

STATEMENT OF EFSA

on the scientific evaluation of two studies related to the safety of artificial sweeteners¹

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ABSTRACT

The European Food Safety Authority was asked to provide scientific advice on two studies, namely a carcinogenicity study in mice (Soffritti et al., 2010) and a prospective cohort study on the association between intakes of artificially sweetened soft drinks and preterm delivery (Halldorsson et al., 2010) and to conclude on the need to revise previous evaluations of aspartame or of the other sweeteners authorised in the European Union. The study by Soffritti et al. (2010) is a long-term carcinogenicity study in mice with transplacental exposure to the artificial sweetener aspartame. The authors concluded that, based on their results, aspartame induces cancer in the livers and lungs of male Swiss mice. EFSA has evaluated this carcinogenicity study and has concluded that, on the basis of the information available in the publication, the validity of the study and its statistical approach cannot be assessed and that its results cannot be interpreted. Furthermore, in view of the generally recognised lack of relevance for human risk assessment of the type of tumours observed in Swiss mice when they are induced by non-genotoxic compounds, EFSA concluded that the results presented in Soffritti et al. (2010) do not provide a sufficient basis to reconsider the previous evaluations by EFSA on aspartame. Halldorsson et al. (2010) investigated preterm delivery in a cohort of 59 334 pregnant women. The authors concluded that their results show an association between intake of artificially sweetened soft drinks and preterm delivery in the cohort. EFSA assessed this study and concluded that there is no evidence available to support a causal relationship between the consumption of artificially sweetened soft drinks and preterm delivery and that additional studies are required to reject or confirm an association. Overall, EFSA concluded that the information available from the Soffritti et al. (2010) and Halldorsson et al. (2010) publications do not give reason to reconsider the previous evaluations of aspartame or of other food additive sweeteners authorised in the European Union.

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KEY WORDS

Sweeteners, aspartame, cancer, liver, lung, preterm delivery, soft drinks

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority was asked to deliver a scientific statement on a carcinogenicity study in mice with transplacental exposure to aspartame, as reported by Soffritti et al. (2010), and on a prospective cohort study on the association between intakes of sugar-sweetened and artificially sweetened soft drinks and preterm delivery in pregnant women, as reported by Halldorsson et al. (2010).

Aspartame has been authorised for use in foods and as a table-top sweetener by several Member States since the 1980s. The European legislation harmonised its use in foodstuffs in 1994 following thorough safety evaluations by the Scientific Committee on Food (SCF) in 1984 and 1988. Further reviews of the aspartame data were carried out by the SCF in 1997 and 2002. No concerns regarding possible reproductive and developmental toxicity, genotoxicity or carcinogenicity were identified. In 2006 and 2009, the Scientific Panels on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) and the Food Additives and Nutrient Sources added to food (ANS) assessed two long-term carcinogenicity studies in rats orally exposed to aspartame performed by the European Ramazzini Foundation (ERF). In both studies the authors reported a significant dose-related increase of malignant tumours in male and female rats. The AFC and ANS Panels concluded in their opinions that on the basis of all the evidence available, there was no indication of any genotoxic or carcinogenic potential of aspartame and that there was no reason to revise the previously established Acceptable Daily Intake (ADI) for aspartame of 40 mg/kg body weight. In a report published in 2010, National Experts nominated by the EU Member States reviewed the scientific literature on aspartame that had become available since 2002 and concluded that there was no requirement to reconsider the previous opinions on aspartame published by the EFSA Panels and the SCF.

In the Soffritti et al. (2010) study on aspartame in mice, dietary concentrations of 2 000, 8 000, 16 000 and 32 000 mg aspartame/kg diet equivalent to doses of 242, 987, 1 919 and 3 909 mg aspartame/kg bw/day were used. The details provided by the authors include the following. The maternal animals were orally exposed to aspartame from the 12th day of gestation throughout lactation and their offsprings were subsequently exposed to aspartame until natural death or for 130 weeks. Food intake, weight gain and clinical signs were monitored throughout the study. Unhealthy or moribund animals were sacrificed and necropsied as were all the mice that were alive at week 130. All tissues and organs were microscopically examined.

