French agency for food, environmental and occupational health & safety

Maisons-Alfort, 14 March 2011

The Director General

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on a publication reporting the incidence of cancer in male mice after administration of aspartame in their feed, and a second publication on a prospective cohort study of pregnant women reporting an association between the consumption of carbonated (fizzy) soft drinks containing sweeteners and the risk of preterm delivery.

1. REVIEW OF THE REQUEST

The French Agency for Food, Environmental and Occupational Health and Safety responded to an internal request on 24 January 2011 to examine two recent scientific publications on sweeteners, one of which concerned aspartame exclusively. The first is a study of experimental carcinogenesis reporting an increase in the incidence of cancers in male mice fed a diet with added aspartame[1]. The second is a prospective cohort study of pregnant women reporting an association between the consumption of carbonated (fizzy) soft drinks containing artificial sweeteners and an increase in the frequency of induced preterm deliveries[2,3,4].

As part of its continual monitoring of health issues, ANSES examined these publications in order to make possible recommendations to the French authorities and/or refer the issue to the European Food Safety Authority (EFSA), which is responsible for assessing food additives at European level.

2. EXPERT APPRAISAL METHOD

The expert appraisal was carried out by an expert group of toxicologists and epidemiologists on the basis of the data presented in the publications mentioned (without having access to the raw data) and the resulting Opinion was adopted by the Expert Committee (CES) on “Additives, flavourings and processing aids”, consulted on 24 February 2011.

The French Agency for Food, Environmental and Occupational Health & Safety’s justification is based on the opinion of the “Additives, flavourings and processing aids” CES, as well as the advice of the “Nutrition” CES and the interviews held, particularly the one with Morando Soffritti (principal author of the article on aspartame).

[1] Soffritti M. *et al.*, American Journal of Industrial Medicine 53: 1197-206, 2010.

[2] Halldorsson T.I. *et al.*, American Journal of Clinical Nutrition 92: 626-33, 2010.

[3] La Vecchia C, followed by the response from T.I. Halldorsson. American Journal of Clinical Nutrition 92: 1540-1542, 2010.

[4] Bursey R.G., Watson, M.L., followed by the response from T.I. Halldorsson, American Journal of Clinical Nutrition 92: 1278-1280, 2010.

2.1. Publication by Soffritti *et al.*[1]

This study was carried out at the Ramazzini Foundation in Bologna, Italy. Swiss mice divided into five groups of 62 to 122 animals of both sexes were fed a diet supplemented with 0, 2000, 8000, 16000 and 32000 ppm of aspartame (reported purity: 98.7%) which corresponds to doses of approximately 0, 250, 1000, 2000 and 4000 mg/kg body weight/day. After mating, the females were administered this diet from the 12th day of gestation until the offspring were weaned. The offspring were then fed the same diets until they were sacrificed at the age of 130 weeks.

Their consumption of feed and water was measured at regular intervals. The body weight of each animal was recorded weekly for 13 weeks and then fortnightly until the 110th week. All the surviving animals were sacrificed at the age of 130 weeks. A full histological analysis was made of about forty organs or tissues.
The purpose of the experiment was to assess the potential of aspartame to induce carcinogenic effects in mice.

The results show an increased incidence of hepatocellular adenomas and carcinomas in male mice. The increase in the incidence of adenomas was not statistically significant at any dose. For carcinomas, an increased incidence only became statistically significant at the highest dose tested (about 4000 mg/kg body weight/day). A statistically significant increase in the accumulated incidence of adenomas and carcinomas was only observed at the intermediate dose (about 2000 mg/kg body weight/day). No increased incidence in liver tumours was detected in the female mice at any of the doses of aspartame tested.

An increase (at the limit of statistical significance) in the combined incidence of bronchoalveolar carcinomas was observed in the males at the highest dose tested (about 4000 mg/kg body weight/day). No increased incidence in bronchoalveolar tumours was detected in the female mice at any dose of aspartame tested.

Comments

During the interview, the principal author of the study stated that the Foundation where the study was carried out was certified as following good laboratory practice (GLP). According to the author, this study was carried out in the spirit of GLP requirements, but without applying them strictly. It should be noted that the study's GLP status is not specified in the publication.

