAFOOD TOXINS	MANY

TABLE 4
STANDARD TOXICOLOGICAL EVALUATION

1. ACUTE (7 DAYS--HIGH SINGLE DOSE)
2. CHRONIC (LIFE TIME--CONTINUOUS EXPOSURE)
3. REPRODUCTION (2-3 GENERATIONS--CONTINUOUS EXPOSURE)
4. MUTAGENESIS (SPECIAL PROTOCOLS INVOLVING BOTH IN VITRO
AND IN VIVO TESTING -- (?))
5. TERATOGENESIS (EXPOSURE DURING CRITICAL PERIODS OF
FETAL DEVELOPMENT)
6. CARCINOGENESIS (RELATED TO MUTAGENESIS STUDIES BUT ALSO
SPECIAL TISSUE EXAMINATION DURING CHRONIC STUDIES)



DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

NATIONAL CANCER INSTITUTE

FEB 11 1977

The Honorable Gaylord Nelson
United States Senate
Washington, D.C. 20510

Dear Senator Nelson:

Thank you for your letter of January 24 concerning research by Dr. Raymond J. Shamberger on the possible carcinogenic effects of malonaldehyde.

The National Cancer Institute (NCI) has not yet tested malonaldehyde for carcinogenicity. However, in November 1976, the chemical was tentatively selected for testing in the Institute's bioassay program. According to scientists in the Carcinogen Bioassay and Program Resources Branch, it will be 2 to 3 years before data are available on this chemical.

As you may know, the NCI's Division of Cancer Cause and Prevention conducts an extensive bioassay program to test environmental and industrial chemicals for carcinogenic activity. Several hundred suspect chemicals are being investigated, including pesticides, food additives, drugs, and natural substances. A description of the chemicals under test and selection of compounds for bioassay is found on pages 24 and 25 of the enclosed book, The Carcinogenesis Program, Fiscal Year 1976. I have also enclosed a list of drugs currently under study in the bioassay program. Malonaldehyde is listed on the last page.

The NCI is currently supporting a study by Dr. Kelly H. Clifton of the University of Wisconsin in Madison dealing with the role of malonaldehyde on radiation-induced cancer. This investigation is part of a larger study entitled "Multidisciplinary Program in Radiation Oncology (grant number CA 19278)." The principal investigator of the Program is Dr. W. L. Caldwell; grant funds awarded by the Institute total \$746,379.

I hope this information is helpful. Please let me know if I can be of further assistance at any time.

Sincerely yours,

Robert G. Schonfeld
Chief, Program Liaison Branch
Office of Cancer Communications

Enclosures

GI Tract Ca Linked to Carcinogen in Beef, Poultry, Pork

By KURTIN WHITE
Special Tribune Correspondent

CLEVELAND—A formidable carcinogen, present in many of the mainstay foods in the average American's diet, can be reduced by cooking and storing foods properly, and its carcinogenic action may be thwarted when the diet includes enough foods high in anti-oxidants, according to Cleveland Clinic biochemist Raymond J. Shamberger, Ph.D.

Dr. Shamberger has found that malonaldehyde, a 3-carbon aldehyde produced by peroxidative fat metabolism, is present in disturbing quantities in many common foods, cheeseburgers to peanut butter. He estimates that the typical American consumes about 1.1 grams of the chemical every year.

Since Dr. Shamberger's experiments have shown that 6 mg of malonaldehyde applied to the skin of a 30-gram mouse produced cancer, he believes that the 75 grams of malonaldehyde consumed in a human lifetime may be a key factor in causing cancer in the gastrointestinal tract. Proportionally, it's a much higher dose, he notes.

Beef Is Highest

"Beef is the food with the highest level of malonaldehyde, while poultry, pork, and non-oily fish contain less," says Dr. Shamberger. "Except for American cheese, most dairy products are free of malonaldehyde while fresh, fresh-frozen, and canned fruits and vegetables contain little or none."

But the cancer-causing activity of malonaldehyde is apparently blocked when the diet contains enough antioxidants, such as vitamins C and E, or traces of selenium, says Dr. Shamberger, whose extensive research on the cancer-preventing properties of antioxidants has attracted much attention.

Malonaldehyde, which is a chemical cousin of glycolaldehyde and beta-propiolactone, both recognized carcinogens, forms when air breaks down polyunsaturated fats. The process is encouraged by warm temperatures. Thus, freshly-slaughtered meat contains less malonaldehyde than aged meat, frozen food develops less malonaldehyde than that which is simply refrigerated, and food in air-tight wrapping contains less malonaldehyde than food exposed to air.

Dr. Shamberger found that a sirloin tip roast, uncooked, contained 7.4 mg. of malonaldehyde per gram of meat. When roasted, the level jumped to 27.0 mg./gram. Ground round steak, raw, contained 3.8 mg./gram; broiled, it contained 10.4 mg./gram. A freshly opened jar of peanut butter contained no malonaldehyde, but after it had been opened and in use for some time, it contained 1.2 mg./gram.

"It's unpredictable stuff. We know enough about malonaldehyde to know that it's a threat, but we need to do a lot more research on the food-science aspects of malonaldehyde before we can understand how to deal with the threat," says Dr. Shamberger. Among other puzzles, he found that while raw chicken and turkey are low in malonaldehyde, cooking increases the concentration of the carcinogen.



Beef is the food with the highest level of malonaldehyde, a formidable carcinogen, according to Cleveland Clinic biochemist Raymond J. Shamberger, Ph.D. Carcinogenic action may be thwarted when diet contains foods high in anti-oxidants.

Concentrations varied among different samples of the same foods, and small changes in cooking methods made great differences in the amount of malonaldehyde present in the food when preparation was complete. An uncooked pork chop containing 1.2 mg of malonaldehyde per gram contained only 0.4 mg./gram after it was cooked an hour at 425 degrees F, but a virtually identical chop, containing 1.3 mg of malonaldehyde per gram when raw, contained 8.1 mg per gram after being cooked for the same length of time at the same temperature but with a crumb coating.

These differences are small, but, repeated throughout a lifetime, they may determine whether an individual will develop cancer or not. "Malonaldehyde is present in feces, so we know that the entire human digestive system is exposed to it," says Dr. Shamberger. "Furthermore, we know that the incidence of many types of human cancer increases with age. Possibly digestive tract cancers are due to a dose-response effect, that is, an accumulation of malonaldehyde's actions in the body."

In his experiments with mice, Dr. Shamberger discovered that malonaldehyde reacts quickly with air and is oxidized to malonic acid. Within 10 minutes after it had been applied to the shaved and prepared skin of the animals, the chemical had turned the skin a bright orange color, probably reacting with protein and DNA to form Schiff base complexes which could start the cancer process. After an hour, less than 2% of the malonaldehyde remained on the skin.

"Since malonaldehyde is so reactive with the air, the mice we used in these experiments were actually exposed to very little of the chemical," Dr. Shamberger says. "In the body, of course, malonaldehyde is not exposed to the air, thus the entire GI tract is continually in contact with much higher concentrations of the chemical." Dr. Shamberger wants to proceed with additional experiments to determine the direct effect of malonaldehyde on the GI tract.

Malonaldehyde may turn out to be the link between GI cancer and diet

which has been strongly suggested by epidemiologic evidence, Dr. Shamberger believes. For example, the incidence of death from GI cancer is much higher among people who eat a lot of meat than among vegetarians and groups like the Mormons and Seventh Day Adventists whose meat intake is limited. Countries with high beef consumption, such as New Zealand and Argentina, have the highest rates of bowel cancer. European countries which have less refrigeration have a higher rate of death from stomach cancer than countries where refrigeration is widely used, thereby inhibiting the development of malonaldehyde in meat. And in the U.S., where northerners eat much more beef than southerners, northerners suffer from higher rates of bowel cancer. In each of these cases, the higher rates of cancer could be due to greater exposure to malonaldehyde.

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'Overwhelming' Evidence

Is malonaldehyde carcinogenic in humans?

"The evidence is really overwhelming, although I think more testing certainly needs to be done," says Dr. Shamberger.

"It really looks rather bad."

Surprisingly, although Dr. Shamberger's paper, "Initiating Activity of Malonaldehyde as a Carcinogen," was published in the *Journal of the National Cancer Institute* in December, 1974, the NCI has neither included the chemical on its list of carcinogens nor, says an NCI spokesperson, are there plans for further testing of its possible carcinogenicity in humans.

"Dr. Frank H. Mukai, at New York University, established that malonaldehyde is a mutagen, and that strongly suggests that it's a carcinogen right away, since over 90% of mutagens are also carcinogens," says Dr. Shamberger.

"Malonaldehyde is similar in structure to glycolaldehyde and propiolactone, but it has aldehyde groups on both ends of the molecule. It just screams, 'I'm a carcinogen!'"

GI Tract Cancer Laid to Substance In Meat, Poultry

Continued from page 13

dehyde, Dr. Shamberger believes

The carcinogenic action of malonaldehyde is inhibited by anti-oxidants, such as vitamins C and E, the element selenium, and the food preservatives BHT and BHA, says Dr. Shamberger. A study by Saxton Graham, Ph.D., of the University of Buffalo, showed that people who regularly ate raw vegetables such as cabbage and sliced tomatoes were significantly less likely to develop GI cancer. Since both foods are high in vitamin C, Dr. Graham says that Dr. Shamberger's findings "fit right in."

After extensive experiments using malonaldehyde on the skin of laboratory mice, Dr. Shamberger concluded that on its own, the chemical is a complete carcinogen on mouse skin—albeit a relatively weak one. Five of 30 mice treated with malonaldehyde developed cancers of the liver and rectum. But, Dr. Shamberger warns, "Because of the large quantities of malonaldehyde we consume, it should be considered a potential human carcinogen."

Potentiated Effect

Its action as a co-carcinogen is even more alarming. In another experiment,

Dr. Shamberger applied 6 mg of malonaldehyde to the skin of mice, then followed up with applications of croton oil, a substance which cannot, in itself, cause cancer, but which can render cells susceptible to the cancer-causing properties of other chemicals. When used in combination with croton oil, malonaldehyde caused tumors to develop in 53% of the test animals.

The carcinogenic action of malonaldehyde could be potentiated by other co-carcinogens to which the individual is exposed. Prime suspects, in Dr. Shamberger's opinion, are the bile acids—breakdown products of cholesterol in the digestive tract. "Bile acids have been shown to produce tumors in rat intestines, and we know that bile acids appear in higher concentrations in the feces of meat eaters than vegetarians," Dr. Shamberger notes. "A correlation between large bowel mortality and the consumption of dietary fat and oil has been observed. Malonaldehyde may be the link between fat consumption and carcinogenesis."

Dr. Shamberger says that he and his family have drastically reduced the amount of meat they eat since he began investigating malonaldehyde, while his collaborator, Dr. Charles E. Willis, has not reduced his own meat consumption at all.

"Life is a series of risks," says Dr. Shamberger. "If we have adequate information we can knowingly choose those we wish to encounter or avoid."



DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
ROCKVILLE MARYLAND 20852

5 JAN 1971

James S. Turner
Center for Study of Responsive Law
1156 19th Street, N.W.
Washington, D. C. 20036

Re: Petition to Halt the Use of the Food
Additive Brominated Vegetable Oil.

Dear Mr. Turner:

Brominated vegetable oils, derived from olive, corn, sesame, cottonseed, or soybean oil, have been used in food for almost fifty years. They are used to adjust the density of the flavoring oils in the manufacture of ice cream and bakery products and to stabilize the cloudy suspension in fruit-based beverages. In 1959, BVO was included on the GRAS list (21 CFR 121.101) because scientific studies and experience based on its common use in food provided a basis on which the Food and Drug Administration concluded that the substance would be generally recognized among qualified experts as safe for its intended use.

In 1969, new toxicity studies with BVO at the 0.5% and 2.5% level were reported by the Canadian Food and Drug Directorate. These studies revealed a variety of deleterious effects including degenerative myocardial changes. From that data we concluded that the then current level of use, approximately 150 parts per million (ppm) in beverages, could no longer be generally recognized as safe. On January 27, 1970, notice was published in the Federal Register deleting BVO from the GRAS list (35 F.R. 1049).

Thereafter, a food additive petition, submitted pursuant to 21 U.S.C. 348(b) by the Flavor and Extract Manufacturers' Association of the United States, proposed the issuance of a regulation permitting the use of BVO in a quantity not to exceed 15 ppm. The Food and Drug Administration carefully reviewed and evaluated the petition and the available scientific data. The data included the results of two unpublished studies that were conducted by the Canadian Food and Drug Directorate subsequent to the original study. In these studies, rats were fed brominated cottonseed, corn, olive, and sesame oils at 0.5%, 0.1%, and 0.02% of the diet for 105 days. At the 0.1% level, enlargement of the heart was not observed for any of the oils fed. In the case of the brominated sesame

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oil, lesions that could be classified as degenerative myocardial changes were seen in 2 of 15 rats. For the other brominated oils, no lesions were seen at the 0.1% level. There is question that a statistically significant effect could be attributed to this treatment, and, in fact, the Canadians have considered 0.1% as being without significant effect. At 0.02%, there was no noteworthy effect reported. Based on their tests, the Canadians have established a tolerance of 15 ppm.

The FDA also received reports of studies in England using rats and pigs. These studies do not reveal effects on the heart. However, studies of the animal tissue revealed tightly bound storage of organic bromine residues which did not disappear even upon starvation of the animals. Similar organic bromine residues were found in human tissues at levels much higher in countries using brominated vegetable oils (England and Holland) as compared to countries where these oils are not used (Germany). This tight binding in the fat depots prevented mobilization during starvation and indicated probable physiological inertness of this residue.

On the basis of the available evidence, together with BVO's long history of human use without report of harm and its anticipated levels and patterns of consumption, the Agency concluded that a "no effect level" could be established at approximately 0.1% (1000 ppm). Even if 0.02% is used as the "no effect level", an intake of one ten (10) ounce bottle of a beverage containing 15 ppm BVO would provide a safety margin of approximately 50. Considering the conservative interpretation of the available data and the long history of human use without report of harm, the Agency concluded that the additive is safe in an amount not to exceed 15 ppm in the finished beverage. A tolerance at that level was promulgated on an interim basis (21 CFR 121.1234), and notice was published in the Federal Register on July 28, 1970 (35 F.R. 12062).

In accordance with your petition, our Bureau of Foods has re-examined regulation 21 CFR 121.1234. They have again concluded that the available scientific evidence, together with BVO's long history of human use without report of harm and its anticipated levels and patterns of consumption, establish the additive to be safe when used as specified by the regulation.

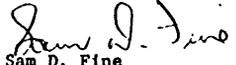
We have recently received an interim report from the Food and Drug Research Laboratories concerning a study where rats and dogs were fed brominated sesame and soybean oil for 90 days at levels of 40, 80, and 160 mg/kg body weight. This equates to approximately 800-3200 ppm for the rat and 1600-6400 ppm for the dog. This study has revealed no adverse effects to this date.

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We do not agree with the statement that the Federal Food, Drug, and Cosmetic Act does not permit the interim use of a food additive pending further toxicological studies. Section 409(c)(1) of the Act, 21 U.S.C. 348(c)(1), specifically permits the Secretary to establish regulations that prescribe the conditions under which the additive may be safely used. This allows the Agency to consider the safety of short term use while more definitive data to support long term use is in development. The Act does not require proof beyond any possible doubt that no harm will result under any conceivable circumstances. All that is required is proof of a reasonable certainty that no harm will result from the use of an additive. Here, the evidence available to us demonstrates with reasonable certainty that no harm will result from the interim use of brominated vegetable oil and that the public health is being protected.

Based on the foregoing reasons, your petition has been denied by the Commissioner.

Sincerely yours,


Sam D. Fine
Associate Commissioner
for Compliance

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The Delaney Anticancer Clause: A Model Environmental Protection Law

James S. Turner*

I. INTRODUCTION

In October 1969, the artificial sweetener cyclamate was banned from sale in the United States by Secretary of Health, Education, and Welfare Robert Finch. To justify his action legally Finch chose to rely¹ on the so-called Delaney Anticancer Clause of the Food, Drug, and Cosmetic Act of 1938. Consequently, the Delaney Clause, with its requirement that any substance producing cancer in animals be removed from the American food supply,² became an immediate center of controversy. The Secretary himself criticized the Clause as an undue restriction on administrative decision making and as an unscientific limitation on scientific discretion.³ When asked if the Delaney Clause should be modified, Food and Drug Administration Commissioner Charles C. Edwards reflected Secretary Finch's view in replying:

I think the scientific community is rather well split on this issue. There are those who feel that it is just what it ought to be right now. My personal view and that of the FDA is that we have to have more flexibility of interpretation or we are put into the position we were with cyclamates—all or nothing.⁴

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1. "I have acted under the provisions of . . . the so-called Delaney Amendment, enacted eleven years ago, which states that any food additive must be removed from the market if it has been shown to cause cancer when fed to humans or animals . . . because I am required to do so." Announcement of cyclamate ban, Press Release of Secretary Finch, Oct. 18, 1969, at 3.

2. "[N]o additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal . . ." Food Additives Amendment of 1958, § 409(c)(3)(A), 21 U.S.C. § 348(c)(3)(A) (1964).

3. "But who is to say that using Fresca or some other diet drink . . . isn't better for you than the problems of overweight or diabetes." *Finch Takes Position Against Delaney Clause*, FOOD CHEMICAL NEWS, Nov. 10, 1969, at 3.

4. Interview with Charles C. Edwards, Commissioner, Food & Drug Administration, in U.S. NEWS & WORLD REPORT, Apr. 19, 1971, at 52.

The contrary point of view was reported to the Surgeon General in 1970 by an eight-member committee of scientists with a staff of six senior scientists from the National Cancer Institute. After reviewing the state of cancer research and its relation to the Delaney Clause, the committee stated:

It is essential to recognize that no level of exposure to a carcinogenic substance, however low it might be, can be established to be a 'safe level' for man. . . . The current legislation in the field of food additives, with its 'anti-cancer clause', is based on this principle.⁵

Although the Delaney Clause has faced criticism from some quarters, careful analysis of the Clause reveals that it seems to serve well as a vehicle for the proper balancing of administrative discretion and scientific independence on one hand with public protection on the other; because of the analogous policy conflicts that arise in many areas of consumer concern, the Clause represents a valuable model for all environmental protection legislation.