The authors reported a statistically significant dose-related increase in hepatocellular carcinomas in the 16 000 and 32 000 mg aspartame/kg diet dose groups of male mice compared to controls. In addition, a statistically significant increase in the incidence of alveolar/bronchiolar carcinomas in males was reported in the highest dose group compared to controls. No compound-attributed carcinogenic effects were reported by the authors in female mice at any of the doses tested. There was no change in the number of hepatocellular or alveolar/bronchiolar adenomas or the total incidence of carcinomas in male mice compared to controls and female mice compared to controls; however, a dose-dependent yet marginal and not statistically significant increase in the total number of adenomas in female mice was noted. Based on these observations, the authors conclude that aspartame induces cancer in the livers and lungs of male Swiss mice, thereby reiterating their previous conclusions that aspartame is a carcinogenic agent in rodents.

For the Soffritti et al. (2010) publication, EFSA noted that the descriptions of the statistical analyses and of the study design (e.g., outcome variable, covariates and assumptions, power calculation, blinding) are not sufficiently detailed, and thus, the relevance of the statistical analyses presented by the authors cannot be assessed at present.

In relation to the design of the study, EFSA also noted that it is generally accepted that life time studies until or close to natural death can lead to erroneous conclusions because of the following limitations. Older animals are more susceptible to illness and have increased background pathology, which includes spontaneous tumours and have a higher probability of autolysis than younger animals.

These attributes can differ between treated and control animals; thus, it is very difficult to causally link tumours in treated animals to treatment or some intervening factor.

EFSA observed that the hepatic and pulmonary tumour incidences reported by Soffritti et al. (2010) all fall within their own historical control ranges for spontaneous tumours. EFSA also noted that Swiss mice are known to have a high background incidence of spontaneous hepatic and pulmonary tumours and that hepatic tumours in mice are generally considered as irrelevant for human risk assessment.

EFSA noted that Soffritti et al. (2010) suggested that the metabolism of aspartame leading to the formation of methanol might have played a role in the development of hepatocellular tumours. However, no evidence to support this hypothesis was provided.

Halldorsson et al. (2010) examined the association between intakes of sugar-sweetened and artificially sweetened soft drinks and preterm delivery in a cohort of 59 334 pregnant women. The authors used data from the “The Danish National Birth Cohort” and the objective of their study was to investigate whether there was a link between sugar-sweetened and artificially sweetened soft drinks and an increased incidence of preterm delivery.

The authors identified seven non-dietary factors that are well-recognized determinants of preterm delivery, namely, maternal age, height, pre-pregnancy Body Mass Index (BMI), cohabitant status, parity, smoking during pregnancy and familial socio-occupational status. To separate out the effects of food and energy intakes the authors also included total energy intake as a covariate. To establish a dose response relationship soft drink intake was also modelled (ranging from never to ≥ 4 servings per day). Secondary analyses were conducted by three categories of pre-pregnancy BMI.

The authors report that there was no association for the sugar sweetened carbonated and noncarbonated soft drinks. A monotonically increasing association was observed for the carbonated artificially sweetened soft drinks. A similar but more modest association was observed for noncarbonated artificially sweetened soft drinks. Similar results were observed from the secondary analysis, for carbonated artificially sweetened soft drinks, for late preterm and moderately preterm although for the early preterm no association was observed. The results were consistent for the three categories of BMI (i.e., an increase in risk for the intake of artificially sweetened carbonated with a further increase in risk with an increase in servings). The authors also presented some results for medically induced and spontaneous deliveries and found that their results were driven by the medically induced deliveries.

Halldorsson et al. (2010) conclude from their prospective cohort study that the daily intake of artificially sweetened soft drinks may increase the risk of preterm delivery in pregnant women.

