REBUTTAL OF THE EFSA DRAFT ON ASPARTAME SAFETY 2013

Sent in 6 parts to the EFSA via specific link for comments by Marià Alemany on January 21, 2003; 13:00 to 13:05 Spain Winter time.

I present my allegations in 6 queries. This is the first

L54 If DKP (a metabolite of Phe) is included, then add HCHO (a metabolite of methanol), since both metabolites take up most of the ensuing discussion.

L59-60 This statement is not based in fact, in fact, there is a number of animal studies showing that in part aspartame is excreted in feces as such, other results of label incorporation into proteins and DNA in tissues are inconsistent with absorption of free methanol and the logical filtering-out by the powerful liver alcohol dehydrogenases, impeding their arrival to tissues other than liver, which has been found experimentally not to be the case. The statement that hydrolysis of aspartame in the gut is very efficient has not been proven either, and could not explain most of the results found in animals using aspartame (as such) with radiolabelled methanol. In fact, hydrolysis depends on the dose and the presence of other substrates for peptidases in the gut (lumen and wall).

L69 The assumed lack of toxicity of aspartame does not take into account long-term effects, for which there is abundant bibliography (humans). Toxicity in food components could not be tested in the same way as drugs, since the use of drugs is occasional and is the lesser of two evils (the drug of the disease). For food components (such as aspartame, non-essential, semi-drug compound) the level of safety for long use should be included and tested. Testing should include analysis of the fate of the compound, its metabolization and possible additive effects. None of this has been done under controlled conditions.

L70 This sentence would be true only is the Reviewers (or the Panel) consider that formaldehyde adducts of proteins and DNA are non-toxic. In fact there is abundant proof on the contrary.

L75 There are a number of genotoxic studies done on aspartame, however, the Panel decided that none was accepted only one as valid (it was negative). This part of the abstract is misleading and should indicate -to be fair- the number and origin of studies discarded in the same way that the only one accepted is cited (and its origin stated). Again, no mention is made to the chronic continued use of the compound; this is the main question for a food product consumed continuously for decades. The text in the ensuing lines is convoluted and misleading, in fact not supporting the final conclusion of L81.

L100 The diagnoses of tumor type may be of interest for a scientific paper, but in a study of adverse effects of a food additive the important question is that there were tumors, and these could be traced to aspartame.

L150 Can the Panel explain how formate can enter the 1C pathway AND become a form of iC usable (instead of being finally oxidized to CO2? To assume otherwise is against what we know of the metabolism of 1C in mammals. The “explanation” of formate is just a theoretical approach, not based on experimental studies not even on the present knowledge of its biochemistry.

L155 The Trocho (1998) study provided clear data on that question (DNA adducts), why did not the Panel consider this proven evidence?

Second of 6

L656 (and L18) The text should refer also to methanol and formaldehyde.

L836 The dismissal of papers reporting tumours because of criticisms of other papers (not repeating experimental procedures, in fact) and the FDA or documents generated by the Aspartame producers should take in consideration what is said in L864-865, which prove the impossibility of using direct approaches. Thus, if the direct approaches could not be (logically) done, all epidemiological and anecdotic studies are dismissed, and the animal studies are all dismissed by real or assumed methodological considerations. How can anybody convince the Panel that there is something wrong with aspartame? Biochemical, physiological, cytological, genetic, epidemiological and clinical studies, hundreds of them, all are wrong for one reason or other. The charge of proof has been heavily set