II. THE STRUCTURE OF PROTECTION UNDER THE FOOD, DRUG, AND COSMETIC ACT OF 1938

Prior to the enactment of the Food, Drug, and Cosmetic Act of 1938, a food was considered adulterated, and therefore excluded from interstate commerce, if it contained any added poisonous or deleterious ingredient that might render it injurious to health.⁶ This state of the law proved to be unacceptable because, before a food could be barred from the national market, the Government had the obligation of showing affirmatively that it contained an added poisonous or deleterious substance which might be harmful under normal conditions of use.⁷ In passing the 1938 Act to alleviate this problem of proof, Congress altered food protection law in two ways, changing both essential definitions and basic operating procedures. First, section 402(a) redefined adulteration:

A food shall be deemed to be adulterated. . . . (2) if it bears or contains any added poisonous or added deleterious substance which is unsafe within the meaning of section 406. . . .⁸

An unsafe substance was defined in section 406(a):

5. National Institutes of Health & National Cancer Institute, Evaluation of Environmental Carcinogens, Apr. 22, 1970 (Report to the Surgeon General, USPHS, by the Ad Hoc Committee on the Evaluation of Low Levels of Environmental Chemical Carcinogens).

6. Food and Drug Act of 1906, ch. 3915, §§ 2, 7, 34 Stat. 768.

7. 1933 FDA ANN. REP. 14.

8. Food, Drug, and Cosmetic Act of 1938, ch. 675, § 402(a), 52 Stat. 1040.

Any poisonous or deleterious substance added to any food, except where such substance is required in the production thereof or cannot be avoided by good manufacturing practice shall be deemed to be unsafe for purposes of the application of clause (2) of section 402(a). . . .⁹

Secondly, procedures were prescribed that for the first time allowed poisonous or deleterious substances to be added to the food supply if the amount was within tolerances promulgated as safe by the Secretary.¹⁰ The new definition of adulteration, however, did not resolve the chronic burden-of-proof problem. Under the 1938 Act the evidentiary issue was simply moved back one step, and the Food and Drug Administration (FDA) found itself compelled to show affirmatively in the first instance that a particular chemical was poisonous or deleterious.¹¹

The difficulty in the application of section 406's test to various chemical substances arose because the drafters of the section attempted to define an acceptable level of human risk by utilizing the constructs "safe" and "unsafe." From the legislative history of the Act it clearly is demonstrable that by using the words "poisonous" and "deleterious"¹² Congress sought to designate all unsafe substances. Understood in this way, sections 402 and 406 form a legal non sequitur.

9. *Id.* § 406(a).

10. Food, Drug, and Cosmetic Act of 1938, § 409, 21 U.S.C. § 348 (1964). In approaching the problem of control from this angle, one Senate Committee Report stated: "[T]he amount of added poisons can be so allocated to different foods, in accordance with the practical necessities, that on the basis of the probable consumption of the various foods consumers will not receive an aggregate quantity of poisons sufficient to jeopardize health." S. REP. NO. 493, 73d Cong., 2d Sess. 4 (1934); see C. DUNN, FEDERAL FOOD, DRUG, AND COSMETIC ACT 113 (1938). In addition, the Senate Committee Report commented on the tolerance provisions as follows: "In promulgating such regulations this section requires that there be taken into account the extent to which the use of the poison is required in the production of the article, as for example, poisonous sprays in producing certain fruits and vegetables, and likewise, the other ways in which the consumer may be affected by the same or other poisonous or deleterious substances. This authorization will permit the establishment of comparatively liberal tolerances for any food where poison is unavoidable or is required by the necessities of production, and less liberal tolerances or complete prohibitions where it is practicable to limit the amount of poison in a particular food to [very] small quantities, or to eliminate it completely. It will likewise afford adequate control of those situations where irresponsible manufacturers, for some fancied or real commercial advantage, add dangerously toxic substances to foods, as, for example, the addition of maleic acid to fats and oils to prevent rancidity when preservation can be accomplished by observance of sanitary conditions in manufacture and packaging and by use of refrigeration for the finished product." S. REP. NO. 493, 73d Cong., 2d Sess. 4 (1934).

11. "Under the law as it was . . . [after 1938] the FDA could not stop the use of a chemical simply because it was questionable, or had not been adequately tested. It was necessary to be able to prove in court that the chemical was poisonous or deleterious." T. CHRISTOPHER, CASES AND MATERIALS ON FOOD AND DRUG LAW 468 (1966).

12. WEBSTER'S NEW INTERNATIONAL DICTIONARY (2d ed. 1957) defines "poisonous" as "[h]aving the properties or effects of poison;" *i.e.*, "[a]ny agent which, introduced . . . into an organism, may chemically produce an injurious or deadly effect." It defines "deleterious" as "hurtful," "noxious;" *i.e.*, "unwholesome."

The circular nature of the food protection device becomes evident when the word "unsafe" is substituted for the terms "poisonous" or "deleterious" as they occur in the Act. Section 402(a)(2) would read: "A food shall be deemed to be adulterated . . . if it bears or contains any added unsafe substance which is unsafe within the meaning of section 406." Section 406 would read: "Any unsafe substance added to any food, except where such substance is required in the production thereof or cannot be avoided by good manufacturing practice shall be deemed to be unsafe for the purposes of the application of clause (2) of section 402(a)." Manifestly, Congress attempted to devise a formula for establishing tolerances for poisonous—unsafe—ingredients in food. Just as clearly, however, by defining circularly the term "unsafe," Congress forced the FDA to prove in each instance the poisonous or deleterious nature of the chemicals. Often this placed the FDA in the position of attempting to answer legally, scientific questions unanswerable in the laboratory. The Food Safety Panel of the 1969 White House Conference on Food, Nutrition, and Health underscored the problem, stating: "It is not possible to determine with absolute certainty the safety of the ever-increasing number of chemicals added to or present in our foods."¹³ Because of its definitional difficulties, the 1938 Act, like its predecessor, proved to be ineffective and food protection problems increased.¹⁴

Faced with the nearly impossible task of establishing safety for every controversial chemical, the FDA once again sought changes in the law. Between 1950 and 1953 New York Congressman James J. Delaney conducted a series of hearings into the nature and use of chemicals added to the food supply.¹⁵ From these hearings three major pieces of legislation resulted: the Pesticide Amendments of 1954,¹⁶ the Food Additives Amendment of 1958,¹⁷ of which the Delaney Clause is

13. WHITE HOUSE CONFERENCE ON FOOD, NUTRITION AND HEALTH, FINAL REPORT 130 (1969).

14. The definitional problems could have been obviated if the section had been drafted without reference to the notion of safety. For example, it could have read "no chemical substance shall be added to any food, except where such substance is required in the production thereof or cannot be avoided by good manufacturing practice." The tolerance-setting procedure under this language would be used to determine whether a chemical was avoidable or was required in food production. This was apparently the very concept that Congress intended to introduce into the law. At this stage the FDA could defer to scientific judgments of safety when they existed.

15. See *Hearings on H.R. 74 Before the House Select Comm. To Investigate the Use of Chemicals in Food Products*, 81st Cong., 2d Sess. (1951).

16. Act of July 22, 1954, ch. 559, 68 Stat. 511 (now 21 U.S.C. § 346a (1964)).

17. Act of Sept. 6, 1958, Pub. L. No. 85-929, 72 Stat. 1784 (codified in scattered sections of 21 U.S.C.).

a part; and the Color Additive Amendments of 1960.¹⁸ The originally straightforward prohibition of unnecessary or avoidable poisonous or deleterious substances from food became the complicated prohibition of:

(A) . . . any added poisonous or added deleterious substance (other than one which is (i) a pesticide chemical in or on a raw agriculture commodity; (ii) a food additive; or (iii) a color additive) which is unsafe within the meaning of section 346 . . . or (B) if it is a raw agricultural commodity and it bears or contains a pesticide chemical which is unsafe within the meaning of 346a(a) . . . or (C) if it is, or it bears or contains, any food additive which is unsafe within the meaning of section 348¹⁹

Each piece of inserted language, covering pesticides, food additives, and color additives, represents an involved regulatory system spelled out in detail within the Act. The administrative discretion granted by this machinery requires the FDA to weigh the value of each proposed chemical use on a scale that balances the rights of the chemical producer against those of the general public; however, proof of safety remains the objective of each part of the Act.

The pesticide, food additive, and color laws all contain essentially the same regulatory structure, consisting of a chemical-by-chemical analysis by "the Secretary." This authority has been delegated to the Commissioner of Food and Drugs for food and color additives and to the Administrator of the Environmental Protection Agency for pesticide chemicals. In each case the process begins by the filing of a petition seeking a ruling by the Secretary that either allows the chemical to be used, or bars its use, in the ways sought by the petitioner. The decision of the Secretary comes in the form of an order that specifies the ways in which the chemical may be properly used. Detailed procedural rules govern the process that the Secretary and all interested parties must follow from the time the petition is filed until the time of a final order and dictate the way in which the appeals from the final order are to be brought to the attention of the courts.²⁰ It should be noted that the complex statutory apparatus leaves unsolved the definitional problems inherent in the use of the word "unsafe"—the same problem that caused

18. Act of July 12, 1960, Pub. L. No. 86-617, 74 Stat. 397 (codified in scattered sections of 21 U.S.C.).

19. 21 U.S.C. § 342(a)(2) (1964).

20. 21 C.F.R. § 120 (1971) (pesticides); 21 C.F.R. § 121 (1971) (food additives); 21 C.F.R. § 8 (1971) (color additives).

the 1906 and 1938 food protection laws to founder.²¹

III. THE PROOF-OF-SAFETY PROBLEM—UNSUCCESSFUL ATTEMPTS TO SOLVE IT

The Food Additives Amendment of 1958 contains three distinct attempts to alleviate the FDA's burden-of-proof problem: (1) the Generally Recognized As Safe (GRAS) approach that resulted in the GRAS list of chemicals approved by the FDA for addition to foods;²² (2) the Delaney Anticancer Clause that bans from food any substance which causes cancer when fed to animals;²³ and (3) the administrative structure that emanated from FDA regulations designed to evaluate item-by-item any chemicals which do not fall into either category one

21. One commentator described the Food Additives Amendment—and would probably say the same about the other 2 amendments—as “an example of law seeking to meet the problems that arise as side effects of scientific, economic and technological progress.” T. CHRISTOPHER, *supra* note 11, at 130. Actually it might be more accurate to say that these 3 amendments are examples of legislation seeking desperately to deal with the problems created by poor legislative drafting.

22. Section 201(s) of the 1958 Act reads: “The term food additive means any substance . . . not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures . . . to be safe under the conditions of its intended use” A parenthetical insert into this section set up a different standard for substances used prior to Jan. 1, 1958, saying “or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food.” 21 U.S.C. § 321(s) (1964). Essentially the same provision exists in both the Pesticide Amendment and the Color Additive Amendments. Section 408(a) of the 1954 Act reads: “Any poisonous or deleterious pesticide chemical, or any pesticide chemical which is not generally recognized, among experts qualified by scientific training and experience to evaluate the safety of pesticide chemicals as safe for use . . . shall be deemed unsafe . . . unless” 21 U.S.C. § 346a(1) (1964). Section 606(b)(4) of the 1960 Act reads: “[A] color additive shall be deemed to be suitable and safe for the purpose of listing under this subsection for use generally in or on food, while there is in effect a published finding of the Secretary declaring such substance exempt from the term ‘food additive’ because of its being generally recognized by qualified experts as safe for its intended use, as provided in Section [321(s)]” 21 U.S.C. § 376(b)(4) (1964).

23. The Delaney Clause for food additives is contained in § 409(c)(3)(A) of the Food Additives Amendment of 1958, 21 U.S.C. § 348(c)(3)(A) (1964). It is also repeated in the Color Additive Amendments of 1960, § 706(b)(5)(B), 21 U.S.C. § 376(b)(5)(B) (1964) that reads: “a color additive (i) shall be deemed unsafe, and shall not be listed, for any use which will or may result in ingestion of all or part of such additive, if the additive is found by the Secretary to induce cancer when ingested by man or animal, or if it is found by the Secretary, after tests which are appropriate for the evaluation of the safety of additives for use in food, to induce cancer in man or animal” Whether the Delaney Clause applies to pesticide chemicals is a more difficult question about which there is considerable controversy. The Secretary's Commission on Pesticides wrote as if the clause could be interpreted to apply to pesticide chemicals; however, the definition of food additives expressly excludes “a pesticide chemical in or on a raw agricultural commodity” Food Additives Amendment of 1958, § 201(s)(1), 21 U.S.C. § 321(s)(1) (1964). Since there is no anticancer clause in the Pesticide Amendment, it would appear that pesticides do not fall under the prohibition of the Delaney Clause.

or two.²⁴ Each of these three legal stratagems endeavored to circumvent the problem of scientific uncertainty, but only the Delaney Clause succeeded. Before detailing the accomplishments of the Delaney Clause, the reasons for the failure of the other two mechanisms should be outlined for comparative purposes. Legislation that effectively controls chemical contamination of the environment must seek to block the use of substances that present undue risk without putting unreasonable restraints on chemicals that provide important benefits to the public. To initiate the GRAS procedure, the Food and Drug Administration asked 900 scientists to comment on the safety of the first substances proposed for the GRAS list. Rather than achieving the scientific consensus assumed possible by the GRAS theory, the FDA harvested a scattering of opinions. Of the 900 scientists questioned, 350 replied with only 194 or 21 percent of the total group ratifying the entire list. The performance of the FDA in accurately predicting the safety of specific chemicals, even after some doubt had been raised, was similarly imperfect. The FDA, for example, dismissed the complaints of a number of scientists against safrole,²⁵ vitamin D, and most notably cyclamate, only to find it necessary to act against the challenged chemicals in subsequent years. Thus the GRAS list mode of procedure proved to be ineffective in discriminating between safe and unsafe substances because the system presented the same problem of scientific choice that the earlier acts had been unable to deal with. Where the Agency earlier had tried to choose which chemicals and which foods were safe, it now foundered trying to choose which scientists were the best judges of safety. An FDA memorandum spelled out the guiding principle of this choice.

In our final evaluation of the safety of a substance we have taken cognizance of the fact that all opinions are not of equal value and thus have weighed most heavily the opinions of scientifically recognized and often world-renowned experts.²⁶

Under this pick and choose procedure the basic GRAS list grew to approximately 700 items with various loopholes and exceptions allowing as many as another 1,000 items to be treated as on the GRAS list by the FDA.²⁷ Food manufacturers, faced with a minimum of an estimated

24. This is the regulatory procedure outlined above and is essentially the same for pesticide chemicals, food additives, and color additives.

25. "Safrole" is the ingredient used for flavoring in root beer.

26. FDA Div. of Pharmacology & Food Memorandum, Sept. 2, 1959.

27. The details of this situation are spelled out in J. TURNER, *THE CHEMICAL FEAST, THE RALPH NADER STUDY GROUP REPORT ON FOOD PROTECTION AND THE FOOD AND DRUG ADMINISTRATION* 153-59, 162-63 (1970).

two years of study²⁸ before gaining permission to market a new additive, sought to achieve recognition of their chemicals through the loopholes in the GRAS list procedure. By the end of 1970 the situation had become so unwieldy that the Agency moved to revise the entire GRAS procedure by attempting to reintroduce suspect chemicals currently on the GRAS list into the chemical-by-chemical investigation.²⁹

As previously noted, the chemical-by-chemical procedure relies on the ability of scientists to distinguish safe from unsafe substances. That portion of the Act authorizing this approach states: "No . . . regulation shall issue if a fair evaluation of the data before the Secretary—(A) fails to establish that the proposed use of the food additive, under the conditions of use to be specified in the regulation, will be safe. . . ."³⁰ All parties to the discussion of the 1958 Food Additives Amendment accepted the assumption that safety or the lack of it could be established in each case, and accordingly, the FDA issued a regulatory definition of safety that said, "'Safe' means that there is *convincing evidence* which establishes with reasonable certainty that no harm will result from the intended use of the food additive."³¹ Faced with reviewing the GRAS list that contained many items for which scant, controversial, or no evidence existed, the FDA, interestingly enough, moved to redefine "safe." "'Safe' must be understood to connote that the Food and Drug Administration, after reviewing *all available evidence*, can conclude there is no significant risk of harm from using the substance as intended."³² This second definition allows untested or only partially tested chemicals to be added to the food supply, while the former definition required the initial presentation of some convincing evidence of safety. The change in definition represents a significant erosion of the safety concept, one of the unfortunate side effects that results when a regulatory agency expected to enforce policy is required to resolve scientific conflicts. The Surgeon General's committee on low-level

28. *Hearings on H.R. 8112 Before a Subcomm. of the House Comm. on Interstate and Foreign Commerce*, 85th Cong., 1st & 2d Sess. 60 (1957-58) (remarks of FDA Comm'r Larrick).

29. Food Additives, 35 Fed. Reg. 18,623 (1970).

30. Food Additives Amendment of 1958, § 409(c)(3)(A), 21 U.S.C. § 348(c)(3)(A) (1964).

31. 21 C.F.R. § 121.1(i) (1971) (emphasis added). Commenting on the safety provision, Charles Wesley Dunn, the General Counsel for the Grocery Manufacturers of America stated: "Such [a] requirement is basically a pretesting one for new food additives. . . . Whereas the FDC Act now prohibits a food that is unsafe, this prohibition normally applies after the food is sold and consumed, and its enforcement may be long delayed for various reasons. . . . [m]oreover in such an enforcement proceeding the Government has the burden of proving that the food is unsafe, whereas this requirement would instead compel the manufacturer of a food to prove in advance that it is safe." *Hearings on H.R. 8112 supra* note 28.

32. Food Additives, 35 Fed. Reg. 18,623, 18,624 (1970) (emphasis added).

carcinogens demonstrated the folly of the FDA's new safety definition. It reported that bioassays are incapable of detecting carcinogenic effects below the ten percent level, and therefore so-called negative data are grossly inadequate to give assurances of safety for man.³³ More importantly, leading scientists³⁴ are increasingly making this same argument about the chemicals related to genetic problems, birth defects, and mental retardation. The current FDA attempt to revise the GRAS list and its redefinition of safety concede the difficulty of giving empirical meaning to the term "unsafe" while the whole area is the subject of scientific controversy. This difficulty is further demonstrated by the FDA's new interim regulation policy.