EFSA considered that the study design, set up, conduct and quality of the Halldorsson et al. (2010) study were adequate to meet the defined objectives. However, as stated by the authors, due to the very nature of this type of studies, only an association between intake of artificially sweetened carbonated and noncarbonated soft drinks and an increased risk of preterm delivery can be identified. This is the first study on this subject, and EFSA noted that the study cannot be interpreted to support a causal relationship between the consumption of artificially sweetened soft drinks and preterm delivery in pregnant women. The authors put forward the hypothesis that the aspartame metabolite methanol might be associated with the observed preterm delivery but no direct supporting evidence was provided. In addition, EFSA observed that several sweeteners are used alone or in combination in soft drinks and that there is no justification to focus on any specific sweetener.

EFSA shared the view of the authors that further studies would be needed to reject or confirm their findings. In particular, EFSA wished to highlight that future studies should take into account potentially important confounders that could have affected the outcome of the study. For example, since the association identified by the authors was mainly driven by medically induced delivery, medical history and criteria on which the medical decisions to induce anticipated delivery are factors

that should be investigated further. Also, differences in levels of the different artificial sweeteners between carbonated and non-carbonated drinks, and potential exposure to artificial sweeteners from sources other than soft drinks should be taken into consideration in the analysis. Other potentially important confounding factors such as exposures from soft drinks or other dietary sources to substances that might have an effect on pregnancy (e.g. caffeine) should be investigated. Furthermore, in view of the fact that studies can contribute directly to the risk assessment of a specific food additive only when they can ascribe the effects to this additive, EFSA advised that studies aiming at confirming an association should focus on a specific food additive.

In conclusion, EFSA has assessed the carcinogenicity study with transplacental exposure of mice to aspartame as reported by Soffritti et al. (2010). EFSA concluded that, on the basis of the information available in the publication, the validity of the study and its statistical approach cannot be assessed and its results cannot be interpreted. Furthermore, in view of the generally recognised lack of relevance for human risk assessment of the tumours observed in Swiss micewhen they are induced by non-genotoxic compounds, EFSA concluded that the results presented in the publication by Soffritti et al. (2010) do not provide sufficient scientific evidence to reconsider the previous evaluations by EFSA on aspartame that concluded on the lack of genotoxicity and carcinogenicity of the sweetener.

EFSA also assessed the prospective cohort study on the association between intakes of sugar-sweetened and artificially sweetened soft drinks and preterm delivery by Halldorsson et al. (2010). Since the intention of this study has been to generate hypothesis, that such studies are exploratory in nature and given the fact that there is no evidence available to support a causal relationship between the consumption of artificially sweetened soft drinks and preterm delivery, additional studies are required to confirm the association. For such studies, potential confounding factors should be investigated. Therefore, EFSA concluded that there is no evidence available to support a causal relationship between the consumption of artificially sweetened soft drinks and preterm delivery and that additional studies are required to reject or confirm an association.

Overall, EFSA concludes that the information available from the Soffritti et al. (2010) and Halldorsson et al. (2010) publications do not give reason to reconsider the previous evaluations of aspartame or of the other food additive sweeteners authorised in the European Union.

EFSA will continue monitoring the scientific literature in order to identify new scientific evidence on sweeteners that may indicate a possible risk for human health or which might otherwise affect the safety assessment of these food additives.

This EFSA statement follows the scientific statement adopted by the Food Additives and Nutrient Sources added to food Panel (ANS) on 3 February 2011 (EFSA, 2011). In its scientific statement, the ANS Panel has indicated that it will undertake a detailed analysis of the study results and conclusions reported by Soffritti et al. (2010), including the suggested implication of methanol.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

In 2010, two articles were published in the scientific literature, describing potential adverse health effects of sweeteners. The paper by Soffritti et al. (2010)⁴ reports that aspartame is a carcinogenic agent in mice. The publication by Halldorsson et al. (2010)⁵ suggests an association between consumption of artificially sweetened soft drinks and increased risk of preterm delivery in pregnant women.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission would like to request technical assistance from EFSA (in line with Article 31 of Regulation (EC) No 178/2002) to undertake a scientific evaluation of the studies presented in these two articles and to indicate whether these studies should trigger a revision of the existing opinions of EFSA related to the safety of food additive sweeteners, including the recent EFSA opinions of May 2006, and March 2009 which reaffirmed the safety of aspartame^{6,7}.