upon the shoulders of independent researchers, with the full weight of the EFSA Panel devoted to find (assumed) errors in their work. I know about what I am saying here. In a separate piece I included my comments on how my work on the biochemistry of the 1C moiety of aspartame induces biochemical damage. Judging from how the results have been misread, distorted and dismissed because somebody said so without experimental backing, I am afraid that the utmost reason of the Panel to exist: the defense of Europeans from possibly toxic compounds, in this case aspartame is retorted to the reverse. Suspicion, based on facts, on peer-reviewed papers by independent scientists looking for the common good deserve a better treatment. Doubts should be completely cleared, if necessary by EFSA sponsored studies, but the enormous amount of negative evidence is not a pure accident, nor a campaign against a product (why this one?), in fact the reverse may be true. The Hippocratic mandate, do not harm, should combine with the need to act cautiously in case of doubt (yours, I assume, not mine), and never allow harm to continue. I suggest you clarify the situation, ask the actual scientists and look less at previous official reports than to the actual data and simply, make projections. Also keep in mind your responsibility towards Europeans and their health and well being, your consciences and your responsibility with scientific truth.

L877 The Soffritti study has been dismissed (obviously by methodological or design limitations), but the tumors were there as the consequence of aspartame administration. This cannot be dismissed. By the way, why the Panel has not dismissed the paper by Tephly (1999) because of flaws in the methodology: there is none, there are no results, nor believable basic Biochemistry? This pseudo-article was used to "dismount" one of the most damaging (for aspartame use) studies (Trocho et al, 1998). The exigencies of methodology and quality used by the Panel are not uniform and seem to point towards proving the safety of aspartame even against contrary evidence.

Third of 6

L1474. The Trocho Stydy used single dose and repeated doses (showing cumulative effects, not cited in the text). Dose detected was in liver as high as 2% of dose administered. Hydrolysis of proteins was filtered because this is the procedure used to analyze amino acids from proteins. In any case, the “dismissal” of spots in protein hydrolysates as Maillard remnants may be true, but the only labelled aldehyde available was formaldehyde. All other Maillard adduct forming compounds were not labelled and thus could not appear in the chromatograms. All labelled artifacts could come only from the label (initially the methanol part of aspartame), thus reinforcing the fact that label arrived to the cells and was not as 1C carbon incorporated into amino acids or nucleic acids.

In fact this is not the first time methanol-labelled aspartame has been found in tissues of experimental animals (Opperman et al. J Nutr 1973; 103:1454-1459. However, in that case, the label was attributed (without further analysis) to the preferred tale of Tephly and associates: incorporation in the 1C pool and transfer to protein amino acids. Now we know that this was not the case.

One-carbon metabolism can use small fragments via the THF pathway, which uses them for synthesis purposes. The only amino acids that could incorporate a 1C unit are methionine (and to a very lower extent serine). None of these amino acid spots showed label. Thus proving without doubt that the label from 14C-(methanol)-aspartame was not incorporated into amino acids but adducted to proteins. The same can be said of RNA and DNA, citrulline was not marked, but undefined adducts contained all the label. The analysis of label was done by exposing the chromatograms to plates not as indicated in the text. The analysis of DNFB-AA was used only to “locate” the exact spots for AA.

The incorporation of 1C to amino acids is done only via THF (and in nucleic acids also via Met methylation). There is no other way (I refer the Reviewers to any elementary book of Biochemistry to check that) to physiologically transfer the label of methanol to amino acids or nucleic acid bases.

L1490. Nowhere in the Trocho paper is said that there was a single radiolabelled species in plasma after hydrolysis and derivatisation, but a number of indeterminate adducts of formaldehyde (they were not in amino acids, but present in protein; the label came from methanol and methanol nor formic acids bind to protein, then they were adducts of formaldehyde). Presence of adducts was higher in tissues with active alcohol dehydrogenases, which convert methanol to formaldehyde.

Fourth of 6

L1493. The paper by Tephly (1999) is the only criticism (other than documents from the producers and official statements) about the Trocho study. However, the paper of Tephly is not experimental, but some sort of criticism, biased and not based on hard experimental data. It is unbelievable that such a text will be taken seriously instead of direct, perfectly repeatable experimental data, in which the thesis are proven without doubt.

L1498. Are the Reviewers aware that Lys-formaldehyde adducts would not appear in the same spot of Lys? In that study, the search was not for a specific compound, but to prove that there was no 1C incorporation into Met, in fact the adducts did not coincide with any amino acid.