If after a responsible and substantial question of safety has been raised regarding a substance previously listed as GRAS the main weight of the scientific evidence still indicates safety (at least within certain limits), an interim food additive regulation will be proposed. This will permit further scientific investigations to define the conditions of safe use for a food additive regulation of indefinite duration.³⁵

This statement seems to be at variance with the provision of the Act that requires that "no such regulation shall issue if a fair evaluation of the data before the Secretary—(A) fails to establish that the proposed use of the food additive, under the conditions of use to be specified in the regulation, will be safe"³⁶ The FDA, however, argues that an interim time period serves merely as one more condition of use under the law, and this interpretation has been upheld in federal district court.³⁷ The practice of issuing interim regulations further erodes the assumption that the food supply contains only safe chemicals.

The FDA, after 65 years of failure, still struggles to solve scientific controversies about safety with legal tools. One apparently overlooked fact underlies this struggle. When scientists agree that a chemical is either safe or unsafe, no controversy about its use erupts. Only when a scientist challenges the label of "safe" attached to a chemical or class of chemicals by other scientists does the FDA engage its balancing mechanism. Otherwise chemicals enter the food supply virtually

33. National Institutes of Health & National Cancer Institute, *supra* note 5.

34. Examples of scientists who are concerned with chemicals causing birth defects and genetic damage include Dr. Samuel Epstein of Case Western Reserve University, Dr. James Crow of the University of Wisconsin, Dr. John W. Olney of Washington University, and Dr. Marvin Legator of the FDA.

35. Food Additives, 35 Fed. Reg. 18,623, 18,624 (1970).

36. Food Additives Amendment of 1958, § 409(c)(3), 21 U.S.C. § 348(c)(3) (1964).

37. The oral opinion of Judge Gerhard Gesell was reported in FOOD CHEMICAL NEWS, July 12, 1971, at 17.

unnoticed. As a result, whenever it enters a controversy the FDA overrules one set of scientifically supported arguments with a legal or regulatory judgment.

The twisting and turning of the food and drug laws since 1906 resulted from using the word "safety" to denote two distinct concepts. First, it includes the scientific observation that a chemical additive or food does not cause damage to humans. Secondly, it includes the policy judgment that even though a chemical might cause injury to a human, the damage it causes is outweighed by the benefits it imparts. Only the Delaney Clause of the Food and Drug Act escapes this pitfall by avoiding any reference to either concept of safety. Instead, it allows scientists to ascertain the degree of risk presented by the use of a particular chemical and assigns policy makers the task of judging whether the scientifically defined risk is acceptable to society. For this reason it serves as a model for all other environmental protection legislation. Despite the simple logic underlying the Clause, and despite its ready applicability to other regulatory fields, this Clause has often been misunderstood by regulators and the public alike.

IV. THE DELANEY CLAUSE: A MODEL FOR ENVIRONMENTAL PROTECTION LEGISLATION

A. *Misunderstanding the Delaney Clause*

Food and Drug Commissioner Charles C. Edwards restated accurately the misunderstanding of the Delaney Clause when he said of it:

My personal view and that of the FDA is that we have to have more flexibility of interpretation or we are put into the position that we were with cyclamates—all or nothing. And it becomes a highly emotional issue at that point, allowing no discretion on our part or anyone else's.³⁸

This statement implies that but for the Delaney Clause the FDA would have allowed cyclamates to remain in the food supply in some amount even though this chemical causes cancer in rats. The Commissioner's characterization of the Delaney Amendment as a usurpation of administrative discretion is incongruous because other parts of this food protection law, although operating more slowly than the anticancer clause, also would have required cyclamates to be completely banned from the food supply. At the onset of the cyclamate controversy, the chemical was generally recognized as safe by the FDA. After a

38. See Interview with Charles C. Edwards, *supra* note 4.

substantial safety question was raised, the Secretary officially removed cyclamates from the GRAS list. At this point the law, absent the Delaney Clause, requires that the chemical be shown to be safe before a petition can be granted allowing its addition to food.³⁹ In view of the state of scientific knowledge about cancer causing substances, it is unlikely that cyclamate could have met this burden of proof; therefore, the chemical could have been removed from the food supply without reference to the Delaney Clause. In fact, some of the most vigorous critics of the Delaney Clause call it an unnecessary duplication of already existing authority.

When the Commissioner asks for "discretion" to decide when a chemical that causes cancer in animals can still be used in food for man, he is asking for the discretion to decide an issue that thousands of cancer researchers have been unable to resolve. The dangers of this position were put forth accurately by former Secretary of Health, Education and Welfare, Arthur S. Flemming:

The rallying point against the anticancer provision is the catch phrase that it takes away the scientist's right to exercise judgment. The issue thus made is a false one, because the clause allows the exercise of all the judgment that can safely be exercised on the basis of our present knowledge. The clause is grounded on the scientific fact of life that no one, at this time, can tell us how to establish for man a safe tolerance for a cancer-producing agent.

.

As I pointed out in my original testimony, the opposition to inclusion of an anticancer clause arises largely out of a misunderstanding of how the provision works. It allows the Department and its scientific people full discretion and judgment in deciding whether a substance has been shown to produce cancer when added to the diet of test animals. But once this decision is made, the limits of judgment have been reached and there is no reliable basis on which discretion could be exercised in determining a safe threshold dose for the established carcinogen.⁴⁰

The fact that the country's highest food and drug officials still believe that this kind of discretion should be granted demonstrates the need for more effective policy setting by Congress.

39. Food Additives Amendment of 1958, § 409(c)(3), 21 U.S.C. § 348(c)(3)(A) (1964).

40. *Hearings on H. R. 7624 Before a Subcomm. of the House Comm. on Interstate and Foreign Commerce*, 86th Cong., 2d Sess. 501 (1960). The members of the committee that reported to the Surgeon General on low levels of environmental carcinogens considered the arguments made by Secretary Flemming so important that they inserted the entire statement of the former Secretary in their report. Following the statement they added this note: "The scientific basis on which the

B. Expanding the Delaney Clause to Other Areas of Environmental Protection Legislation

From the FDA's experience in attempting to differentiate between safe and unsafe substances, it seems apparent that in order to shield the environment from further chemical contamination, the policy issues and the scientific issues, although interrelated, must be approached separately. The report to the Surgeon General on environmental carcinogens clearly defined the problem and divided the scientific and policy responsibility. "While science can provide quantitative information regarding maximum risk levels, the task of ultimately selecting socially acceptable levels of human risk rests with society and its political leaders."⁴¹ The role of the scientist is to describe physical phenomena—this chemical caused lesions in mouse brains under these conditions; that chemical caused cancer when fed to mice in certain quantities; those chemicals caused birth deformities when injected into chickens in designated amounts at certain ages. Scientists can offer less definite but still important scientific opinions on the degree to which damage to man can be predicted from damage to animals. Without knowing the levels of risk that society will tolerate, however, scientists cannot effectively differentiate between "safe" and "unsafe" substances.

Congress, on the other hand, taking into consideration the certainty or relevancy of the scientific findings, must set broad policy guidelines. Several issues suggest themselves as important for the consideration of the nation's policy-makers. Which purposes served by chemicals are worth the apparently increasing risk of their use in foods? Resolving this issue involves a reassessment of the "required for" or "unavoidable in" food production concept of section 406. If additional uses of chemicals are found necessary to improve the food supply, these concepts could be expanded.⁴² In addition, Congress must determine

Government's position was established in 1960 remains valid. The progress of knowledge in carcinogenesis in the last decade has only strengthened the points made in Secretary Flemming's testimony." National Institutes of Health & National Cancer Institute, *supra* note 5.

41. National Institutes of Health & National Cancer Institute, *supra* note 5, at 14.

42. The Food Safety Panel of the White House Conference suggested some additional criteria that Congress might consider: "[That] no additional chemicals should be permitted in or on foods unless: They have been shown with reasonable certainty to be safe on the basis of the best scientific procedures available for the evaluation of safety and meet one or more of the following criteria:

1. They have been shown by appropriate test to be significantly less toxic than food additives currently employed for the same purpose.
2. They significantly improve the quality or acceptability of the food.
3. Their use results in a significant increase in the food supply.
4. They improve the nutritive value of the food.

which extrapolations from animals can be made to man. In the cancer area it is policy that if a chemical affects animals it will not be given to humans.⁴³ This practice was adopted because under the present state of scientific knowledge a safe tolerance for man of a substance that produces cancer in animals cannot be established.⁴⁴ What chemicals should be added to the "zero tolerance" list now containing only carcinogens? Already chemicals causing birth defects and genetic damage in animals have been suggested for addition to the list. Congress must collect and review the evidence that other irreversible biological damage can be caused by chemicals and set a "zero tolerance" policy for these areas where necessary.

The Delaney Clause can serve as a model for environmental protection legislation because it delegates to scientists the responsibility for making scientific judgments and to Congress the task of making policy decisions. The scientist, after an analysis of all technical data, specifies the degree of risk that would result if any amount of known carcinogens were allowed in the nation's food supply; Congress, after considering all other relevant information, determines that the risk is unacceptable. The FDA is then charged with the responsibility of removing carcinogenic chemicals from the food supply. The procedure outlined for developing a new food protection or any other environmental protection law should not include any effort to define "safety." Rather, scientists should describe a degree of risk as accurately as science allows. Congress then should decide whether that risk is worth taking. To begin the development of a more effective food protection law, the report to the Surgeon General enunciated one additional fundamental point. "Chemicals should be subjected to scientific scrutiny rather than given individual 'rights': they must be considered potentially guilty unless and until proven innocent."⁴⁵ The authors of that report directed their comment at carcinogens, but the same observations may now be made for chemicals relating to genetic damage or birth defects.

V. CONCLUSION

The nearly uninhibited addition of chemicals to the environment for the last several decades lies at the heart of the so-called

5. Their use results in a decrease in the cost of food to the consumer." WHITE HOUSE CONFERENCE ON FOOD, NUTRITION AND HEALTH, *supra* note 13.

43. Food Additives Amendment of 1958, § 409(c)(3), 21 U.S.C. § 348(c)(3)(A) (1964).

44. National Institutes of Health & National Cancer Institute, *supra* note 5, at 15.

45. *Id.* at 15.

environmental crisis. To control this use of chemicals requires a new combination of scientific expertise and legal policy. The drafters of the Delaney Clause of the current food protection law were successful in writing into that legislation a proper balancing of the policy function and the scientific function. Congress heard scientists describe the level of known and unknown risk associated with cancer causing chemicals. It set the policy that no chemical known to cause cancer in animals would be allowed in the food supply. The regulatory agency was assigned the scientific task of distinguishing those chemicals that cause cancer in animals from those that do not. The Delaney Clause sets clear public policy and allows complete scientific freedom.

Congress, by setting the public policy concerning cancer causing chemicals itself and by assigning the scientific implementation of that policy to the agency that regulates food, established a procedure for effectively weighing environmental dangers and acting to prevent them. All chemicals—whether they be pesticides in or on foods, industrial chemicals that contaminate the water or air, hazardous substances that are used in the home, or any one of hundreds of other environmental pollutants used in this society—must be subjected to a rationalized policy. Congress, guided by the state of scientific knowledge, must place limits on the risks to be assumed by society; the appropriate regulatory agency, again guided by scientific research, must not allow that established risk to be exceeded. This is the principle of the Delaney Clause, and for this reason the Delaney Clause serves as a model for other environmental legislation.

Center for Science in the Public Interest

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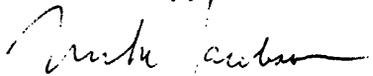
December 21, 1976

Ms. Judy Robinson
c/o Senator Gaylord Nelson
U. S. Senate
Washington, D.C. 20510

Dear Judy:

As per your request, enclosed is a self-explanatory document that lists current problems and suggested remedies in the area of food additives. I hope this will be helpful as you develop plans for hearings on food additives.

Sincerely,



Michael Jacobson, Ph.D.
Co-Director

Enclosure

Center for Science in the Public Interest

1757 S Street, N.W. • Washington, D.C. 20009
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Food Additives: Current Problems and Suggested Statutory Changes

1. Particularly questionable food additives include: sodium nitrite (carcinogen), Red No. 40 coloring (carcinogen), Orange B (inadequately tested), caffeine (possible teratogen), Yellow No. 5 coloring (allergen), saccharin artificial sweetener (possible carcinogen).
2. 3rd Party testing of additives: a major problem of food additive regulation is that the company that seeks to profit from the manufacture of the additive is also in charge of the testing of the chemical; the natural incentive is to do minimal testing and overlook possible problems. Inserting an intermediary between the manufacturer and the testing laboratory, and developing appropriate standards for laboratories and their personnel, would help insure that the testing is done more honestly and without as great a conflict of interest.
3. Jurisdiction of regulations: Though FDA has jurisdiction over the bulk of food additive regulation, USDA and BATF (Bureau of Alcohol, Tobacco, and Firearms; Department of the Treasury) regulate the use and labeling of additives in meat-containing products and alcoholic beverages, respectively. FDA should be given specific statutory jurisdiction over all food additive regulations, inasmuch as, in theory at least, only FDA has the qualifications and independence to regulate fairly on behalf of the public's best interests. FDA can do little to get USDA to tighten regulations on the use of nitrite in cured meats, and BATF/industry have stymied FDA's desire to require all ingredients to be listed on alcoholic beverage labels.

- * 4. Further testing of approved food additives: Once a chemical is approved for use as a food additive, it is difficult for FDA to require further testing of it or to limit or ban its use. Orange B is a case in point: it is an abysmally tested synthetic food coloring, yet FDA cannot ban it, because it has no evidence of hazard. In a letter, FDA said it would not require further testing unless someone provided evidence of hazard. In other words, once a chemical is listed, the manufacturer is home scot free. There are several possible ways of handling this problem. (1) FDA should be given the legal authority to require manufacturers to conduct further tests as new potential problems are recognized and as testing standards are raised; further testing would not be considered an indictment of a chemical, but merely an updating of the information on hand. (2) A much more effective way of solving this problem would be to license a food additive for a given period, say 5, 7, or 10 years, at which time the manufacturer would have to renew its application, supplying any new test data that would be needed to bring the test data up to current standards; this would insure that long-used additives are as well tested as new ones. This is probably the most important new legislation that could be adopted.
5. Ingredient labeling: Many foods for which standards of identity have been adopted are not required to bear full ingredient listings on their labels; additionally, a Federal Court in Kentucky held that BATF, not FDA, had jurisdiction over the labeling of alcoholic beverages, so such foods do not bear ingredient listings. Artificial colorings, flavorings, and spices are generally not listed by name. Current laws should be

changed to require all ingredients to be listed on standardized foods and alcoholic beverages. Artificial colorings should be listed by specific name (Red No. 3; beet juice extract; etc.).

6. Artificial Coloring labeling: In the past several years it has become quite clear that artificial colors are the most questionable category of food additives. Violet No. 1, Red No. 2, Red No. 4, and Red No. 40 have all been called into question. There is also some evidence that dyes contribute to hyperactivity in children. The presence of synthetic dyes should be called to the buyer's attention more effectively than simply by being listed in small type on the ingredient listings. All foods containing synthetic dyes should bear a prominent symbol, perhaps a black square with the words "artificial coloring" placed above or below the symbol. This informative mark would make it easier for people to select or avoid foods colored with synthetic dyes.

7. Imminent hazard: If the Commissioner of FDA determines that a particular chemical is carcinogenic and proposes a ban under the DeLooney amendment, industry can employ a variety of legally provided delaying measures to postpone the actual ban. The delaying measures include several 30-60 day periods for comment, having a committee of the National Academy of Sciences evaluate the evidence, holding an administrative hearing. This lengthy process could be short-circuited, if FDA chose to consider the presence of a carcinogen in our food an "emergency" and used the appropriate portion of section 706(b) to outlaw the chemical immediately. FDA apparently believes that a carcinogen is not an "imminent hazard" because the carcinogenic effect is not observable for many years, or even

decades, after exposure. The Court of Appeals has held that carcinogenic pesticides do represent imminent hazards. The law should define a carcinogen as representing an imminent hazard to the public.

8. The Delaney amendment: The Delaney amendment currently applies only to carcinogens. It is possible that rodent testing systems are now available that would permit this section of the law to be applied to mutagens. The Delaney amendment should not be revised to include teratogens.

9. The Courts have upheld FDA's authority to put additives in an "interim approval" category that is not specified in the law. The problem with this category is that it permits the public to be exposed to chemicals that are not adequately tested and for which some evidence of hazard might exist. If FDA is going to continue using this questionable legal status, the law could require interim status additives to be identified as such on food labels. A label might read: "sodium saccharin (U. S. Gov't. Does Not Consider This Additive To Be Adequately Tested)."

10. Consumer advocate within FDA: In the last ten years, many inadequately tested or apparently hazardous food additives (nitrite, Violet No. 1, Red No. 2, Red No. 40, caffeine, BVO) and pesticides (aldrin, dieldrin, heptachlor, 2, 4, 5-T) have been identified and brought to the public's attention not by government or industry, but by privately funded public interest groups. The reason these groups have been effective is because they have seen their goal as ferreting out dangers to the public's health, and because they have not been encumbered by lumbering bureaucracies or by the need to cover-up past mistakes. It would make an interesting, inexpensive experiment to

have a full-time "food additive cop" within FDA; this person would be independent in the sense that he or she would work directly in the office of the Director of the Bureau of Foods and would be charged with bringing important or suppressed findings to the public's attention.

CENTER for SCIENCE in the PUBLIC INTEREST

1757 S Street, Northwest; Washington, D.C. 20009 - (202) 332-4250

May 6, 1976

Alexander Schmidt, M.D.
Commissioner, Food and Drug Administration
Parklawn Bldg. - 5600 Fishers Lane
Rockville, MD 20852

Dear Dr. Schmidt:

Thank you for taking the time to respond to my letter urging FDA to ban two inadequately-tested food colorings, Orange B and Red No. 40. It seems, though, that the Bureau of Foods and you completely ignored or missed the point I was making. Your second paragraph begins: "I would agree with your evaluation that the data available to support the safety of these dyes do not meet present day standards." In fact, what I contended in my letter of February 25th was that "Red No. 40 was not tested in the number of species used by FDA itself or recommended by several authoritative committees. Some of these recommendations had been made prior to the testing of Red No. 40."