The methodology used in this study seems unusual insofar as it consists in exposing a very large number of animals throughout their entire lifespan. This contrasts with the recommendations issued by international bodies such as the OECD[5] and the ICH[6] for long-term carcinogenesis experiments, which advise exposing adult mice (aged from 5 to 6 weeks) for 2/3 of their lives, i.e. 18 months to 2 years. Indeed, it is generally accepted that, beyond this age, the physiological condition of the animals markedly degrades, which can distort the results. Furthermore, these recommendations require that all groups be subjected to an identical period of treatment in order to allow reliable comparative analysis between spontaneous lesions (presented by the unexposed control groups) and those presented by the exposed groups of animals. The purpose of limiting the monitoring period is not only to minimise analytical bias related to the significant reduction of numbers by the end of the study, but also to avoid difficulties in interpretation that could result from age-related pathologies.

The authors of the aspartame study state in their publication that the survival rate of the groups at 130 weeks was lower than 10%, but detailed data describing the general condition of the animals are lacking. Available data consist only of a few graphs showing a sharp reduction in the

[5] Organisation for Economic Cooperation and Development (OECD) http://www.oecd.org
[6] The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). http://www.ich.org/home.html

consumption of feed beyond 88 weeks, in both males and females. No information was given on non-neoplastic pathologies.

Considering the sweet taste of the substance, it was probable that there would be some change to the feed consumption of the exposed animals, but this does not seem to have significantly changed in animals exposed to the highest doses of aspartame.

No details were given regarding the course of gestation for the gravid females tested, especially in terms of litter size, their viability at birth, and during lactation. Considering that the protocol involves forming groups from series of parent pairs exposed to the different doses tested, the number of animals monitored in the first generation differs considerably from one group to the next (ranging from 117 to 62 animals).

The method used in the study for compiling an inventory of tumours in mice is unusual and not fully explained. The following types of tumour are listed separately: multiples tumours of different types occurring in different organs, tumours of different types occurring in the same organ, tumours of the same type in bilateral organs, tumours of the same type occurring on the skin, in subcutaneous tissue and mammary glands or at remote sites consisting of “diffuse” tissue (muscle and bone). Only multiple tumours of the same type occurring in the same tissue and/or organ are considered as single events. The validity of this method for compiling an inventory of tumours is not explained in the publication. During the interview, the principal author of the study agreed that this method is unusual.

The expert group also noted that different statistical models were applied in different cases without the choice being explained.

The tumours described in this study are mainly adenomas and carcinomas but no hyperplasia was reported (this type of lesion is an important intermediate stage in the transformation of normal tissue into neoplastic tissue). According to information provided by the principal author, hepatocellular lesions develop directly into carcinomas, which would explain the absence of hyperplasia in the affected organs and, in his opinion, would also explain the absence of any link between the effect observed and the doses of aspartame. Such a hypothesis remains to be confirmed. Furthermore, the incidence of benign tumours, of any type, was higher in the females exposed to aspartame than in the control females (not exposed to aspartame), while no increase was observed in the incidence of malign tumours. Lastly, the authors do not advance any explanation for the absence of mortality among males treated with the highest doses, despite their presenting a significant increase in the incidence of tumours. In addition, the publication by Soffritti *et al*. includes no data for evaluating the hepatotoxicity markers (such as the ratio of AST to ALT enzymes or the level of bilirubin), or about the weight of the organs affected.

It should be emphasised that a high rate of spontaneous liver and lung tumours is generally observed in the strain of mice used in this study[7].

In their observations, the authors point out that the rates of hepatocellular and bronchoalveolar carcinomas in contemporary male controls (5.1% and 6.0% respectively) are in the lower part of the range of historical controls (0 – 26.3% and 0 – 14.3% respectively). The authors observed an increased incidence of tumours in animals exposed to aspartame, which was 2 to 3 times higher in exposed animals than in contemporary control animals. Nonetheless, no comment was made about the higher rate of bronchoalveolar carcinomas in contemporary female controls (6.9%) compared with historical female controls (0 – 2.1%). It therefore seems likely that there is some variation in these data.

[7] Gopinath C. Spontaneous tumour rates: their use to support rodent bioassays. *Toxicol. Pathol.* 22: 160-164, 1994; Prejean JD. *et al*. Spontaneous tumours in Sprague-Dawley rats and Swiss mice. *Cancer Res*. 33: 2768-2773, 1973.

Lastly, the authors suggest that the increased incidence of hepatocellular tumours could be due to the partial metabolism of aspartame into methanol. However, no measurements were taken of plasma concentrations of aspartame metabolites (aspartic acid and phenylalanine or methanol), which would have made it possible to verify that systemic and dose-dependant exposure of the animals to the aspartame metabolites really occurred. The absence of any increase in the incidence of hepatocellular tumours in female mice, reported by the authors in their publication, is explained by a "gender-related resistance" without providing any experimental proof.