Even assuming that the methodology were defective (it has not been proven such thing, anyway), the presence (and removal) of Maillard adducts (I repeat, labelled-Maillard adducts) could come only from a labelled aldehyde, and the only source of 14C in the whole system was orally-given aspartame's methanol moiety. The reason adduced in the draft for dismissing the study is, in consequence further proof of its validity, showing only that the transfer of the label from aspartame was probably higher than reported.

The use (L1504) of the word "unequivocally" is inadequate, and show a predisposition of the Panel. The Re-evaluation is done to prove the safety of the compound, not to reverse the chrge of the proof and have other prove "unequivocally" its lack of safety. But indeed, the Trocho study is the only one arriving at the biochemical level and proving UNEQUIVOCALLY that label from the methanol moiety of aspartame damages (in an additive way) both proteins, and DNA in most rat tissues.

The modification of DNA is a cause of mutation, and the basis of the mutagenic effect of formaldehyde. The presence of label in DNA proves that it has been modified by aspartame feeding. This could not be dismissed because of reasons other than scientific analysis of the results or the demostration (by experimentation) that they were wrong.

It is suggested that the Panel actually read the Trocho paper before dismissing it because of absurd reasons such as the formation of Maillard adducts (labelled), which is part of the proof. By the way, how can the Panel explain the presence of label in DNA. Is there any bias in the way this paper was analyzed? As responsible Author for the Trocho study, I am not sure whether somebody with minimal knowledge of Biochemistry has read the paper or even the conclusions, since they are self-conflicting, and open doubts about how other similar "negative" studies have been treated by the Panel. The panel cites an study by Karikas et al (1998), which actually shows that aspartame has a clear and direct interaction with DNA, but the citation is out of context.

Fifth of 6

L1358 (and others) The presence of Phe in plasma and tissues is no proof in itself that all aspartame is hydrolyzed in the gut. The use of individual amino acids and Phe-methanol are no substitutes for aspartame.

L1392 In this key study, hydrolysis of aspartame was not measured.

L1404 The assertion of aspartame hydrolysis was not been proven, the cite is a review from the Company producing aspartame. They also assumed that methanol gave yield to 1C fragments which incorporated into protein, but this was not studied neither proven.

1412 The label from aspartame was not detected in plasma, but this is in contradiction with the finding of significant amounts of label from methyl-labelled aspartame in plasma (Opperman et al. 1973, Trocho et al. 1998), as well as from the studies on hydrolysis of aspartame in the gut reported in the gut aspartame hydrolysis studies. The differences in methodology should be explained, especially when all the references supporting this entry correspond to documents from the Aspartame producers.

L1421 The "total hydrolysis" of aspartame has not been proved experimentally if the strict considerations of the Panel are taken into account. The references to hydrolysis (Oppermann 1984 and Hoooper et al. 1994) in the gut may represent a conflict. The work of Oppermann 1984 is a

review, it does not present experimental data. In the study of Hooper et al. 1994, the main product of peptidase activity is Phe-methanol, which they also indicate showed a slow degradation. These data contrast with those of Burgert et al. 1991 (also cited in L1430) who proved that Phe-methanol was not absorbed in significant amounts and no whole aspartame was found in absorption via portal vein (after direct intestinal infusion). This is not sufficient proof, since aspartame is not stable in the acidic medium of the stomach and alcohols are absorbed through the stomach wall (I am not suggesting this is a way for aspartame methanol to enter the bloodstream, but the reasoning used here is at the same level as that used by the panel). In any way, if aspartame is completely hydrolyzed in the gut, and methanol is rapidly eliminated (if absorbed), how can methanol moiety label be found in significant amounts in animal tissues?