As I noted in my letter the Food Protection Committee of the National Academy of Sciences-National Research Council recommended back in 1952--long before Red. No. 40 was even a gleam in a chemist's eye--that tests involve feeding the test substance to rats and mice for their normal life spans and to dogs for at least five years.

Although the tests on Orange B started out well, they ended up a disaster. So many animals died that the experiment was stopped prior to the intended two years. Only four (!) rats per test group were examined at the end of 22 months. This was a lousy study by today's standards or by 1950s' standards.

I believe that if FDA wished to protect the consumer it could and should outlaw these dyes, though to do so would require admitting past mistakes. A further reason for banning Red No. 40 immediately is the fact that in the current study on Red No. 40, as you wrote, "six of the animals had developed premature and unexpected malignant lymphomas." The fact that other animals sacrificed by Allied Chemical did not have tumors did not make the six tumor-laden animals healthy again.

Sincerely yours,

Michael F. Jacobson, Ph.D.
Co-Director

CENTER for SCIENCE in the PUBLIC INTEREST

1757 S Street, Northwest, Washington, D.C. 20009 - (202) 332-4250

February 25, 1976

Alexander Schmidt, M.D.
Commissioner
Food and Drug Administration
Parklawn Bldg.
5600 Fishers Lane
Rockville, MD 20852

Dear Dr. Schmidt:

An FDA study revealed that the food coloring Red No. 2 may be linked to an increase in malignant tumors in laboratory animals. FDA has banned the coloring pending substantiation of its safety. It is now urgent that FDA re-examine the safety and regulatory status of two other artificial food colorings, Red No. 40 and Orange B.

Red No. 40

Red No. 40 is the most recent artificial coloring to be permitted in the food supply (1971). It was not widely used until studies suggested that Red No. 2 might not be harmless, at which time many manufacturers switched to this coloring. Until 1973, Red No. 40 was mixed with Violet No. 1 to create a color quite similar to that of Red No. 2, but FDA banned Violet No. 1 in 1973 because of a carcinogenic hazard. Red No. 40 is now mixed with other colors and is one of the major colorings in our food supply. In fiscal year 1975, 788,000 pounds were certified for use, making it the fourth most widely used coloring. Barring unforeseen developments, this poundage can be expected to increase dramatically in the next year as food manufacturers shift from Red No. 2 to Red No. 40.

Although Red No. 40 was one of the first, if not the first, coloring to be tested for its effects on the reproductive process, tests for possible carcinogenesis are woefully inadequate. In fact, so inadequate are the tests that the Canadian government has never authorized the use of Red No. 40 and the manufacturer, Allied Chemical, has had to initiate further long-term feeding tests.

Red No. 40 was tested for carcinogenic effects on only one species, the rat, despite the recognition that testing only one species does not provide an adequate basis for evaluating safety. A two-year study was conducted on dogs, but this is not a lifetime feeding study that could be relied upon to detect carcinogenesis. The "Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health" (page 467) recommended in 1969

that tests involve:

Adequate numbers of animals of at least two species and both sexes with adequate positive and negative controls, subjected for their lifetime...

In 1959 the Food Protection Committee of the National Academy of Sciences-National Research Council recommended:

Continuous feeding of various levels of the test substance to rats and mice for their normal life spans and to dogs for at least 5 years ("Problems in the Evaluation of Carcinogenic Hazard from Use of Food Additives," Publication 749).

Similarly, the Joint FAO/WHO Expert Committee on Food Additives recommended in its "Evaluation of the Carcinogenic Hazards of Food Additives" (Technical Report Series No. 220; 1961):

Both sexes of each of at least two species of animals should be used in the tests throughout their life span.

FDA itself conducted safety evaluations of several food colorings in the early 1960s, long before Red No. 40 was tested by its manufacturer. As summarized in "Food Colors," a pamphlet published by the National Academy of Sciences, FDA conducted short-term feeding studies, which, in turn, were

used to determine dosage levels for the examination of chronic effects. These latter studies, performed with two or three species...All animals in the chronic study were maintained on experimental or control diets for a minimum of 2 years...

It is clear that Red No. 40 was not tested in the number of species used by FDA itself or recommended by several authoritative committees. Some of these recommendations had been made prior to the testing of Red No. 40.

In addition to the deficiency of having tested only one species of animal in tests designed to detect carcinogenesis, many of the rats developed respiratory disease beginning in the sixth week of the test, resulting in the premature death of many of the rats in control and experimental groups. An antibiotic was administered to the rats in the 56th week, a further disturbance of the protocol. Because so many animals succumbed to the disease, the "two-year" study was terminated at 21 months. Apparently, only about 19 animals in the high dosage group and 20 animals in the other groups were still alive at that time--half as many as planned. Thus, the safety of this coloring is based on 19 animals of one species tested for 21 months. Considering that Red No. 40 will be one of the two or three major colorings in our food supply and that over the years artificial colorings have been the most hazardous kind of direct food additive (butter yellow, Green 1, Orange 1, Orange 2,

Red 1, Red 2, Red 32, Sudan 1, Yellow 1, Yellow 2, Yellow 3, Yellow 4, and Violet 1, which were once permitted in our food supply, have been deemed unsafe by FDA and banned), the manufacturer's tests supporting the dye's safety are woefully inadequate.

The inadequacy of the "long-term" feeding study has been recognized by FDA's Acting Director of Toxicology, H. Blumenthal, who recommended to Allied Chemical, that if the company conducts additional tests, it should concentrate on chronic feeding studies, rather than reproduction studies. According to a memo (January 23, 1974) written by Blumenthal regarding a conference he had with Allied Chemical representatives:

We all were aware, for example, that their chronic rat study had been plagued by disease and, therefore, the rats did not live as long as one might have hoped. I would rather see resources put into repeat of this kind of study or new kinds of work rather than to back track over data which has been done by the FDA and found to raise no questions.

Prior to final approval of Red 40 by FDA, Allied Chemical was touting its new color in the trade literature, and leading industry to believe that its safety testing was extraordinarily thorough. For example, according to a May, 1971 article in Softdrinks,

According to Allied sources, Allura Red AC has undergone one of the most extensive batteries of testing ever used for a food colorant. The screening included two series of feeding tests (one lasting two years; another lasting five years).

The "two-year study" apparently refers to the 21-month rat study. No reference to the five-year study was included in FDA's February 7, 1972, "FD & C Red No. 40 - FDA's Summary of Toxicological Evaluation," and it must be concluded that no such study was done. At a time of great concern about the safety of food additives, this kind of information in the trade literature probably contributed to the food industry's quick acceptance of this new coloring.

If the safety of the American public were the paramount consideration, the most prudent course of action would be for FDA to begin proceedings to rescind its previous approval of Red No. 40 and to warn the public to avoid artificially colored foods.

Orange B

Orange B is a coal tar dye that is permitted at levels up to 150 parts per million in sausage casings. Approximately 20,000-30,000 pounds are certified for use each year. Orange B is an azo dye, and closely related to Red No. 2. In fact, one of the two primary metabolites of Orange B and Red No. 2 are identical. If FDAC Red No. 2 is carcinogenic, there is a fifty percent chance that Orange B is also carcinogenic.

The tests on which the safety of Orange B was judged were conducted

twenty years ago, and although they undoubtedly looked adequate at the time and may have seemed good according to the contemporary standards, they look woefully inadequate in light of current standards and the new findings regarding Red No. 2.

Long-term feeding studies were conducted on rats, two strains of mice, and dogs. The value of these studies is severely reduced, because very few of the rodents survived to the desired two-year mark. For example, of the rats whose diet contained 2% Orange B, none survived to two years. At 22 months, the following numbers of animals in the various dosage levels were given a histologic examination, which itself was not thorough:

<u>dosage level</u>	<u>number of animals examined at 22 months</u>
controls	4
0.5%	4
1%	3
2%	4
5%	4

Carcinogenicity would be undetected in this rat study, if as many as one out of five animals develops cancer, when fed Orange B at a level of 5% of their diet. One would have liked at least ten times as many animals to survive to at least two years.

In the 8th quarterly progress report filed with the manufacturer of Orange B (Stange Company), Prof. K.B. DuBois wrote:

The feeding of Orange B to rats at levels up to 5% does not appear to have produced any adverse effects on the histopathological appearance of the tissues studied. It should be emphasized, however, that both the number of tissues examined and the number of animals sacrificed in each group are relatively small and do not, therefore, permit definitive conclusions. (page 149 of petition)

The long-term feeding studies on mice suffer the same limitation as the rat study: very few animals survived to two years. Thus, after eighteen months, the mice whose diet contained 5% dye, only 8 were examined microscopically, and only 4 mice from the 1% group and control groups were examined. This is a wholly unsatisfactory number, particularly in light of Orange B's close structural relationship to Red No. 2 and the cloud of doubt which surrounds all coal tar dyes.

The inadequacy of the Orange B studies was apparently recognized even by FDA. In 1971 I talked with FDA's Dr. Charles Kokoski about Orange B. A January 19, 1971, memo prepared by Dr. Kokoski after the meeting noted:

Dr. Jacobson asked why there were so few rats and mice actually examined histologically. I explained that these studies were completed in the 1950s, a time when most investigators examined microscopically only a representative sample of tissues unless unusual toxicity or lesions became evident. All animals... were examined grossly for tumors or unusual lesions.

In that same memo, Dr. Kokoski noted that:

Further support of safety is the fact that the two metabolites (products of azo reduction) are the same with FD & C Red No. 2 and FD & C Yellow No. 5, both extremely studied and found to be safe.

Now that an FDA study has shown that Red No. 2 may cause tumors in rats, this same reasoning argues against, rather than for, the safety of Orange B.

In determining to what extent a food coloring shall be permitted in the food supply, Congress has instructed HEW. (section 706-b-8 of the Food, Drug and Cosmetic Act) to consider "the relative marketability of the articles involved as affected by the proposed uses of the color additive...and the relative dependence of the industries concerned on such uses." The non-essentiality of Orange B is indicated both by its new-ness and by the fact that most sausage manufacturers do not find it necessary to dye the surface of their sausage products.

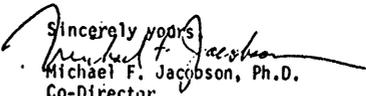
Considering the inadequacy of long-term tests, the chemical similarity to Red No. 2, and the fact that Orange B serves only a cosmetic function, FDA can best protect the public's health by outlawing this coloring, pending better substantiation of its safety.

So many artificial colorings have been outlawed after once having been admitted to the food supply, that prudent people would reduce their exposure to these chemicals to an absolute minimum. That colorings are used primarily in relatively non-nutritious foods, such as candy, soda pop, hot dogs, and other foods high in sugar or fat, is a further (and perhaps more important reason) to avoid this questionable group of additives. Also, colorings serve only a cosmetic function in food. More narrowly, as described in this letter, there is particular reason to ban--until further testing is done--Orange B and Red No. 40. Section 706 (b)(4) of the Federal Food, Drug, and Cosmetic Act states that a color additive shall not be permitted in food, if the data do not establish that the use of the coloring will be safe. The data available are so inadequate, as judged by criteria set out by authoritative committees, that they cannot be considered to establish the safety of Orange B and Red No. 40. I urge you to investigate this matter and commence proceedings to ban these colorings, under section 701 of the Act.

If you choose not to initiate proceedings to ban Red No. 40 and Orange B, I hope you will consider mounting a publicity campaign urging people to avoid artificially colored foods for the two reasons cited above: there is inadequate data to substantiate the safety of three colorings (Red No. 40, Red No. 2, and Orange B) used in our food supply; and colorings are used primarily in foods of relatively low nutritional value.

I hope you take this opportunity for leadership and encourage the public to consume a safer, more nutritious diet.

Sincerely yours


Michael F. Jacobson, Ph.D.
Co-Director

TESTING METHODOLOGY

Prepublication Copy

**REPORT OF
THE SECOND TASK FORCE
FOR RESEARCH PLANNING IN
ENVIRONMENTAL HEALTH SCIENCE**

for submission
to the

Subcommittee on Labor, Health, Education, and Welfare
Committee on Appropriations
U.S. House of Representatives

December, 1976

Chapter 11
MUTAGENESIS

- I. Introduction
- II. Research Needs and Recommendations
 - A. Screening
 - 1. Nonmammalian Test Systems
 - a. New Test Systems
 - b. Validation of Extant Systems
 - 2. Mammalian Test Systems
 - B. Population Monitoring
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MUTAGENESIS*

I. INTRODUCTION

The results of exploratory experiments, initiated in the late 1960s, to determine whether chemicals in widespread distribution might possess mutagenic activity has revealed a variety of active compounds in all chemical classes (Environmental Mutagen Society, 1975). Tests on a wide variety of organisms under experimental conditions clearly indicate a problem of magnitude sufficient to necessitate screening on a much broader scale, as exemplified in the numerous papers in the journal, Mutation Research. The recent development of short-term tests for mutagenicity provides a battery of techniques that can be used on large numbers of chemicals to provide a rapid, inexpensive and comprehensive screen (de Serres, 1975, 1976). The high correlation between carcinogenic and mutagenic activity revealed by these short-term mutagenicity tests further indicates that positive results may signal potential carcinogenic risk to man (Brookes and de Serres, 1976; McCann, et al., 1975; McCann and Ames, 1976). Short-term tests can also be used to establish priorities for further testing of specific chemicals in higher organisms. Such additional testing is required both to confirm and extend the original data from screening programs and to provide an appropriate base for extrapolating such data to man and for conducting associated risk assessments.

Rapid developments in this field clearly indicate the presence of hazards for the present human population as well as for succeeding generations. The major problem areas have been clearly identified and resources must now be allocated to provide additional test

*This chapter was prepared under the direction of Frederick J. de Serres. Other contributors were John W. Drake and Thomas H. Roderick.

methods that can be extended to humans. There is also a need to increase our capability to extrapolate from experimental test systems to man and to evaluate the risk of exposure in terms of birth defects, genetic disease and cancer. The number of trained workers, however, is totally inadequate to cope with a rapid resolution of these broad problem areas, and serious thought needs to be given to various mechanisms for stimulating and facilitating research in important problem areas.

A potential increase in the frequency of various genetic diseases in man as a result of exposure to mutagenic environmental agents is sufficiently important to justify extensive investment of resources in each of the following areas: screening, population monitoring, interrelations between mutagenic and other toxic effects, basic mechanisms, extrapolation of laboratory data from organisms to man, risk assessment and training of research scientists.

II. RESEARCH NEEDS AND RECOMMENDATIONS

A. Screening

1. Nonmammalian Test Systems

a. New Test Systems

The development of new nonmammalian test systems should be encouraged to make it possible to detect all types of genetic damage. The specificity of chemical mutagens necessitates the development of highly sensitive indicators of gene mutations, chromosome breaks and rearrangements, and non-disjunction. Even though the mechanisms are poorly understood, similar attempts should be encouraged with regard to mitotic recombination and sister-chromatid

exchange. In addition, studies on unscheduled DNA synthesis and differential growth-inhibitory effects on wild-type and repair-deficient strains need further exploration.

To further maximize the detection of genetic damage leading to gene mutations at specific loci, the development of forward-mutation systems at specific loci should be encouraged (especially those which can detect both point mutations and interstitial deletions) in order to more closely mimic the human situation. In addition, research should be encouraged to detect specific-locus mutations (in the form of recessive lethals) over a substantial fraction of the entire genome.

A variety of techniques should be explored to maximize the sensitivity of such assays; these include increasing the permeability of strains as well as determining the effect of various repair-deficient mutations.

Since nondisjunction is a major factor in producing birth defects and genetic disease, development of specific short-term tests should be encouraged. Environmental chemicals which cause nondisjunction may not cause other types of damage such as gene mutations or chromosome breaks/rearrangements.

Finally, new test protocols must be developed to permit evaluation, not only of chemically pure compounds but also of technical-grade mixtures and of formulated products as well as product constituents. Separation of product ingredients into artificial categories such as "active" and "inert" has led to numerous mistakes in the past and has shown that toxicologic evaluation on the basis of chemical structure alone can be highly unreliable. To en-

sure the highest possible safety to the human population, testing protocols should be designed to evaluate chemicals as they are commonly used in order to discover possible synergistic and antagonistic effects.

Recommendation 11-1: Research should be encouraged to develop new nonmammalian screening tests for mutagenicity, such as those with mutational endpoints of mitotic recombination, sister chromatid exchange, and especially tests which can detect both point mutations and interstitial deletions.

b. Validation of Extant Systems

An effort should be made to validate existing assay systems by using them to test large numbers of chemicals from different classes which are known from other evidence to be mutagenic. This approach, which is similar to that used with Ames' Salmonella system (McCann, et al., 1975; McCann and Ames, 1976), will demonstrate whether there are marked differences in the level of ascertainment as a function of chemical class. Comparisons should also be made between different test systems to determine whether there are either quantitative or qualitative differences in response. This approach will provide a data base, derived from tests on chemicals which are known to be mutagenic, which can be used to select those assay systems required to develop a comprehensive screen.

Recommendation 11-2: The general utility of existing assay systems should be validated by testing large numbers of chemicals from different classes which are known from other evidence to be mutagenic.

2. Mammalian Test Systems

The general classes of known mutational events are point mutations (including base-pair substitutions and frameshifts);

chromosomal breaks resulting in deletions, translocations, inversions, ring chromosomes, etc.; and chromosomal nondisjunction resulting in monosomics and trisomics. The class of point mutations is of utmost concern because its genetic damage may be initially invisible and may only show serious consequences many generations later. The specific-locus test is an example of the test needed to determine point mutations in mammals. In this test, mouse coat colors and morphologic markers (seven specific loci) are used to estimate forward mutation rates. Treated wild-type males or females are bred with non-treated mates homozygous for the seven recessive genes. A viable mutation at any of the seven loci can be seen in the offspring and an estimate of the rate of induction of recessives can be made (Searle, 1975). This test provides a direct measure of recessive mutations in mammals and is the basis for most of the present risk estimates in mammals, including man. Another specific-locus test in mice uses isozyme loci (Valcovic and Malling, 1973).