⁴ Soffritti M, Belpoggi F, Manservigi M, Tibaldi E, Lauriola M, Falcioni L, Luciano Bua L, 2010. Aspartame Administered in Feed, Beginning Prenatally Through Life Span, Induces Cancers of the Liver and Lung in Male Swiss Mice. *American Journal of Industrial Medicine* 53, 1197-1206.

⁵ Halldorsson TI, Strøm M, Petersen SB, Olsen SF, 2010. Intake of artificially sweetened soft drinks and risk of preterm delivery: a prospective cohort study in 59,334 Danish pregnant women. *American Journal of Clinical Nutrition* 92, 626-633.

⁶ 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 (Question number EFSA-Q-2005-122) Adopted on 3 May 2006. *The EFSA Journal* (2006) 356, 1-44.

⁷ Updated Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food 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* (2009) 1015, 1-18.

ASSESSMENT

1. Introduction

Following a request from the European Commission, EFSA initiated a scientific evaluation of a carcinogenicity study in mice with transplacental exposure to aspartame, as reported by Soffritti et al. (2010), and of a prospective cohort study on the association between intakes of sugar-sweetened and artificially sweetened soft drinks and preterm delivery in pregnant women, as reported by Halldorsson et al. (2010).

The study by Soffritti et al. (2010) is a long-term carcinogenicity study in mice with transplacental exposure to the artificial sweetener aspartame, performed by the Cesare Maltoni Cancer Research Centre of the European Ramazzini Foundation (ERF). The authors state, based on the results of their study, that aspartame induces cancer in the livers and lungs of male Swiss mice, reiterating their previous conclusions that aspartame is a carcinogenic agent in rodents (Soffritti et al., 2006, 2007).

Halldorsson et al. (2010) investigated preterm delivery in a cohort of 59 334 pregnant Danish women. Their results are based on an assessment of the consumption of soft drinks (sugar-sweetened carbonated, artificially sweetened carbonated, sugar-sweetened noncarbonated and artificially sweetened noncarbonated drinks) in this cohort and birth outcomes, and point towards an association between artificially sweetened soft drinks and preterm delivery in the cohort.

EFSA based its evaluation of the ERF carcinogenicity study with transplacental exposure of mice to aspartame as reported by Soffritti et al. (2010) and of the prospective cohort study by Halldorsson et al. (2010) on the information provided in these published papers. This EFSA statement follows the scientific statement adopted by the Food Additives and Nutrient Sources added to food Panel (ANS) on 3 February 2011 that advised EFSA for further work to be undertaken for the evaluation of these two studies (EFSA, 2011). In its scientific statement, the ANS Panel has indicated that it will undertake a detailed analysis of the study results and conclusions reported by Soffritti et al. (2010), including the suggested implication of methanol.

2. Previous evaluations on aspartame in EU

Aspartame has been authorised for use in foods and as a table-top sweetener by several Member States since the 1980s. The European legislation harmonised its use in foodstuffs in 1994 following thorough safety evaluations by the Scientific Committee on Food (SCF) in 1984 and 1988. Further reviews of aspartame data were carried out by the SCF in 1997 and 2002. No concerns regarding possible reproductive and developmental toxicity, genotoxicity or carcinogenicity were identified in these evaluations. In 2006, the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) assessed a long-term carcinogenicity study in rats exposed to aspartame performed by the ERF and published by Soffritti et al. (2006). At the same time, the AFC Panel reviewed the genotoxicity studies on aspartame and concluded that the artificial sweetener had no mutagenic or DNA damaging potential *in vitro* or *in vivo* (EFSA, 2006). Based on all the evidence available from the 2006 ERF study and other recent studies and previous evaluations, the AFC Panel concluded in 2006 that there was no reason to revise the Acceptable Daily Intake (ADI) for aspartame of 40 mg/kg body weight as established by the Scientific Committee for Food in 1984 (EFSA, 2006).