2.2. Publication by Halldorsson *et al.*[2] and complementary elements[3,4]

This publication reports the results of a study carried out originally on 91,827 pregnant Danish women, recruited on a voluntary basis between 1996 and 2002. About 35% of all women who were pregnant during the recruitment period took part; a comparison undertaken with a sample of non-participants shows no major difference in the rate of preterm delivery. Among the participants, 62,374 women (68%) completed a dietary questionnaire providing information about beverage consumption. Only singleton pregnancies (i.e. excluding twins) were considered. Among the women who completed the questionnaire (final population of 59,334 women), primiparous women (those giving birth for the first time) were proportionately over-represented, as were those with slightly more favourable social characteristics (a frequent observation in this type of study).

Information on the consumption of sweetened beverages was gathered at 25 weeks of pregnancy using a classic food-frequency questionnaire (FFQ) on consumption that covered the preceding 4 weeks. According to the authors, the questionnaire included questions on the different types of beverage such as "How many servings of the following beverages have you consumed during the last four weeks?" The study concerns the consumption of 4 types of beverages: artificially-sweetened carbonated soft drinks / colas, sugar-sweetened carbonated soft drinks / colas, artificially-sweetened non-carbonated soft drinks and sugar-sweetened non-carbonated soft drinks.

The results of the survey show that 67% of the Danish women surveyed never drank artificially sweetened carbonated soft drinks / colas, 29% drank less than one serving per day and 4% at least one serving per day. Concerning artificially-sweetened non-carbonated soft drinks, 66% of the women surveyed never drank them, 21% drank less than one serving per day and 13% at least one serving per day. Consumption of sugar-sweetened soft drinks, carbonated or non-carbonated, was much more common, with 16% and 36% of women, respectively, never drinking them.

Preterm delivery was defined according to the criteria of the World Health Organization as having a gestational age of less than 37 weeks (i.e. ≤ 36 complete weeks since the first day of the last menstruation). The overall rate of preterm deliveries in the sample of women studied was 4.6%, a lower rate than the national figures for Denmark or France during the same period, which were 7% and 6.3% respectively[8] (including multiple pregnancies). In the Danish study, 33% of preterm deliveries were induced as the result of a medical decision, because of maternal or foetal complications, and 67% were spontaneous, with labour starting unexpectedly.

The results of the study[2,3,4] show a statistically significant dose-effect relationship between the consumption of artificially-sweetened carbonated soft drinks and the frequency of preterm deliveries. This relationship mainly concerns induced preterm deliveries, and is stronger in the case of artificially-sweetened carbonated soft drinks than artificially-sweetened non-carbonated soft drinks.

If all artificially-sweetened soft drinks are taken into account (carbonated and non-carbonated), the results remain practically the same: a dose-effect relationship with preterm delivery, mostly from induced preterm deliveries, but fewer than with artificially-sweetened carbonated soft drinks alone.

No relationship was found between the consumption of sugar-sweetened drinks, whether

[8] Europeristat. European Perinatal Health Report. 2008. http://www.europeristat.com/

carbonated or non-carbonated, and preterm delivery, either induced or spontaneous.

Comments

This study took the major known risk factors for preterm delivery into account, to control for possible confounding effects. However, considering that the increased risk mainly concerns induced preterm deliveries, it would have been useful to know which complications led to the decisions to induce delivery: pregnancy-induced hypertension, retarded intrauterine growth, haemorrhages or premature rupture of the membranes. An analysis of retarded intrauterine growth (low weight for gestational age) as a function of the consumption of artificially-sweetened beverages would have provided additional information. A knowledge of these complications could help identify the mechanisms responsible for the results observed.

The only mechanism mentioned by the authors is the exposure to aspartame, assumed to be present in larger quantities in carbonated than in non-carbonated artificially-sweetened soft drinks. They suggest the possible role of methanol, resulting from the degradation of aspartame, and a possible link with hypertension. However, the data do not show any modification of the results after adjustment for hypertension or preeclampsia, but the information on hypertension and preeclampsia was obtained only from the women surveyed and these complications may have been under-declared.