Recommendation 11-3: The Subtask Force recommends that efforts be made to develop a more efficient indicator for point mutations in mammals.

Such tests do exist in sub-mammalian species and have been widely exploited, for example, in Drosophila. The availability of similar tests in mammals would permit: (1) measurement of recessive mutations, which are a significant fraction of the genetic damage of consequence to human health and fitness; and (2) detection of these effects over a substantial fraction of the genome. The ability to detect these effects would not only increase the sensitivity and efficiency of the test but would also permit accurate generalizations because the mutational effects can be ascertained

at many loci and chromosomes simultaneously. Such systems in other organisms employ inversions which reduce recoverable recombinants and thus permit the tracking over generations of a sizeable segment of the genome from the mutagenized ancestor. Several paracentric inversions are now available in mice (Roderick and Hawes, 1974; Evans and Phillips, 1975) which may lead to useable test systems. But other recombination inhibitors could be used as well, either separately or in combination, including translocations, deletions, Robertsonian metacentrics, and certain systems such as the T-locus mutations, all of which are available in mice.

Recommendation 11-4: More research effort should be directed to the development of tests that will detect recessive detrimental and lethal mutations over a sizeable portion of the mammalian genome.

An implicit assumption in present risk estimation is that all individuals have an equal probability of incurring a mutation depending solely on the dose of mutagen received and the duration of exposure. But this assumption is undoubtedly not true because (1) individuals differ in their metabolism and thus probably differ in their ability to activate or break down chemical mutagens in their bodies prior to the delivery of the toxins to the germinal tissue; (2) furthermore, some individuals have specific genetic diseases, such as xeroderma pigmentosum, which make them less able to repair certain kinds of induced genetic damage. These are only two possible mechanisms that would make different individuals respond to mutagens in different ways; there could be many more. It is possible that this heterogeneity of response is large enough to reduce significantly the value of present estimates of risk. Many genetically independent inbred mouse strains as well as single

gene mutations exist where such studies could be done.

Recommendation 11-5: Efforts should be made to determine the extent to which certain mammalian genotypes are more sensitive to mutagenic effects.

Previous human mutagenesis risk assessments have been based primarily on males with the assumption that the female contribution to the mutational load is negligible because of the low frequency of mutations recovered after irradiation of the dictyate oocyte. Recent studies cast doubt on this assumption and suggest a greater contribution from females (Abrahamson and Wolff, In press). Because the meiotic resting stage in mice is different from that in humans, other mammalian species should be studied as well.

Recommendation 11-6: More studies should be initiated to investigate mutagenesis in female germ cells.

Mutation-detection systems employing somatic cells either in vivo or in vitro will not by themselves provide estimates of mutational risk to succeeding generations, but they can be used together with tests which detect heritable mutations to provide important information on risks. Most importantly, since such somatic cell systems simultaneously screen large numbers of cells, they provide an efficiency and sensitivity in detection that sexually transmitted mutation tests cannot now provide. Somatic tests, therefore, could provide an important first-level assessment of the mutagenic potential of a larger number of compounds.

Recommendation 11-7: More work should be devoted to devising mutagen-detection systems in mammalian somatic cells.

B. Population Monitoring

There is a great need for knowledge of the naturally occurring genetic burden and the spontaneous mutation rate in humans and for

the detection of increased mutation rates in unknown but high-risk subpopulations. The British Columbia and Irish studies (Trimble and Doughty, 1974; Stevenson, 1959) make a significant first contribution to the study of the naturally occurring disease burden of genetic origin. Some new work (Neel, In press) is underway to assess mutational events at several loci which specify chromographically detectable gene products. If this study is successful, it may provide one method needed not only for assessing human mutagenesis in high-risk populations but also for monitoring the population as a whole. The nationwide standardization of vital records of easily recognized birth anomalies and causes of death would be a significant further step in assessing the burden and monitoring population groups.

Recommendation 11-8: Efforts should be expanded to survey genetic characteristics and mutational changes in large segments of the human population.

C. Disease Consequences of Mutation

1. Carcinogenesis

Due to the correlation which has been observed between carcinogenic and mutagenic activity in tests for mutation-induction (McCann, et al., 1975; McCann and Ames, 1976), unscheduled DNA synthesis (Stich and Laishes, 1973) and differential inhibition of growth of wild-type and repair-deficient strains (Kada, et al., 1974; Slater, et al., 1971), further studies are required to validate the use of mutagenesis assay for predicting carcinogenic activity. Experiments should be encouraged to determine the mutagenicity of large numbers of carcinogens in different chemical classes to determine whether the level of ascertainment varies markedly as a function

of chemical class (e.g., nitroso compounds vs. halogenated hydrocarbons). Since the level of ascertainment may well vary as a function of class, the development of different effects should be supported. These data will make it possible to determine which test systems should be used to develop a comprehensive in vitro screen for carcinogenic activity.

Recommendation 11-9: The correlation between carcinogenic and mutagenic activity which has been observed in exploratory experiments for mutation-induction, unscheduled DNA synthesis and differential inhibition of growth of wild-type and repair-deficient strains should be further validated with research on compounds from different chemical classes.

Although a high correlation has been observed between carcinogenic and mutagenic activity in selected samples of known chemical carcinogens, scant data are available on the reverse correlation. As mass screening programs are developed, selected samples of compounds which give positive test data should be evaluated for carcinogenic activity in mammals. This approach will provide the data required for a critical and objective evaluation of the efficacy and general utility of applying various short-term tests for mutagenicity for the detection of carcinogenic activity in mammals, including humans.

Recommendation 11-10: Selected compounds which have shown positive results in in vitro tests for mutagenicity should be tested in animals for carcinogenicity.

The development of new test systems, which can not only detect the mutagenic activity of chemical carcinogens but also predict quantitative relationships, should be explored. Comparative studies, using different assay systems, should also be supported to determine whether there is a qualitative relationship between mutagenic and

carcinogenic activity for certain classes of chemical compounds. The general use of highly sensitive assay systems requires further evaluation of the effect of mutations on both the quantitative and qualitative relationships determined with respect to carcinogenicity.

Recommendation 11-11: The development of new test systems to relate potency of mutagenic activity to carcinogenicity should be given high priority.

2. Contribution of Other Disease Categories

While the apparent role of somatic mutation in carcinogenesis is now under vigorous investigation, the further possibility of a strong contribution by somatic mutation to the etiology of other major classes of disease should be explored. There is some overlap, for instance, between mutagenic and teratogenic agents; and while mutagens are unlikely to constitute the major class of teratogens, they may form a substantial minority. The steady accumulation of somatic mutations has long been considered a likely contributor to the process of aging, and the technologies required to establish or refute this hypothesis are rapidly becoming available. The results of very recent investigations (Benditt and Benditt, 1973; Pearson, et al., 1975) also suggest that mutation may contribute critically to the initiation of atherosclerosis. Looking further ahead, it is clearly possible that somatic mutation, particularly in embryogenesis, may also contribute to other diseases of obscure etiology.

Recommendation 11-12: The role of somatic mutation in the etiology of diverse types of human disease should be explored, with special emphasis on teratogenesis, aging, heart disease and mental disease.

D. Fundamental Mechanisms

The technologies, necessary for protection of the public from environmental mutagenic hazards and for possible future reversal of genetic damage already present in the population, require a thorough understanding of the fundamental aspects of the mutation process and of the structure and expression of the gene itself.

1. Eukaryotic Gene

The successes of molecular biology during the past three decades have revealed many properties of gene structure and function in simple bacterial and viral systems. Technologic advances now make similar analyses possible in higher animal systems, including man.

Recommendation 11-13: Research on the analysis of eukaryotic gene structure and function should be expanded.

2. General Nature of the Mutation Process

Although considerable information is now available concerning the interactions of certain simple mutagens with the genetic apparatus, this knowledge does not extend very far in the case of numerous potential environmental mutagens. It is also becoming clear that the mutation process depends not only upon the primary genetic lesion but even more importantly upon the biochemical fates of such a lesion.

Recommendation 11-14: Research into the nature of spontaneous and induced damage to DNA should be expanded.

Recommendation 11-15: Research into the biochemical fates of premutational lesions should be expanded, with particular emphasis on the relevant enzymologies of DNA replication and repair.

3. Special Aspects of the Mutation Process Relevant to Environmental Mutagenesis

a. Mutagen Activation and Disposition

Evidence indicates that many chemicals only become mutagens when biochemically transformed within the body, while other chemicals which are mutagenically active in simple microbial test systems are far less active in higher eukaryotes. Evidence also indicates that certain organs and tissues are at greater risk than others and that these patterns vary among different chemical agents, perhaps because of specificities of mutagen transport and biochemical processing.

Recommendation 11-16: Research should be expanded into the nature and genetic determination of the biochemistry of mutagen activation and deactivation, with special emphasis on human tissues.

b. Mutagen Interactions

Experiments performed in refined microbial systems have revealed a number of complexities of the mutation response, such as occasional multiple-hit kinetics and interactions between mutagens and other agents which may or may not themselves be mutagens. These interactions may occur at low exposure levels and can be either synergistic or antagonistic; they may involve perturbations of enzymologic avoidance and repair processes.

Recommendation 11-17: Increased research should be directed to evaluations of the extent and nature of mutagen interactions, with special emphasis on fundamental interaction mechanisms and on their significance for environmental mutagenesis.

c. Gene Specificity

Some genes are more mutable than others, and such patterns can vary with different mutagens. The consequences of mutation to

the organism also vary markedly among different genes. A reasonably good understanding of the underlying basis of this variability is now available for simple model systems, but not for higher organisms.

Recommendation 11-18: Research should be undertaken to analyze the molecular basis of differential gene mutability in eukaryotic systems.

d. Fundamental Aspects of Specific Screening Systems

A variety of mutagen screening systems are now in actual use, but not all of these are well understood. There is great concern about the abilities of some screening systems, particularly those applied to the mammalian systems, to detect a broad spectrum of mutational damages. The mouse specific-locus system, for instance, displays a suspiciously high spontaneous mutation rate and appears insensitive to certain chemical mutagens active in numerous other systems; furthermore, the nature of the genes employed in this system remains obscure. Rodent dominant-lethal "mutations" are even more poorly understood and may represent a special class of genetic or pseudogenetic damage of minimal significance for humans.

Recommendation 11-19: Genetic structure, regulation and expression should be elucidated in detail for specific-locus systems designed to be employed in mutagen screening. The ability of any proposed screening system to detect the several known classes of mutational damage should be fully assessed.

A number of mutagen screening systems have been proposed which depend upon the detection not of heritable mutation but of induced recombinational events which may also arise from DNA damages. The applicability of such systems will depend not only upon empirical correlations with mutagenicity tests but also upon an understanding of their basic mechanisms.

Recommendation 11-20: Research should be encouraged into the molecular basis of recombinational processes relevant to mutagen screening, with special emphasis on mitotic recombination and sister-chromatid exchange.

E. Extrapolation and Risk Assessment

The detection of heritable mutations in humans is presently a difficult matter. The reason for this difficulty is not because there is doubt that mutations can be induced in humans, but rather because the studies which have been possible in humans have either been retrospective and therefore not critically designed to make unequivocal inferences or have involved insufficient numbers of individuals to demonstrate a statistically significant change in mutation rate. Defining a human mutagen will, therefore, require experimental systems with strong homologies to the human condition. This will require increased comparative mutagenic studies employing several species, including mammals, and knowledge of the correlation of effects from different kinds of mutagenesis tests.

Recommendation 11-21: Continued support should be encouraged for studies which permit quantitative extrapolation to man of induced mutation rates measured in lower systems and which further permit assessment of risks due to these extrapolated rates.

For assessment of mutagenic consequences in mammals, it would be extremely difficult to test every new compound in a mammalian system designed to detect sexually transmitted mutations. Therefore, it is important to determine if certain classes of compounds have a similar spectrum of mutagenic consequences in lower forms. Such studies will not only reduce the need for testing in more time-consuming and costly mammalian systems, but they may well lead to an understanding of the relationships between chemical structures and mutagenic effects.

III. TRAINING

It is clear that there will be a marked increase in the need for scientists well trained in mutagenesis research, both in test development and in test application. Since new programs at the undergraduate or graduate levels cannot meet this need in the immediate future, postdoctoral retraining is probably the most efficient mechanism for developing the necessary manpower. Because there are now fewer professional opportunities for scientists educated to the doctoral level, there may be a number of well-qualified individuals who would be receptive to this type of training and the subsequent professional challenge.

Recommendation 11-22: New postdoctoral fellowships should be made available to train scientists in the areas of mutagenesis and mutation testing.

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METHODS FOR DETECTING CARCINOGENS AND MUTAGENS WITH THE SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST*

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INTRODUCTION

We have previously described a very sensitive and simple bacterial test for detecting chemical mutagens^{2-4,7,11} which has recently been reviewed¹². The compounds are tested on petri plates with several specially constructed mutants of *Salmonella typhimurium*^{4,7,11} selected for sensitivity and specificity in being reverted from a histidine requirement back to prototrophy by a wide variety of mutagens.

The test has been adapted for use in detecting chemicals which are potential human carcinogens or mutagens by adding homogenates of rat (or human) liver directly to the petri plates thus incorporating an important aspect of mammalian metabolism into the *in vitro* test. In this way, a wide variety of carcinogens requiring metabolic activation can be detected easily as mutagens^{4,13,14}.

There is considerable evidence, much of it obtained using this test^{2-5,7,9,14,15,16,17,18,20,21}, that with few exceptions carcinogens are mutagens. This supports the desirability of using this type of rapid and economical test system as a screening technique to pinpoint potentially dangerous chemicals among the thousands of chemicals to which humans are exposed.

The test is highly efficient in detecting carcinogens as mutagens. Many carcinogens and non-carcinogens have been tested and we are currently compiling results obtained using the test in this and many laboratories throughout the world (McCANN *et al.*, in preparation). So far about 85% of the carcinogens tested (135/158) have been detected as mutagens. These include a wide variety of carcinogens such as direct alkylating agents, nitrosamines, polycyclic hydrocarbons, fungal toxins, aromatic amines, nitrofurans, a variety of antineoplastic agents, and antibiotic carcinogens such as adriamycin, daunomycin, and mitomycin C. Also the known human chemical carcinogens which have been tested are positive. These include β -naphthylamine, benzidine, cigarette smoke condensates, bis-chloromethylether, aflatoxin B₁, vinyl chloride, 4-aminobiphenyl. This and other evidence that a high percentage of carcinogens are mutagens is most easily explained if these carcinogens cause cancer by somatic mutation²⁻⁵.

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Abbreviations: MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; furylfuramide, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide.

To date 106 non-carcinogens (chemicals negative in tests for carcinogenicity) have been tested, many of which are close relatives of carcinogens. Extremely few (< 10%) are mutagenic in the test, and for several of these carcinogenicity tests have been extremely limited and there is some doubt as to the classification of these chemicals as non-carcinogens. Many hundreds of common chemicals of unknown carcinogenicity have also been tested, and in general very few chemicals are positive. We feel that chemicals to which humans are exposed which are clearly positive in the test should be considered potential human health hazards, and should be thoroughly tested in animal systems, and where extensive human exposure has occurred, appropriate epidemiologic studies should be done.

The economy of the bacterial/mammalian-microsomal assay suggests its usefulness as a tool in rapidly obtaining information about the potential mutagenic/carcinogenic activity of uncharacterized compounds in complex mixtures. Because quantitative information is obtained from the test (linear dose-response curves are usually observed) the test is quite valuable as a bioassay in identifying and purifying mutagenic components in complex mixtures. This has been useful in a detailed study that has been made of the mutagenic activity of cigarette smoke condensate and 12 standard smoke condensate fractions¹⁸. (The condensate from less than 0.01 cigarette could easily be detected). Recently, considerable mutagenic activity has been found in most commercial hair dyes⁶, and several of the individual mutagenic components in the hair dyes have been identified. We have also shown that there is considerable mutagenic activity in soot from city air (with D. STREITWIESER).

We describe in this paper the general methods for using the Salmonella/microsome test as a mutagenesis screening system. The methods described include the standard plate test, the use and storage of the bacterial tester strains, preparation and use of the liver homogenates (S-9), and the methods of inducing the rats for elevated microsomal enzyme activity. The application of this test system to screening large numbers of compounds, and the interpretation of test results is also discussed.

METHODS

The bacterial tester strains

The strains used for mutagenesis testing are shown in Table I. There are several standard bacterial tester strains containing different types of histidine mutations. One strain (TA1535) can be used to detect mutagens causing base-pair substitutions and two (TA1537 and TA1538) to detect various kinds of frameshift mutagens. The molecular basis of the frameshift mutations in these strains has been investigated. Frameshift mutations occur by shifted pairing in repetitive sequences of DNA and frameshift mutagens can be very specific for the particular sequences they mutate. TA1538 has a repetitive -C-G-C-G-C-G-C-G- sequence near the site of the histidine mutation¹⁹, and is reverted particularly well by many carcinogens, such as 2-nitrosofluorene. The other frameshift tester strain, TA1537, appears to have a run of C's at the site of the mutation⁷ and, is reverted well by carcinogens such as 9-aminoacridine. In addition to the histidine mutation, each tester strain contains two additional mutations that greatly increase its sensitivity to mutagens: one causes loss of the excision repair system and the other loss of the lipopolysaccharide barrier that coats the surface of the bacteria⁷.