In April 2009, EFSA's ANS Panel published an opinion (EFSA, 2009) on a further ERF study investigating the effects of a life-span exposure to low doses of aspartame in rats (Soffritti et al., 2007). This opinion also took into consideration additional data submitted by the Ramazzini Foundation in February 2009. In the 2007 study, rats were exposed to aspartame from the 12th day of gestation until natural death at dietary concentrations of 400 and 2000 mg aspartame/kg diet equivalent to doses of 20 and 100 mg aspartame/kg bw/day. The authors reported a dose-related increased incidence of males bearing malignant tumours, an increase in the incidence of

lymphomas/leukaemias in males from the high-dose group, a dose-related increase in the incidence of lymphomas/leukaemias in females and a dose-related increased incidence of mammary carcinomas in females. The ANS Panel concluded that, on the basis of all the evidence available, including the data presented in Soffritti et al. (2007), there was no indication of any genotoxic or carcinogenic potential of aspartame and no reason to revise the previously established ADI for aspartame of 40 mg/kg body weight/day.

In 2009 a series of meetings of National Experts nominated by the EU Member States was organised with the support of EFSA to review the scientific literature on aspartame including non-peer reviewed and anecdotal evidence that had become available since 2002. The report of these meetings was published in 2010, and included a re-evaluation of data on the potential carcinogenicity (including cancer epidemiology) and genotoxicity of aspartame (EFSA, 2010). The conclusion by the National Experts was that there was no new evidence that required a recommendation to EFSA that the previous opinions on aspartame adopted by the EFSA AFC and ANS Panels and the SCF should be reconsidered.

3. The study by Soffritti et al. (2010)

3.1. Description

In the Soffritti et al. study on aspartame in mice, published in 2010, dietary concentrations of 2 000, 8 000, 16 000 and 32 000 mg aspartame/kg diet equivalent to doses of 242, 987, 1 919 and 3 909 mg aspartame/kg body weight/day were used. The details provided by the authors include the following. The maternal animals were exposed to aspartame from the 12th day of gestation throughout lactation and the offsprings subsequently until natural death or for 130 weeks. The group sizes of the offspring were 117 male and 102 female mice in the control group whereas the treated groups ranged from 62 animals/sex to 122 animals/sex. Food intake, weight gain and clinical signs were monitored throughout the study. Unhealthy or moribund animals were sacrificed and necropsied as were all the mice that were alive at week 130. All tissues and organs were microscopically examined.

The authors reported a, statistically significant dose-related increase in hepatocellular carcinomas in the 16 000 and 32 000 mg aspartame/kg diet dose groups of male mice compared to controls ($p < 0.05$ and $p < 0.01$, respectively, using Cox regression model). In addition, a statistically significant increase in the incidence of alveolar/bronchiolar carcinomas in males was reported in the highest dose group ($p < 0.05$). No compound-attributed carcinogenic effects were reported by the authors in female mice at any of the doses tested. There was no change in the number of hepatocellular or alveolar/bronchiolar adenomas or the total incidence of carcinomas in male or female mice compared to controls; however, a dose-dependent yet marginal and not statistically significant increase in the total number of adenomas in female mice was noted.

3.2. Comments on the study by Soffritti et al. (2010)

The following issues were noted on the design, the conduct of the study and the interpretation of the experimental data.

3.2.1. Design and conduct of the study

Although the objectives of the study are stated by the authors, the specific hypotheses they intended to test are not described. The authors appear to present their findings as confirmatory but the analyses

suggest an exploratory study type approach. If the approach were intended to be confirmatory then the primary hypothesis (or hypotheses) to be tested should be clearly stated in advance. However, if the approach was exploratory or to generate hypotheses then the conclusions of the paper should encourage independent replication of the results in confirmatory studies.