The role of the microbiota in the disposal of methanol should be taken into account and cited, since the microbiota can use efficiently as substrate the methanol it pries out of pectin. This is the main reason because we don't have methanol poisoning when eating most pectin containing fruits (in addition to the main way of disposing fo this form of fibre: unaltered in stool). In fact, most of the methanol attributed to pectin (and other methanol ester of uronic acids in fibre) is simply used by the biota as energy substrate, or, at least, oxidized to formaldehyde which attaches itself to whatever protein, or DNA strand (including microorganisms) ir can found in the large intestine contents.

Sixth of 6

I suggest the Panel, to analyze (and include) in the study, the following papers (and book):

Abdel-Salam OME, Salem NA, El-Shamarka MES, Hussein JS, Ahmed NAS, El-Nagar MEE. Studies on the effects of aspartame on memory and oxidative stress in brain of mice. European Review for Medican and Pharmacological Sciences. 2012; 16 2092-2101.

Aune D. Soft drinks, aspartame, and the risk of cancer and cardiovascular disease. American Journal of Clinical Nutrition. 2012; 96 (6): 1249-1251.

Collison KS, Makhoul NJ, Zaidi MZ, Saleh SM, Andres B, Inglis A, Al-Rabiah R, Al-Mohanna FA. Gender dimorphism in aspartame-induced impairment of spatial cognition and insulin sensitivity. PLoS One. 3-4-2012; 7 (4): e31570

Schernhammer ES, Bertrand KA, Birmann BM, Sampson L, Willett WC, Feskanich D. Consumption of artificial sweetener- and sugar-containing soda and risk of lymphoma and leukemia in men and women. American Journal of Clinical Nutrition. 2012; 96 (6): 1419-1428.

Monte WC. Methanol: A chemical Trojan horse as the root of the inscrutable U. Medical Hypotheses. 2010; 74 493-496.

Monte WC. While Science Sleeps. Monte WC Lexington KY, 2011 [Book: a complete and actualized analysis of methanol chemistry and toxicology]

Portari GV, Mathias MGM, Almeida BB, Marchini JS, Jordao AA. Effect of the temperature and pH o methanol release in coffee brew sweetened with aspartame. Acta Alimentaria. 2009; 38 (3): 303-307.

Humphries P, Pretorius E, Naudé H. Direct and indirect cellular effects of aspartame on the brain. European Journal of Clinical Nutrition. 2008; 62 451-462.

Gombos K, Varjas T, Orsós Z, Polyák É, Peredi J, Varga Z, Nowrasteh G, Tettinger A, Mucsi G, Ember I. The effect of aspartame administration on oncogene and suppressor gene expressions. In Vivo. 2007; 21 89-92.

Belpoggi F, Soffritti M, Padovani M, degli Esposito D, Lauriola M, Minardi F. Results of long-term carcinogenicity bioassay on Sprague-Dawley rats exposed to aspartame administered in feed. Annals of the New York Academy of Sciences. 2006; 1076 559-577.

Lau K, McLean WG, Williams DP, Howard CV. Synergistic interactions between commonly used food additives in a developmental neurotoxicity test. Toxicological Sciences. 2006; 90 (11): 178-187.

Hall WL, Millward DJ, Rogers PJ, Morgan LM. Physiological mechanisms mediating aspartame-induced satiety. Physiology and Behavior. 2003; 78 (4-5): 557-562.

Soffritti M, Belpoggi F, Lambertini L, Lauriola M, Padovani M, Maltoni C. Results of long-term

experimental studies on the carcinogenity of formaldehyde and acetaldehyde in rats. Annals of the New York Academy of Sciences. 2002; 982 87-105.

Karim A, Burns T. Metabolism and pharmacokinetics of radiolabeled aspartame in normal subjects. 1996; 5 57-66.

Olney JW, Farber NB, Spitznagel E, Robins LN. Increasing brain tumor rates: is there a link to aspartame? Journal of Neuropathology and Experimental Neurology. 1996; 55 (11): 1115-1123.

Thanks for your attention. Please let me know if I can help you further.