TABLE I

GENOTYPE OF THE TA STRAINS USED FOR MUTAGEN TESTING*

Histidine mutation			Additional mutations			Use
<i>hisG46</i>	<i>hisC3076</i>	<i>hisD3052</i>	LPS	Repair	R factor	
TA1535	TA1537**	TA1538	<i>rfa</i>	<i>AuvrB</i>	-	Standard tester strains and R factor strains; recommended for general mutagenesis screening
TA100		TA98	<i>rfa</i>	<i>AuvrB</i>	+R	
(TA1975)	(TA1977)	TA1978	<i>rfa</i>	+	-	Used in combination with the standard tester strains in the <i>repair test</i> ***
<i>hisG46</i>	<i>hisC3076</i>	<i>hisD3052</i>	+	+	-	Additional related strains available
TA92	TA2420	TA2420	+	+	+R	
TA1950	TA1952	TA1534	+	<i>AuvrB</i>	-	
TA2410			+	<i>AuvrB</i>	+R	
TA1530	TA1532	TA1964	<i>Agal</i>	<i>AuvrB</i>	-	
TA2631		TA2641	<i>Agal</i>	<i>AuvrB</i>	+R	
	TA2637		<i>rfa</i>	<i>AuvrB</i>	+R	

*All strains were originally derived from *S. typhimurium* LT2. Wild-type genes are indicated by a +. The deletion (*Δ*) through *uvrB* also includes the nitrate reductase (*chl*) and biotin (*bio*) genes. The *Agal* strains (and the *rfa uvrB* strains) have a single deletion through *gal chl bio uvrB*. The *rfa*, *repair*⁺ strains have a mutation in *galE*. R = pKM101. The standard tester strain TA1536, originally included in the tester set⁷ and all other strains containing the histidine mutation *hisC207* have been deleted as they are reverted by only very few mutagens and these can be detected well by the other tester strains.

**A new tester strain is under development which will, with its R factor derivative, replace TA-1537.

***The TA1538/TA1978 pair is recommended.

We have recently developed²² two new tester strains (TA100 and TA98) by transferring a resistance transfer factor (R factor) to our standard tester strains TA1535 and TA1538 respectively. These new strains are extremely effective in detecting classes of carcinogens that we previously had not detected with our original strains, and they are much more sensitive to a number of carcinogens we had previously detected only weakly²² (MCCANN *et al.*, in preparation). These carcinogens include aflatoxin B₁, sterigmatocystin, furylfuramide (the nitrofur food additive AF-2) and other nitrofur carcinogens, acetylenic diarylcarbamates, methyl methanesulfonate, nitroquinoline-*N*-oxide, benzo(*a*)pyrene, 7,12-dimethylbenzanthracene, *benzyl chloride*, 1'-acetoxy safrole, and a variety of polycyclic hydrocarbons.

For general mutagenesis testing we recommend that the three standard tester strains, TA1535, TA1537, and TA1538 be used in combination with the two new R factor strains, TA100 and TA98. For screening purposes TA1538 may be deleted, as TA98 appears to be more sensitive than TA1538 for the detection of all mutagens we have tested so far on the two strains²². The other frameshift tester strain, TA1537, is still useful, though we are developing a considerably more sensitive strain and its R factor containing derivative as a replacement. We recommend that TA1535 be used in addition to TA100 because it has a considerably lower spontaneous mutation rate and is thus more convenient and sensitive for the detection of mutagens that do not preferentially revert TA100²².

Strain TA1978 can be used in the *repair test* in combination with TA1538^{9,7}. This test is not a mutagenicity test, but indicates whether an agent is killing bacteria by damage to DNA that can be repaired by the *uvrB* excision repair system^{9,7}.

We are also developing several new tester strains which will be useful for the detection of special classes of mutagens: (1) a new tester strain with normal excision repair designed to detect certain DNA cross-linking agents such as the carcinogen mitomycin C²⁸ (with E. Cho1), and (2) new tester strains lacking nitro-reductase enzymes. Many nitro carcinogens, such as nitrofurazone and furylfuramide, are activated directly to mutagens by bacterial nitro reductases. Mammalian nitro reductases can also activate this class of carcinogens. Bacterial nitro reductase mutants have been isolated by others (Ref. 22; T. KADA, personal communication from T. MATSUSHIMA), and in collaboration with M. VORE we are isolating nitro reductase mutants in our tester strains. These new strains will be useful for comparisons between bacterial and mammalian nitro reductase activities:

We would prefer that tester strains be obtained directly from us. We keep a list of people who are using the strains so that new developments in the test can be communicated. We also request that results obtained using the strains be sent to us, as we keep a running compilation of the chemicals tested on the strains.

Checking out the tester strains

We recommend that upon receiving a strain that a nutrient broth (8 g Difco-Bacto nutrient broth, 5 g NaCl/l) culture be inoculated and grown up. (The culture should also be streaked on a nutrient agar plate for storage until the liquid culture is checked out and found to be satisfactory.) A frozen permanent should be made from the nutrient broth culture as described in the next section, and the culture should also be checked in the following ways:

- 1) Histidine requirement. Streak the cultures on minimal plates both with and without histidine (spread 0.1 ml sterile 0.1 M L-histidine on the agar surface). For strains containing the *uvrB* deletion, biotin (0.1 ml of 0.5 mM per plate) is also required for growth.
- 2) Strains having the deep rough (*rfa*) character should be tested for crystal violet (and/or deoxycholate) sensitivity⁷. A sterile filter paper disc containing crystal violet (10 μ l of 1 mg/ml) (or 2 mg deoxycholate) is placed on a nutrient agar petri dish containing 0.1 ml (about 10⁸ bacteria) of the nutrient broth culture to be tested in a thin overlay of agar (*top agar*). After 12 h incubation at 37°, a clear zone of inhibition around the disc (about 14 mm diameter) indicates the presence of the *rfa* mutation, which permits large molecules such as crystal violet to enter the bacteria and inhibit growth. Wild-type strains, or strains containing the *gal* deletion (Table I) are not inhibited.
- 3) The new tester strains with R factors (TA100 and TA98) should be checked routinely for the presence of the ampicillin resistant R factor, as R factors are somewhat unstable and can be lost from bacteria²⁸. A simple way to do this is to streak a small amount (10 μ l of 8 mg/ml in 0.02 N NaOH) of an ampicillin solution across the surface of a nutrient agar plate. After the streak is dry, cultures to be checked are cross-streaked against the ampicillin, and after incubation for 12-24 h at 37°, strains which do not contain the R factor will show a zone of growth inhibition around the ampicillin streak, whereas R factor containing strains will not. Experiments confirming increased mutagenesis (with methyl methanesulfonate and aflatoxin B₁) compared to TA1535 and TA1538 are also recommended²⁸. We also routinely include such positive mutagenesis controls when using these strains.

4) The *uvrB* deletion is quite stable and is not easily lost from bacteria, however its presence can be easily confirmed by checking the UV sensitivity of strains containing the *uvrB* deletion¹. Cross-streak the culture to be checked on a nutrient broth agar plate and irradiate half of the streak for 6 sec (8 sec for strains containing the R factor) with a G.E. 15 W germicidal lamp at a distance of 33 cm. After incubation at 37° for 12–24 h the UV-sensitive strains will grow only on the un-irradiated side of the plate.

5) The spontaneous reversion rate should be checked and should be approximately as indicated in the section on *Mutagenesis assays on plates*. An abnormally high spontaneous reversion rate may indicate contamination. We discuss diagnostic mutagens for each tester strain in a later section (*Interpretation of Results*). These can be used for confirming the reversion properties of each strain.

To reisolate a strain, streak out on a nutrient agar plate and after incubation pick 5–10 single colony isolates and grow them up in nutrient broth. A new permanent can be prepared (see below) from the isolate with a low spontaneous reversion rate and with a good growth rate which should then be checked further as described above.

Storage of R factor and standard tester strains

This is at –80° after freezing on dry ice a fresh nutrient broth culture (0.8 ml) with dimethylsulfoxide (0.07 ml) in 2-ml (0.5 dram) sterile, glass screw-capped vials with rubber-lined screw caps. We prepare duplicate frozen cultures of each tester strain. One is stored as a master copy and is only opened when we need to regenerate our frozen stocks. The other is used routinely to obtain fresh cultures for mutagenesis testing by scraping a sterile wooden applicator stick over the surface of the frozen culture (which is not thawed), inoculating nutrient broth (5 ml), and shaking overnight (16 h maximum) at 37°. Fresh cultures can be kept in the refrigerator for a week. It is not desirable to subculture the tester strains because of selection for *rfa* revertants to wild-type and loss of the R factor. Strains not carrying R factors or *rfa* mutations are usually stored at room temperature as permanent stab cultures in 2-ml glass vials containing 1.2 ml soft agar (Difco-Bacto nutrient broth, 2 g; agar, 1.5 g; distilled H₂O, 250 ml) sealed with paraffin. This method can also be used for the tester strains (where a freezer is not available) if the cultures are checked out frequently.

Induction of rat liver enzymes for carcinogen activation

For general mutagenesis screening we recommend using liver homogenates (S-9) (9000 g supernatant) from rats induced with a polychlorinated biphenyl (PCB) mixture (Aroclor 1254)^{1,13,14,16,17}. The induction procedure¹⁸ is similar to the method of CZYGAN *et al.*¹². We use male rats of about 200 g each (Sprague-Dawley/Bio-1 Strain, Horton Animal Laboratories). A single i.p. injection of Aroclor 1254 (diluted in corn oil to a concentration of 200 mg/ml) at a dosage of 500 mg/kg is given to each rat five days before sacrifice. The rats are given drinking water *ad libitum* and Purina Laboratory Chow until 12 h before sacrifice when the food is removed. On the fifth day of induction the rats are stunned by a blow to the head and then decapitated. Liver homogenates (S-9) are prepared as described in the following section⁴.

Although we have, on occasion used other tissues (see later section), we find that, in general, rat liver is the most convenient source of activating enzymes. It is essential for efficient detection of a wide variety of carcinogens requiring metabolic activation that S-9 be obtained from induced animals. We have used various induction proce-

dures^{4,10} and find that liver from Aroclor-induced rats is the most efficient for detecting different classes of carcinogen. To illustrate the relative efficiencies of the different induction procedures, we have compared directly uninduced, phenobarbital, 3-methylcholanthrene, and Aroclor 1254 induced rat liver S-9 fractions for the activation of two polycyclic hydrocarbons (benzo(a)pyrene and 3-methylcholanthrene), and 2-acetylaminofluorene, an aromatic amine carcinogen. The results (Table II) indicate

TABLE II
INDUCTION OF RAT LIVER ENZYMES

Induction method	$\mu\text{l S-9/plate}$	Revertants of TA1538 per plate*		
		5 μg Benzo(a)pyrene	50 μg 3-methylcholanthrene	50 μg 2-acetylaminofluorene
Aroclor 1254	10	127	37	750
	20	318	71	3320
	50	443	206	9600
	100	189	349	3420
	150	146	297	4280
Phenobarbital	10	—	85	1050
	20	—	136	3020
	50	45	256	13,700
	100	23	356	14,700
	150	17	207	12,200
3-Methyl-cholanthrene	10	15	—	59
	20	121	—	620
	50	296	49	2045
	100	287	35	7187
	150	223	52	5640
Uninduced** (corn oil)	50	31	10	2344
	100	43	17	5824
	150	3	16	7184

The assays were performed as described in *Mutagenesis assays on plates* using S-9 samples which were prepared from 200 g rats as shown in *Induction of rat liver enzymes*.

*The number of spontaneous revertants (<30) have been subtracted from the appropriate experimental values. Assay plates were incubated for two days at 37° and then scored.

**The controls were 100 g rats which were injected with the vehicle, corn oil, which was used for the Aroclor 1254 and 3-methylcholanthrene induction. Induction with sodium phenobarbital (0.1% in the drinking water) and 3-MC (80 mg/kg; i.p.) was as described previously⁴.

that, for the three compounds tested, induced rats are superior to uninduced animals, and of the three induction procedures, Aroclor induced S-9 is the most efficient, overall, for the detection of all three carcinogens. Although phenobarbital is a generally effective inducer and can be used for the efficient detection of 2-acetylaminofluorene, and many other aromatic amine carcinogens⁴, it is very inefficient for detection of certain polycyclic hydrocarbons.

It is also important to note that for optimum mutagenesis with a particular compound the amount of S-9 per plate is critical and can be different. Too much as well as too little S-9 can drastically lower the sensitivity. We routinely check this variable when optimizing mutagenesis with a particular compound. We also routinely check each new S-9 preparation with several compounds, such as benzo(a)pyrene and an aromatic amine, e.g. 2-acetylaminofluorene or 2-aminofluorene, and aflatoxin B₁ to

find the optimum amount of S-9 for general screening. In general, we find from Aroclor induced rats that 20–50 μ l of S-9 per plate (0.04–0.1 ml of S-9 per ml of S-9 mix) is a good amount for general screening.

Preparation of liver homogenate fraction ("S-9")

We have used basically the procedure of GARNER *et al.*¹⁰. All steps are at 0–4° using cold, sterile solutions and glassware. The livers (rat livers are about 10–15 g each) are placed in beakers (pre-weighed) containing 0.15 M KCl (approx. 1 ml/g wet liver). After weighing, livers are transferred to a beaker containing 3 vol. of 0.15 M KCl (3 ml/g wet liver), minced with sterile scissors, and homogenized in a Potter-Elvehjem apparatus with a Teflon pestle. The homogenate is centrifuged for 10 min at 9000 g (8700 rev./min in SS-34 head of Sorvall RC2-B) and the supernatant, which we call the S-9 fraction, is decanted and saved. 1 ml of S-9 fraction contains microsomes from about 250 mg of wet liver; protein concentrations were fairly constant from preparation to preparation (about 40 mg/ml: LOWRY). The fresh S-9 fractions are distributed in 2-ml portions in small plastic tubes (2-ml liquid nitrogen storage tubes/4-Shore-USA, La Jolla, Calif.), quickly frozen in dry ice, and stored at –80° in a Revco freezer. As required, sufficient S-9 fraction is thawed (at room temperature) and kept on ice; the unused portion is discarded at the end of the day. The extent of bacterial contamination of the S-9 should be determined: if necessary the S-9 mix may be filter sterilized (see following section).

S-9 from other tissues and animals

Microsomal preparations can be made from various tissues of different animals. We have compared rat and human autopsy liver^{4,6} and rat liver and lung¹⁰ and also made preparations from mouse liver and mammary gland (J. RICHARDS, unpublished). The preparations were similar to that from rat liver except for problems of sterility (with lungs) and homogenization difficulties with lungs and human liver. Because of the fibrous nature of lung tissue, the lungs were homogenized at 0–4° for 5 min in a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, N.Y.) rather than the Potter-Elvehjem apparatus used for the liver¹⁰. (This homogenizer would probably be useful for any tissue that is fibrous or difficult to homogenize.) The S-9 fraction was then prepared from the homogenate in the same manner as the liver fraction. Although liver preparations are usually sterile, lung preparations contained bacterial contaminants. These were removed by passage of the lung S-9 mix through a sterile Swinex Filter unit (Millipore Corp. Bedford, Mass.) equipped with a Millipore filter (0.45- μ m pore size). Nalgene disposable membrane filter units are used for larger amounts. (S-9, before dilution into the mix, clogs the filter and it is better to filter the S-9 mix.)

It should be emphasized that in order to compare quantitatively two different S-9 preparations for activation of a carcinogen (this can be for different tissues of the same animal, the same tissue from different animals, or different conditions of induction) a one point comparison is not sufficient (*e.g.* Table II). As in any enzyme assay the activity (in this case histidine revertants) must be proportional to the amount of enzyme (S-9) added in order to determine specific activity. Thus a multi-point comparison is necessary. It is also advisable to do the comparisons at several concentrations of carcinogen. As can be seen from Fig. 1A if one did a one point comparison of

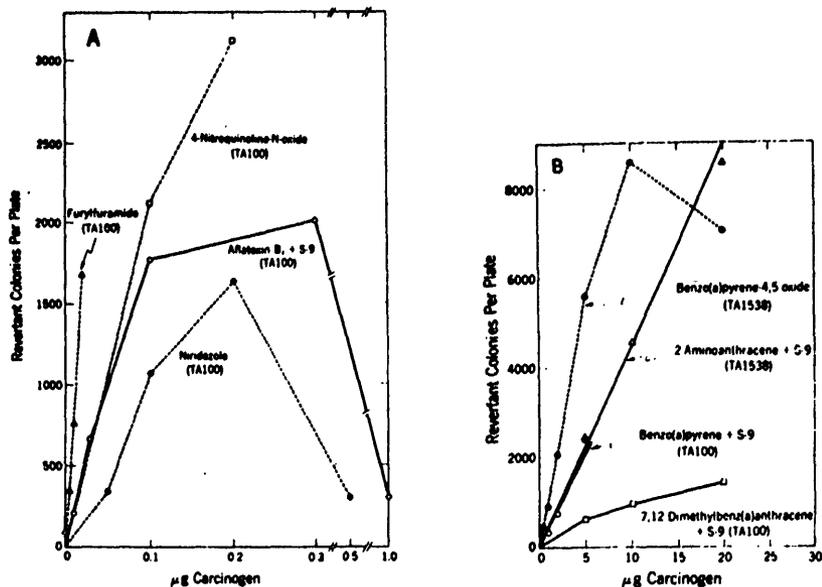


Fig. 1. A and B. The number of spontaneous revertants (varying from <30 for TA1538 to 160 for TA100) have been subtracted from the revertant values plotted against amount of carcinogen. Freshly prepared solutions of the carcinogens, and where indicated, S-9 mix, were incorporated into top agar as described in Methods.

aflatoxin activation at any concentration above $0.3 \mu\text{g}$ of aflatoxin the more active S-9 preparation might appear as less active.

There have been suggestions that in general screening of chemicals several S-9 preparations from different tissues or different animals should be used. We have seen no data so far that convinces us that the considerable extra work involved if this procedure is followed will in fact result in detection of mutagens that would not be detected using rat liver Aroclor-induced S-9 following the standard procedure we have outlined above.

Preparation of S-9 Mix⁴

S-9 mix contains per ml: S-9 (0.04–0.1 ml) (see Induction section), MgCl_2 (8 μmoles), KCl (33 μmoles), glucose-6-phosphate (5 μmoles), NADP (4 μmoles), and sodium phosphate, pH 7.4 (100 μmoles). Stock solutions of NADP (0.1 M) and glucose-6-phosphate (1 M) are prepared with sterile water in sterile tubes and stored at -20° . The stock salt solution (0.4 M MgCl_2 , 1.65 M KCl) and phosphate buffer (0.2 M, pH 7.4) are autoclaved and stored in the refrigerator. S-9 mix is freshly prepared each day and may be kept on ice several hours before use.