The design and conduct of the Soffritti et al. (2010) study in mice was essentially identical to that described for the second study in rats (Soffritti et al., 2007) that also included a transplacental exposure but with a termination after 130 weeks. EFSA noted that the study design does not follow any accepted international test guidelines for conduction of carcinogenicity studies in rodents such as OECD Test Guideline 451. The consequence of extending a carcinogenicity study beyond the recommended 104 weeks is the appearance of age-related pathological changes that may confound the identification of compound-related effects.

In addition, EFSA noted that the group sizes were different between control and treated groups, and that the sample size (power) calculation was not presented. It is not clear why two experimental groups were composed of 30 male and female breeding pairs each whereas the remaining three experimental groups were composed of only 20 male and female breeding pairs per group. Further, it is not clearly stated which groups had 30 breeding pairs and which groups had 20 breeding pairs. The randomization procedure was stated but insufficiently described. Therefore, it is not possible to assess whether it is scientifically sound.

It is not clear how the different tumours were counted and classified as independent tumours in the study.

The description of the statistical analysis is limited, and it is not possible to know exactly which statistical models were used. No details are given on the assumptions made and the hypotheses being tested. The outcome measures for analysis are neither clearly nor adequately described. Covariates used in the modelling are also not detailed. Pups from the same litters are likely to have similar characteristics and responses leading to issues in model estimation. This should have been taken into consideration in the analysis (e.g. via clustering) and should have been clearly described.

3.2.2. Interpretation of the data of the study

EFSA noted that at no time point during the study was there a difference in the survival between tumour bearing and non-tumour bearing male and female animals and between control and treated animals, even though an increase in incidence in liver and lung carcinomas was reported in the highest dose groups.

The authors report that the long-term exposure to aspartame results in an increase in the incidence of hepatocellular carcinomas at the two highest doses. However, EFSA noted that even at the highest dose, the incidence in hepatocellular carcinoma (18.1%) remains within the historical range of hepatocellular carcinomas in control male Swiss mice (0-26.3%) in the laboratory (Cesare Maltoni Cancer Research Centre of the European Ramazzini Foundation). The control incidence of 5.1% reported in this study was also towards the lower part of the range for male mice. In addition, EFSA noted that at the highest dose, the incidence in lung carcinoma (13.3%) in males remains within the historical range of lung carcinomas in control male Swiss mice (0-14.3%) in the laboratory (Cesare Maltoni Cancer Research Centre of the European Ramazzini Foundation). The control incidence of 6.0% reported in this study was also towards the lower part of the range for male mice.

EFSA noted that the descriptions of the study design and of the statistical analyses (e.g. model, outcome variable, covariates and assumptions, power calculation, blinding) are not sufficiently detailed in the paper, and until these issues are addressed, the relevance of the statistical analyses presented by Soffritti et al. (2010) cannot be assessed.

3.2.3. Biological significance of the findings

EFSA noted that there is a general consensus in the scientific community backed up by a considerable body of evidence that hepatic tumours in mice when induced by non-genotoxic compounds can be considered as irrelevant for human risk assessment (Gold and Slone, 1995; Carmichael et al., 1997; Boobis et al., 2006; Holsapple et al., 2006; Billington et al., 2010). The lack of genotoxicity of aspartame has been demonstrated in several standard *in vivo* genotoxicity studies and moreover it was not carcinogenic in several models of transgenic mice, particularly sensitive to genotoxic agents. Furthermore, Swiss mice are known to have a high spontaneous incidence of hepatic and pulmonary tumours (Prejean et al., 1973; Fox et al., 2006). EFSA also noted that the authors did not provide any information on non-neoplastic lesions, including the absence of any documentation of liver changes (e.g. organ weight, hepatocellular injury and hyperplasia).