For a better understanding of the significance of these results, it would have been preferable if the authors of the Danish study had provided greater detail on certain points:

- the exact wording of the questionnaire on beverage consumption, and on the other dietary sources of artificial sweeteners,
- a more detailed description of the women surveyed according to their level of consumption of the beverages studied, especially any obstetric antecedents, chronic illnesses, social characteristics, birth-control practices, dietary habits (particularly concerning the consumption of low-sugar and low-fat products),
- the reasons why delivery was induced prematurely, such as retarded intrauterine growth (low weight for gestational age) for instance, as a function of the level of consumption of the beverages studied,
- complications in pregnancy for each level of consumption of the beverages studied,
- analyses of all the foods that might be sources of aspartame and/or other artificial sweeteners consumed by the women surveyed.

Exposure calculation

French levels of exposure to aspartame have been estimated for the highest consumers of products potentially containing them; this was estimated on the basis of the maximum permitted usage levels by the legislation. These estimates are considerably below the Acceptable Daily Intake (ADI), including for young diabetics (21% of the ADI, i.e. 8.4 mg/kg body weight/day, according to data from a study on young diabetics[9]) and for pregnant women (23% of the ADI, i.e. 9.2 mg/kg body weight/day, according to data from the EDEN cohort monitored by INSERM since 2003[10]). Among the latter (n=1594 pregnant women), the observed rate of preterm deliveries (<37 weeks) is 5.4%.

[9] The data on the consumption by young diabetics are taken from a French study carried out on 227 young diabetics (Garnier-Sagne I., Leblanc J.C., Verger P. Calculation of the intake of three intense sweeteners in young insulin-dependent diabetics. Food Chem. Toxicol. 39, 745 – 749, 2001).

[10] The data on the consumption by pregnant women are taken from the EDEN cohort, a study carried out by INSERM since 2003 on 2000 pregnant women.

3. CONCLUSIONS

On the basis of the information available in the publications (without having had access to the raw data) and also on the basis of interviews with Morando Soffritti (principal author of the article on aspartame), the French Agency for Food, Environmental and Occupational Health & Safety makes the following observations:

On the study by Soffritti *et al.*: the study was carried out using Swiss mice, according to an unusual protocol that does not comply with the international guidelines laid down for this type of study. It is generally accepted that in this type of experimental protocol the physiological condition of the animals declines substantially with age, which can distort the results and cast doubt on the conclusions of the study. Furthermore, the incidences of liver and lung tumours reported in this study are characteristic of, and frequently observed to occur spontaneously in, the strain of mice studied. The statistical analyses carried out do not show a dose-effect relationship for aspartame. In addition, because of the uncertainties and methodological deficiencies, it is impossible to characterise the effects reported in this study so as to extrapolate them to the situation in humans.

On the study by Halldorsson *et al.*: this study shows a positive association between the consumption of artificially-sweetened beverages and the risk of preterm delivery. No causal relationship has yet been established, however. This is the first study published on the subject and the mechanisms potentially responsible for an increase in the incidence of induced preterm delivery have not been identified. As the authors themselves mention, it is necessary to carry out further studies to negate or confirm their results.

It should be remembered that aspartame has been the subject of numerous toxicological studies and scientific publications. The Acceptable Daily Intake (ADI) of aspartame has been reviewed and confirmed several times by European and international health agencies over the last decade or so[11,12,13,14,15]. These two new publications do not provide a sufficient scientific basis to justify a revision of the established ADI for aspartame (40 mg/kg body weight/day).

As part of their continual monitoring of health issues, ANSES's Expert Committees regularly examine new studies produced on aspartame and the other authorised sweeteners with a view to re-evaluating any potential associated risks.

Apart from the specific toxicological issues, these questions fall within the framework of recurrent investigations of authorised sweeteners, the appropriateness of their use and the characterisation of their nutritional benefits and risks. In this context, ANSES will shortly be setting up a working group to evaluate the nutritional benefits and risks of sweeteners and the eventual need to draw up recommendations for the population groups most closely concerned.

[11] AFSSA report on the question of a possible link between exposure to aspartame and brain tumours. Maisons-Alfort 7 May 2002. Request number 2000-SA-0249.

[12] Opinion of the Scientific Committee on Food: Update on the safety of aspartame. Expressed on 4 December 2002, European Commission. 10 December 2002.

[13] Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to a new-long term carcinogenicity study on aspartame. The EFSA Journal 356:1-44, 2006.

[14] Updated opinion on a request from the European Commission related to the 2nd ERF carcinogenicity study on aspartame, taking into consideration study data submitted by the Ramazzini Foundation in February 2009. The EFSA Journal 1015: 1-18, 2009.

[15] Report of the meetings on aspartame with National Experts. Question number: EFSA-Q-2009-00488. May, 2010.

The Director General

Marc MORTUREUX

KEYWORDS

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