Mutagens and carcinogens

Solutions are prepared in disposable sterile polystyrene screw-cap tubes. We generally dissolve compounds which are not water soluble in dimethylsulfoxide

(Schwarz-Mann, spectrophotometric grade, sterile as is). Less than 0.5 ml per plate does not interfere with mutagenesis, or with microsomal activity. We use fairly small bottles of dimethylsulfoxide (100 ml) which we only enter with sterile pipettes and which we keep dry by closing the top immediately after use. *p*-Dioxane or ethanol can also be used as solvents, and up to 100 μ l of either solvent can be incorporated into the top agar overlay without inhibiting the lawn of bacterial growth. Most mutagen solutions can be stored at -20° in the freezer or in the refrigerator for extended periods. Solutions of highly reactive compounds, such as alkylating agents, however, should be freshly prepared.

Top agar and petri plates

Top agar (0.6%, Difco agar, 0.5% NaCl) is autoclaved and stored in bottles in volumes of 100 ml at room temperature. Before use the agar is melted by heating the bottle in a steam bath, and 10 ml of a sterile solution of 0.5 mM L-histidine·HCl-0.5 mM biotin is added to the molten top agar and mixed thoroughly by gentle swirling.

The trace of histidine in the top agar allows all the bacteria on the plate to undergo several divisions; *this growth is necessary in many cases for mutagenesis to occur*. The slight background that grows up also allows any inhibition by the compound to be seen. Further increase in the amount of histidine on the plate enhances mutagenesis, but also causes heavy growth of the background lawn that obscures the revertants.

We use plastic or metal caps (Bellco, Vineland, N.J.) for both the tubes and the agar bottles. These can be removed easily and are more convenient than cotton plugs or screw caps.

The petri plates (100×15 mm style, Falcon Plastics, Oxnard, Cal.) contain 30 ml of minimal-glucose agar medium. We use 1.5% Bacto-Difco agar in Vogel-Bonner Medium E²⁰ with 2% glucose, but other minimal media would presumably also serve. The plates can be stored at room temperature for several weeks. We have been informed (M. NAGAO, personal communication) that plastic petri plates sterilized by the mutagen ethylene oxide can give rise to a high "spontaneous" rate for strains TA1535 and TA100 if there is residual ethylene oxide in the plates, though we have not had difficulty with this problem with the plates we have used.

Mutagenesis assay on plates⁴

The following are added (in order) to 2 ml of molten top agar at 45°: 0.1 ml of an overnight nutrient broth culture of the bacterial tester strain, the sample to be tested (usually in 0.1 ml or less), and 0.5 ml of the S-9 mix (if required). The bacteria can remain at 45° for a few minutes without harm, but the S-9 mix should not be left at this temperature for more than a few seconds. The contents are mixed (by rotating the tube between the palms) and poured on minimal glucose agar plates. Uniform distribution of the top agar on the surface of the plates is accomplished by gently tilting and rotating the uncovered plate and then setting the plates down to harden. The mixing, pouring and distribution should take less than 20 sec and the plates should be left to harden for several minutes. It is important to follow these time limits. If the top agar starts to harden in mid-operation a stippled plate surface will result which makes scoring of revertants difficult. We cover the plates promptly to avoid any light effects on photosensitive chemicals. Within an hour the plates should

be put in a dark, 37° incubator. After 2 days the colonies (revertants to histidine prototrophy) in both test plates and controls are counted, and the presence of the light background lawn of growth (due to the trace amounts of histidine added) is confirmed. Spontaneous revertant colonies on control plates without mutagen and S-9 mix are about (at 48 h): 20 (TA1535), 7 (TA1537), 25 (TA1538), 160 (TA100), and 40 (TA98). Slightly larger numbers of spontaneous revertant colonies arise on plates containing the S-9 mix⁴.

When mutagens are to be tested in a spot test the mutagen is left out of the top agar and instead is applied to the plate surface after the top agar containing the bacteria and S-9 mix is poured. The mutagen can be added to the agar surface as crystals, or a micro drop (about 10 μ l). We also have used sterile 6-mm filter paper discs, though, some chemicals, such as daunomycin, are detected much less efficiently on discs, possibly due to adsorption. The plates can be stored at room temperature for up to an hour before applying the mutagen, but as the bacteria will begin to grow, it is best to add the mutagen promptly after the top agar hardens.

Testing of volatile compounds in the plate test

The testing of volatile, relatively water insoluble compounds, whether gas or liquid, cannot be done quantitatively in the manner described earlier for pour plate incorporation of solutions of chemicals. By a modification of the plate test, RANNUG *et al.*¹⁰ were able to demonstrate the mutagenicity of vinyl chloride, a gas, by exposing petri plates having TA1535 and S-9 Mix in the soft agar overlay to known vinyl chloride/air mixtures in a 10 l dessicator for various lengths of time. BARTSCH *et al.*¹⁰ also tested vinyl chloride using this method, and later showed the method applicable, to the testing of vinylidene chloride and 2-chlorobutadiene¹¹.

Establishing desired concentrations (v/v) of the compound in air can be accomplished in several ways:

(a) Evacuating the dessicator, introducing a known volume of the gas to be tested, then allowing air to enter until atmospheric pressure is reached.

(b) Using a manometer attached to the dessicator by a 3-way valve, remove a known volume of air, then allow the gas to enter until atmospheric pressure is reached.

(c) Using manometers, or flowmeters, measure the flow rates of the gas and compressed air into a mixing flask which then feeds the mixture into the dessicator.

(d) For liquids, the calculated weight to give a known volume of vapor is frozen and placed in the dessicator. The dessicator is partially evacuated and sealed, allowing the solid to vaporize. Then air is introduced until atmospheric pressure is reached.

A magnetic stirrer should be included in the sealed container to maintain a homogeneous mixture. The incubation is carried out at 37°. After the desired time of exposure, the compound is removed by three evacuations and refillings with air. Further quantitation can be attained by analyzing a sample of the gas/air mixture by GLC. After 48 h of total incubation time, the plates should be scored for revertants. (We are indebted to H. BARTSCH for contributions to this section.)

Interpretation of results: plate incorporation assay

In this method the mutagen is added directly to the molten top agar and is poured on the plate along with the S-9 mix and bacteria. This is the standard method that has been used for validating the test using hundreds of chemicals. For initial

screening of a chemical we recommend testing concentrations over a wide dose range (say 0.2, 2, 20 and 500 μg per plate) both in the presence and absence of the standard S-9 mix (see *Induction and preparation of S-9 mix*), using the tester strains TA1535, TA100, TA1537 and TA98. A positive or questionable result should then be confirmed by demonstrating a dose-response effect using a narrower range of concentrations. A few dose-response curves are shown in Fig. 1. In general, we find that for most mutagens a concentration range exists when there is a linear dose-response, and the revertants per plate reported for any mutagen should be taken from this region of the curve. (We have occasionally obtained dose-response curves that are not linear: with 9-aminoacridine, MNNG, diethylsulfate and ethyl methanesulfonate²⁹). Excessive bacterial killing by a mutagen causes a decrease in the number of revertants on the plate, and it can be seen (Fig. 1) that if one were to test just one concentration on the downhill part of a curve one could easily obtain a misleading result as to the quantitative activity of a particular mutagen.

Routine examination of the background lawn of bacterial growth resulting from the trace of histidine added to the top agar (see *Top agar and petri plates*) can be an aid in determining the presence of toxic effects. If massive cell death has occurred, the background lawn on the test plates will be sparse compared to control plates. In this case more histidine is available to the individual surviving bacteria and they undergo more cell divisions, consequently appearing as small colonies which can be mistaken for revertants if the absence of a normal background lawn is not noted.

Although the standard amount of S-9 recommended for general testing should permit detection of a wide variety of chemical mutagens requiring metabolic activation, to maximize reversion it is advisable to determine the optimum amount of S-9 in the S-9 mix required for activation, as this varies with the type of compound tested and its concentration on the petri plate (see *Induction* section).

In each experiment we routinely include positive mutagenesis controls using diagnostic mutagens to confirm the reversion properties of each strain. The characteristic reversion patterns of the standard strains to some diagnostic mutagens are shown in Table III. Positive controls using chemicals requiring metabolic activation confirm that the S-9 mix is active. For this purpose 2-aminofluorene or aflatoxin B₁ can be spot tested, as indicated above, but the polycyclic hydrocarbons, such as benzo(a)pyrene (5 μg should be sufficient for activation) which do not diffuse in the agar must be incorporated directly into the agar overlay. Sterility controls (solutions

TABLE III

DIAGNOSTIC MUTAGENS: FOR CONFIRMING REVERSION PROPERTIES OF TESTER STRAINS

Mutagen	Amount spotted	S ₉	TA1535	TA100	TA1537	TA98	TA1538
Methyl methanesulfonate	2 μl	--	-*	+++	-	-*	-
4-Nitroquinoline-N-oxide	10 μg	--	-*	+++	-	++	-*
MNNG	2 μg	--	+ + +	+++	-*	-	-*
9-Aminoacridine	10 μg	--	-	-	++	-	-
Daunomycin	5 μg	--	-	-	-	++	-
2-Aminofluorene	10 μg	+	-	+++	-	+++	+++
Aflatoxin B ₁	1 μg	+	-	++	-	++	-*

All chemicals were dissolved in DMSO except for 9-aminoacridine which was in ethanol
 -, <20; ++, >100; + + +, >500 colonies in the spot test.

* We have combined slight positives with the negatives for the purpose of this table.

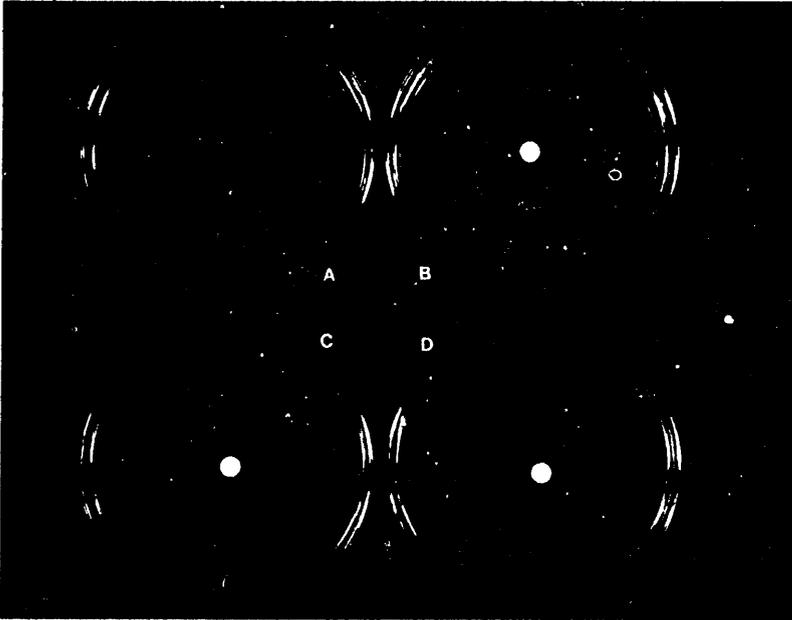


Fig. 2. The spot test. Each petri plate contains, in a thin overlay of top agar, the tester strain TA98, and in the case of plates C and D, a liver microsomal activation system (S-9 Mix) from aroclor-induced rats, (see text). Mutagens were applied to 6 mm filter paper discs which were then placed in the center of each plate: (A) spontaneous revertants, (B) furylfuramide (AF₂-2) (1 μ g), (C) aflatoxin B₁ (1 μ g), and (D) 2-aminofluorene (10 μ g). Mutagen induced revertants appear as a ring of colonies around each disc.

of new test chemicals, and S-9 mix) are also routinely included in each experiment. It is important to do a control plate without the test chemical and with S-9 mix and bacteria as the small amount of histidine in the S-9 mix can influence the number of spontaneous revertants.

We would consider a chemical to have a negative response in the test if at least 500 μ g had been tested on the plate (or the maximum non-inhibitory level) and if the number of induced revertants compared to the spontaneous was less than 2-fold. For compounds of low mutagenicity it is important to obtain reproducible dose-response curves.

Interpretation of results: spot tests

Spot tests are the simplest way to test compounds for mutagenicity and are particularly adaptable for the initial rapid screening of large numbers of compounds in a short period of time. A few examples of spot tests are shown in Fig. 2. We have tested 169 different hair dyes for mutagenicity using this method⁸. We have also used it in student laboratory experiments. There are several advantages of the spot test and it is often useful to test all new compounds by this method before doing the standard plate incorporation test. It is not necessary to prepare solutions of the

chemicals to be tested, as a few crystals (or μ l of a liquid) can be put directly on the agar surface; also, as the compound diffuses out from the central spot a range of concentrations are tested simultaneously. The spot test affords a preliminary indication of the toxicity of the chemical for the bacteria by the size of the zone of inhibition of the background lawn of bacterial growth around the spot and an indication whether the S-9 mix is necessary for mutagenicity, and in the case of a positive result, which tester strain should be used for the dose-response curve. The method can also be used to test samples which are not completely sterile, provided the colonies directly under the spot (or touching the paper disc) are not counted.

The spot test is primarily a qualitative test, and although very useful, has distinct limitations. It can only be used for the detection of chemicals which are diffusible in the agar, and thus most polycyclic hydrocarbons and other water insoluble chemicals are not easily detected by this procedure. It is also much less sensitive than the standard plate test (see above) as only relatively few bacteria on the plate are exposed to the chemical at any particular dose level.

A positive result in a spot test should be considered adequate evidence for mutagenicity only if there is a large increase in colonies (many times the number of spontaneous revertants) well away from the spot. In general, however, we believe that mutagenicity should be confirmed by demonstrating a dose-response effect using the standard plate incorporation assay.

Mutagenesis tests in liquid

The original experiments of MALLING²¹ on dimethylnitrosamine activation and mutagenesis of the *Salmonella* strain *hisG46* (the mutation in TA1535) were in liquid suspension, and in fact detection of dimethyl- and diethylnitrosamine in the *Salmonella* test requires preincubation with the S-9 mix and the bacteria in liquid suspension before plating. An efficient and simple procedure is to follow the protocol described for the standard plate incorporation assay with the slight modification that the top agar is added last, after pre-incubating the other components 20 min at room temperature (T. MATSUSHIMA, D. R. STOLTZ, personal communications). It is important to add the carcinogen in the minimum amount of DMSO, or other organic solvent, so as not to inactivate the bacteria or S-9 during the 20 min. This slight modification of the standard plate test may be included as an addition to the screening protocol for completeness. However, at present liquid suspension procedures have several serious disadvantages for general mutagenesis testing, and should only be used as an adjunct to the standard plate test. Relatively few mutagens and carcinogens have been tested in liquid suspension tests, so the ability of this type of test to detect a wide variety of carcinogens is by no means clear. In fact, of the few chemicals so tested dimethyl- and diethylnitrosamine are more efficiently detected in liquid suspension than in the standard plate assay^{9, 17, 21}, and almost all others tested, of which we are aware, including other N-nitrosamines such as dibutylnitrosamine and polycyclic hydrocarbons (H. BARTSCH, personal communication) which are efficiently detected in the plate test, are very inefficiently or not at all detected in a liquid suspension test.

It is not unreasonable that liquid suspension procedures would be relatively inefficient compared to a plate test for detection of many mutagens. Liquid mutagenesis procedures usually require incubation of the suspension at 37°, and at this temperature the microsomes are active for a relatively short period of time, whereas in the

agar overlay in the plate test they remain active up to 9 h at 37° (ref. 10). We think this is due to stabilization of the immobilized enzymes in the agar layer. In addition, for many frameshift mutagens efficient mutagenesis requires growing bacteria for both induction and expression of the mutation. This is accomplished on the plates by having the bacteria undergo several generations growth on the trace of histidine incorporated in the top agar. Liquid suspension procedures designed to determine mutagenesis relative to killing usually involve exposure to mutagens in the absence of bacterial growth and plating of bacteria directly on medium lacking any histidine¹⁶. Under these conditions the degree of mutagenesis observed can be severely reduced.

Detection of mutagenic metabolites in urine

A wide variety of metabolites of drugs and other ingested compounds appear in the urine and we would like to expand the Salmonella test method as a general screening procedure for the detection of mutagenic metabolites in urine. We, and others, have reported procedures for the detection in urine of mutagenic metabolites of 2-acetylaminofluorene^{12,14}. The addition of commercial β -glucuronidase to the petri plates along with the urine, liver homogenate, and bacteria allows detection of metabolites of these carcinogens which are excreted in urine primarily as β -glucuronide conjugates. By this method mutagenic activity is readily demonstrated with urine of rats administered as little as 200 μ g (1.6 mg/kg) of 2-acetylaminofluorene¹⁴. We have recommended that testing urine for mutagenicity be done routinely in the case of experimental animals given a chemical in toxicology testing¹⁴. This would be a worthwhile addition to any mutagenesis screening program using the Salmonella test system. The urine test has been shown to detect several carcinogens requiring metabolic activation for mutagenic activity which are not efficiently detected using the standard *in vitro* liver microsomal method of activation. These are dimethylaminoazobenzene (butter yellow)¹², isoniazid¹³, and safrole (H. ROSENKRANZ, personal communication). This method is potentially of great value and should be adaptable for the screening of human urines in order to detect mutagenic metabolites of drugs and of dietary components, and for testing urinary metabolites of drugs and food additives in experimental animals. However, as recently discussed¹⁴, there are several technical problems which must be surmounted before any large scale application of the method for humans is justified. Of primary importance is the determination of an optimum extraction procedure for the detection of a variety of metabolites, and the need for, as in the case of liquid assay procedures (see above) validation for efficiency in detecting a large variety of carcinogens.

Disposal of carcinogen and mutagen waste

We recommend using disposable test tubes, petri plates and micropipettes in order to avoid re-circulation of these items with the rest of the laboratory apparatus. The disposal of these mutagen/carcinogen-contaminated materials is by contract with the firm which disposes of radioactive waste from our laboratory.

Safety precautions in the handling of carcinogens/mutagens

Every effort is made in this laboratory to isolate the areas where carcinogens/mutagens are used in order that the entire laboratory is not contaminated unknowing-

handling of solid and volatile liquid compounds is done during the preparation of solutions or during an experiment when the carcinogens/mutagens are incorporated in or spotted on the top agar overlay. The investigator wears disposable plastic gloves for these manipulations and all weighings are done by weight difference determination in order that the container of solid or liquid compound is not opened in the laboratory outside of the hood. A 37° incubator is used only for carcinogen/mutagen-containing petri plates. It has been adapted with a hose connection to a fan which draws a slight flow of air through the incubator to the hood. The fan motor is activated by a microswitch on the incubator door so that it operates whenever the petri plates are moved in or out of the incubator. This precaution is coupled with the use of desiccator jars in which the petri plates are incubated whenever dangerous volatile compounds are assayed.

Safety precautions in the handling of the Salmonella tester strains

Salmonella typhimurium can cause diarrhea and food poisoning. The particular *S. typhimurium* strain, LT2, the parent of all of our tester strains, is used by geneticists all over the world and is not very virulent. The deep rough mutation (*rfa*) present in the standard tester strains (see Table I) lowers virulence by orders of magnitude and these strains should be relatively harmless. The pKM101 plasmid, with one antibiotic resistance marker for ampicillin, and contained in the tester strains TA98 and TA100 should be a minimal hazard. Plasmids are extremely common in the enteric population in nature and most of the RTF plasmids isolated from *Salmonella* in hospitals contain many antibiotic resistance genes. Nevertheless, as a matter of routine with any *Salmonella* strain we use plugged pipettes and autoclave any material containing *Salmonella* before it is washed or disposed of. (The material containing carcinogens as well as *Salmonella* is not autoclaved and is disposed of as previously described.) It is also a laboratory rule not to keep any food material in the same refrigerators or area with carcinogens or *Salmonella*.

It may be prudent to keep the *Salmonella* away from mouse colonies.

Chemicals

Benzo(a)pyrene, MNNG, and 2-acetylaminofluorene were from Aldrich. 3-Methylcholanthrene was from Eastman, and Mallinckrodt was the source of sodium phenobarbital. 2-Amino-anthracene, 2-aminofluorene, and 4-nitroquinoline-1-oxide were from Schuchardt (Munich) and 7,12-dimethylbenzo(a)anthracene, daunomycin, and 9-aminoacridine were from Sigma. Aflatoxin B₁ was from Calbiochem. The following were gifts: furylfuramide [2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide: trade name AF-2] from T. SUGIMURA (National Cancer Center Research Institute, Tokyo); benzo(a)pyrene-4,5-oxide from P. L. Grover and P. Sims (Chester Beatty Research Institute, London); and niridazole from E. BUEDING (The Johns Hopkins University, Baltimore). Aroclor 1254 is available in 50 ml samples at no charge from W. B. PAPA-GEORGE, Monsanto Chemical Co., St. Louis, Mo. 63166, and is also available commercially from Analabs, Inc. (North Haven, Conn).

Cost of determining mutagenicity

We estimate that once set up and working regularly one person ought to be able to test thoroughly several compounds a day and that the cost should be in the

range of \$100 to \$300 a compound counting time and material but not overhead. This would include testing on four tester strains and, after the initial spot tests and set of concentrations, doing dose-response curves with those compounds that are positive. Spot testing only would miss some compounds but would of course be much cheaper.

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February 21, 1977

Judith Robinson
Professional Staff Member
Subcommittee on Employment, Poverty
and Migratory Labor
United States Senate
Washington, D.C. 20510

Dear Judy:

Thank you for the opportunity to review the Food and Drug Administration Appendix I, "State of the Art of Mass Screening for Mutagenicity."

In their presentation there are numerous errors. Their discussion of systems is incomplete, and in many cases inaccurate. Additionally, they do not realize that the methodology we have in this area is probably as good, if not better, than we have in many other areas of Toxicology. The fact that we are always changing, hoping to improve our methodology, is a reflection of the dynamic field of mutagenicity testing, and is one that should not be viewed as a deficiency of this area of toxicology, but something that should also be carried out wherever toxicological procedures are utilized.

I know that time is pressing, and therefore I do not have the time to address myself specifically to the errors in the document and also try to synthesize what I consider a more meaningful critique of the state of the art of mass screening for mutagenicity. I have, however, prepared recently a chapter for an upcoming book entitled "The Chemical Environment and Mutagenesis." The material from page 10 to the end of the manuscript expresses most of my views on the subject. If you could include for the record the material on page 10 under Roman numeral II, "Chemical Mutagenesis Testing", and starting with the second paragraph: "The growing awareness . . .," I believe that this is relevant material which I would like to have inserted into the record.

I would of course be pleased at some future date to appear in Washington at a hearing or, indeed, just simply to discuss chemical mutagenicity testing with you.

Thank you again for giving me the opportunity to present some of my views for the record.

Yours truly,

Marvin S. Legator
Marvin S. Legator, Ph.D.
Professor & Director

MSL/vc

II. CHEMICAL MUTAGENESIS TESTING

A. COMBINED TESTING

The growing awareness that it is no longer possible to introduce new chemicals into the environment without toxicology testing in the area of mutagenesis makes it imperative that we examine the relevance of these existing procedures as compared to those techniques that are commonly used in other areas of toxicology. Mutagenicity studies should not be considered simply as a means to identify a potential carcinogen, but also as a means of preventing genetic abnormalities whose importance to man may well eclipse all other areas of combined testing. The so-called tier approach (42), or preliminary screens,

based on microbial studies are inadequate and toxicologically naive for evaluating potential mutagens. The use of in vitro microsomal preparations combined with microorganisms or other indicators as an exclusive primary screen is an approach whose deficiencies must be recognized. An in vitro microsomal activating system cannot reflect the complex dynamic processes that are carried out in the intact animal. Indeed, it is impossible to devise a standard in vitro activation system that can be used generally to screen potential mutagens and carcinogens. Even if such an in vitro system could activate all compounds that are metabolized by microsomal enzyme systems, the fact that many materials are either potentiated or detoxified by other routes, e.g. intestinal flora, would argue against the use of the system as an initial screen test. Thirdly, an important class of chemicals that induce nondisjunction by affecting spindle mechanism, one of the most important categories of cytogenetic abnormalities, would be missed by bacterial studies.

In every category of chemicals in our environment, there are examples of carcinogens and mutagens that would not be detected by such microbial procedures, with and without activation. Urethane, the insecticidal group represented by Dieldrin, Cycasin (the naturally occurring toxins), and halogenated purines are a few of the many examples of compounds that would not be detected by this simplistic approach (43). In the literature, one can find data suggesting that the correlation between microsomal activation procedures and known mutagens and carcinogens is between 70 and 90 percent (44). These correlations, which in themselves are not adequate to justify in vitro procedures as a primary screen for detecting potential carcinogens and mutagens, are questionable. For instance, quantitative data and levels of significance have not been

stated; in most cases, selected compounds, rather than randomly chosen materials, were evaluated. In government sponsored research projects, a failure to code the materials, i.e. the investigators knew beforehand if the compound was a suspected or confirmed carcinogen or mutagen, has led to an overestimate of this correlation (43). In fact, a recently completed study indicates that only 65% of known carcinogens are active in Salmonella testing strains, with or without activation (45).

All responsible scientists share the goal of identifying mutagens and eliminating them from our environment. It is essential, however, that we do not adopt indefensible, toxicological procedures in attempts to achieve our goal. The crux of the matter is not the identification of active compounds by microbial procedures, with or without activation, but the fact that we may miss potent carcinogens and mutations by screens that cannot be considered as preliminary screens for detecting active materials. Industrial toxicologists and other interested individuals should not be lulled into a false sense of security and assume that the chemicals are not mutagenic when the compound is found not to be active by existing microbial procedures (46). In an in-depth study of chemical mutagens, one would first select those test systems that have the capability of indicating the various types of DNA alternations produced by chemicals that are active per se, or those which are activated by enzymes of the tissues or intestinal flora of the host. In principal, with an unknown chemical, one would start with the best available animal systems, including those tests that evaluate metabolic products of the intact host. Since there is no single test for detecting chemical mutagens, a complete testing protocol would utilize a battery of tests carried out in the intact animal. The integration of the results from these systems should

offer the optimum opportunity of identifying mutagens that are potential hazards to man. The subsequent studies of an active compound would rely on refining procedures to isolate and identify the active compound and, subsequently, to characterize the genetic lesions induced by the chemicals under study. This approach is contrary to tier approach which uses in vitro systems and then advances to animal tests; such an approach should be reserved. In the field of chronic toxicology, available methods are used to evaluate new compounds before and after they are introduced into the market place as well as to evaluate currently used materials. Although, in many instances, these procedures are time consuming and expensive, no one would suggest that they be abandoned until more suitable methods are developed. In like manner, to postpone the evaluation of chemicals for mutagenic activity, or to settle for short-term procedures that may indeed yield misleading information, is to diminish the importance of this area of toxicology. Furthermore, it represents a failure to recognize the fact that currently available procedures for mutagenic evaluation are less time-consuming, less expensive, and probably more meaningful than many tests that are available and presently used in the field of toxicology. Indeed, if one employs a battery of tests that would detect compounds which cause point mutations and chromosomal aberrations, including nondisjunction, the total cost would be only a fraction, approximately one-third, of the \$100,000 that is presently allotted for a single carcinogenic evaluation.

B. TESTING PROCEDURES (47)

Procedures for detecting and characterizing various types of genetic lesions include the following:

- (1) Detection of "premutational lesions": repair studies in experimental animals.
- (2) Detection of "point mutations": Host mediated assay, and body fluid analysis in experimental animals, using various indicator organisms as well as in vitro studies, with and without microsomal activation.
- (3) Detection of chromosomal change in experimental animals:
 - a. Dominant lethal test.
 - b. Translocation studies.
 - c. Micronuclei test.
 - d. Direct cytogenetic analysis with both meiotic and mitotic cells.
 - e. Sister chromatid exchange studies.

While collaborative studies have rarely been conducted in the field of toxicology, it is noteworthy that already in the field of genetic toxicology the dominant lethal test and in vivo cytogenetic analysis have been subjected to collaborative studies (48). The utilization of all or most of the above procedures should characterize the majority of mutagenic agents. Additionally, these studies combined with in vitro procedures will in many instances classify the induced genetic lesion. Existing procedures are as good as, if not better than, existing methods in the field of chronic toxicology; furthermore, we have the ability to gain insight into the molecular basis for the genetic alteration.

C. INDUSTRIAL MONITORING

The industrial environment provides a unique set of circumstances for detecting and characterizing chemicals that induce chronic effects such as the induction of mutations. In many instances, we can identify

employees who are exposed to a variety of chemicals, some of them unique to various industries, while others, representative of exaggerated exposure to what may be occurring in the general population. It is encouraging to note that some of our more progressive corporations have embarked on a comprehensive program to detect chemical mutagens (48).

In the context of an industrial program to characterize mutagenic agents, there are three aspects that one can consider. First, we can deal with experimental compounds that have yet to be introduced into the market place, or those agents that have not been adequately tested for mutagenic activity. These chemicals should be thoroughly investigated by a variety of available mammalian procedures, as previously discussed. Another aspect would be the evaluation of chemicals to which workers are exposed. Again, one can rely on cytogenetic analysis and the investigation of the blood, urine, and semen (when available) of these workers to look for potential mutagens; evaluation of these body fluids can employ the indicator organisms. A third aspect would involve classical epidemiological studies. Unfortunately, the epidemiological studies come rather late in the game; if indeed they are positive, significant adverse effects in the human population have already occurred.

D. INTERPRETATION OF RESULTS

Toxicology as well as pharmacology is often criticized for not being a more quantitative science. (Facetiously, it has been said that conventional procedures for carrying out chronic toxicity studies include counting the dead, weighing everything that can be removed, slicing everything that can be sliced, and feeding everything that could be fed for two years.) Actually, the importance of the quantitative data and

the various factors that can influence a biological response in animals has long been appreciated by toxicologists and pharmacologists. The route of chemical administration, the chemical's distribution in the tissues, the nutritional state of the animal, and, especially, the biotransformations of chemicals entering the circulation are some of the more obvious factors contributing to the variation of biological results in the same, as well as in different, animal species. A given chemical at different rates may be esterified in the rat, conjugated in the dog, and acetylated in man. All of these factors will influence assays for mutagenicity as well as measurements for any other toxicological effect.

The importance of a dose-response curve, the concept of a threshold effect, and the difficulties of extrapolating from animal data to man, especially in the area of carcinogenicity, have been the subject of many reports (49). As far as can be determined, the carcinogenic hazard never disappears with a diminishing dose but rather becomes infinitely small. A further complication of establishing a threshold concentration in carcinogenesis is the possibility of co-carcinogens (50) being present in the environment, further obscuring even the possibility of obtaining a no-effect level. The concept of a threshold response in mutagenesis is hardly different from that in carcinogenesis. In the case of X-rays, a direct linear relationship between dosage and genetic effects in various biological systems gave rise initially to the "single hit" interpretation of X-ray effects (single hit and ionization of DNA). This single hit causes a permanent alteration of DNA (mutation), and the effects are usually additive when doses are applied intermittently. A number of substances (e.g. 1, 2-dithioglycerol) are known to modify the

lethal and mutagenic effects of X-rays. The existence of compounds that can modify the mutagenic effects of X-rays has cast some doubt on the target (single hit) theory and has led to the assumption that the genetic effects of radiation may be a more indirect result. As in carcinogenesis, a direct effect or an effect modified by environmental agents in mutagenesis is possible.

In mutagenic studies, a dose-response curve for interpreting animal results should be a prerequisite. Mathematical models that take into account the size of the animal population studies, as well as the dose-response in extrapolating to a large population exposure, are presently under investigation (51). The precision of the test used for mutagenicity should be such that a doubling of the control level of mutation would be statistically significant at the 5 percent level. A substance should be considered as a potential mutagen if one or more of the procedures carried out in the intact animal are positive. In a given experiment, failure to establish an existing effect with a chemical is generally referred to as a type II or beta (β) error. It is essential that in all mutagenic studies sufficient animals are used, enough slides are read, sufficient implants are analyzed, etc., to minimize the β error. A casual examination of the literature in this field frequently reveals that the studies conducted would preclude assuming a negative response.

IV. PRIORITY FOR TESTING

The assigning of priorities to environmental agents and their subsequent in-depth screening and characterization offers the possibility of eliminating the most important deleterious environmental agents. At first glance, the task of screening environmental agents for mutagenicity

seems overwhelming. No data are available for most of the thousands of compounds introduced into our environment over the last three decades. The appreciation of this formidable task led to a search for a simple, economic screen to detect mutagens. As stated heretofore, it is not logical to utilize simple systems, such as microbial procedures, in such a screening for potentially active compounds; in fact, the most meaningful screen would rely on a battery of tests carried out in intact animals. In view of these considerations, we must establish priorities for testing environmental agents and then proceed to screen the selected compounds in a meaningful manner. A comprehensive screen using a combination of available methods in animals, including testing of metabolites produced in the intact host, is still comparatively rapid and economical when compared to the conventional carcinogenicity screens.

A selection of compounds for testing should be based on the following criteria: (1) usage pattern, i.e. the exposure of a large segment of our population, especially people of child-bearing age, (2) length of exposure, (3) chemical's persistence in the environment, and (4) structure-activity relationship for mutagenesis*. Employing usage pattern as the criteria, what seems like an overwhelming task reduces to a reasonable number of chemicals whose testing does not present an enormous financial burden. Illustratively, 200 prescription drugs control 69% of the market; this translates into 190 separate active ingredients (28). The non-prescription, or over-the-counter (OTC), drug market sports as many as 500,000 products (3). Although Congressmen were told that this plethora boils down to 216 active ingredients (53), our survey indicates that at

*Such a vital relationship is in the process of development at the Environmental Mutagen Information Center, Oak Ridge, Tennessee (52).

least another 30 "real" active ingredients, not of the ilk of oatmeal, cottonseed oil, etc., should have been on that list of chemicals that can appear in OTC's; while a final number of active substances was not determined in our survey, it is felt that the number does not exceed 300 (28). On a positive note, a third of these are probably uninteresting as they are comprised of such presumably non-mutagenic items as vegetable oils, salts, and alcohols.

Once again, a variegated and voluminous market as that of the pesticides can be managed by concentrating on the market leaders: 19 fungicides, 37 herbicides, and 31 insecticides control 94%, 98%, and 93% of their respective markets (6).

With the banning of F. D. & C. Red No. 2 in February 12, 1976, only ten synthetic colorings remain approved for use in food. Regarding food additives in general, a priority list for them undoubtedly exists in the FDA since that body was ordered by the President in 1969 to review the safety of food additives; this was in response to the banning of former GRAS (Generally Recognized As Safe) additives, the cyclamates, due to their carcinogenic property. Disclosure of such a list is of paramount importance if the thinking behind the Toxic Substances Control Act is to work. In general, a lot more statistical information is needed of the government, including the kind that will allow the development of a meaningful priority list for industrial and miscellaneous chemicals.

V. BENEFIT-RISK ANALYSIS

In past cases, concern for the mutagenic activity of a compound surfaced prior to a comprehensive evaluation of the suspected product. In almost every instance, regulatory agencies and various expert committees were asked to make decisions on the continued use of a product with incomplete information. Polarization frequently occurred between consumer advocates and the manufacturer of the product. With a minimal amount of information, consumer groups advocated the restriction of the product, while the manufacturer usually questioned the reliability of the incriminating data and took the view that more definitive work is needed prior to taking any action. The Government usually vacillated between the two extremes. It would be the hallmark of the Toxic Substance Control Act if it reduces this situation. Though it is doubtful that we will ever have enough data to satisfy some manufacturers, on the other hand, we should determine minimal requirements before concluding that a chemical is mutagenic. Regardless of the intended use of the product, no decision on the restriction or the elimination of the chemical should be made solely on the basis of non-animal testing. A compound should not be considered non-mutagenic if the β error is of such magnitude to preclude detecting of a minimal mutagenic effect. The experiment should be constructed so that a doubling over background rate (cytogenetic response, increase in point mutations, etc.) is significant at the 5% level. A positive response in any of the animal systems should be sufficient to characterize the compound as a mutagen. This positive finding will be sufficient to eliminate

a non-nutritive food additive or non-prescription drug from the market place. The elimination or prudent exposure to other positive compounds such as drugs, pesticides, and industrial chemicals will depend on the use-benefits outweighing the potential risks.

Figure III. U. S. Quantity of Sales of Food, Drug, and Cosmetic Colors, 1940-1970 (4).

Insert: U. S. Quantity of Sales of F. D. & C. Colors per Person, 1940-1970.