4. The Study by Halldorsson et al. (2010)

4.1. Description

The objective of the Halldorsson et al. (2010) exploratory study was to investigate the link between intakes of sugar-sweetened and artificially sweetened soft drinks and preterm delivery in pregnant women. The authors used data from “The Danish National Birth Cohort” study (Olsen et al. (2001).

In “The Danish National Birth Cohort” from January 1996 to October 2002, 91 827 pregnant Danish women were registered into the cohort resulting in 101 042 pregnancies (analysis were restricted to the first pregnancy). A total 62 374 women filled out a food frequency questionnaire (FFQ) at around week 25 of pregnancy. After restricting the analysis to singleton pregnancies (61 409) and excluding women who did not answer the questions on soft drinks the final data set of the Halldorsson et al. (2010) study consisted of 59 334 women.

The primary outcome variable was preterm delivery (<37 weeks). Secondary outcome variables were defined as later preterm ($34 \leq \text{wk} < 37$), moderately preterm ($32 \leq \text{wk} < 34$) and early preterm delivery (<32 wks). Dates of birth were extracted from the Danish Civil Registrations System. The gestational age was assessed from the last menstrual cycle and the rules for exceptions are described in Halldorsson et al. (2010).

The authors identified seven non-dietary factors that are well-recognized determinants of preterm delivery, namely, maternal age, height, pre-pregnancy Body Mass Index (BMI), cohabitant status, parity, smoking during pregnancy and familial socio-occupational status. To separate out the effects of food and energy intakes the authors also included total energy intake as a covariate. To establish a dose response relationship soft drink intake was also modelled (ranging from never to ≥ 4 servings per day). Secondary analyses were conducted by three categories of pre-pregnancy BMI. The statistical analysis used univariate and multivariate logistic regression to estimate the association between intakes of soft drinks and preterm delivery. Soft drinks were classified into four categories; sugar-sweetened carbonated, artificially sweetened carbonated, sugar-sweetened noncarbonated and artificially sweetened non-carbonated soft drinks.

The primary analysis investigating preterm deliveries in pregnant women and the intake of soft drinks was presented for each of the four soft drink categories. The authors conclude that there was no association for the sugar sweetened carbonated and noncarbonated soft drinks with preterm deliveries. A monotonically increasing association was observed for the carbonated artificially sweetened soft drinks. A similar but more modest association was observed for noncarbonated artificially sweetened soft drinks. Similar results were observed from the secondary analysis, for carbonated artificially

sweetened soft drinks, for late preterm and moderately preterm although for the early preterm no association was observed. The results were consistent for the three categories of BMI (i.e., an increase in risk for the intake of artificially sweetened carbonated with a further increase in risk with an increase in servings). The authors also presented some results for medically induced and spontaneous deliveries and found that their results were driven by the medically induced deliveries.

Halldorsson et al. (2010) state that a chance finding was unlikely because consistent results were observed for sugar-sweetened and artificially sweetened soft drinks. They considered their results to be robust based on the primary and secondary analyses that were conducted. They further state that the strength of their study comes from the fact that it was prospective and has a large number of pregnant women. They also accept that they cannot exclude that their findings could be as a result of unidentified and unadjusted confounders. However in recognition of the exploratory (hypothesis generating) nature of their analysis they conclude that “the replication of our findings in another experimental setting is warranted”.

4.2. Comments on the study by Halldorsson et al. (2010)

Olsen et al. (2001) describe the background, structure and aim of the Danish National Birth Cohort. The authors discuss the infrastructure, aims, power calculations, enrolment, exposure recording, logistics, end points, recruitment and compliance, pilot testing, ethics, permissions and collaboration, contact with unions and offer perspectives on the long term vision of the study. There were extensive consultations with stakeholders and wider discussions involving public debate.

The main aims for establishing the Danish National Birth Cohort were “*to study pregnancy complications and diseases in offspring as a function of factors operating in early life, fetal growth, and its determinants. ... especially at studying side effects of medications and infections. ... focused not only on diseases in the period from conception to early childhood, but also on all diseases with a possible origin in the fetal time period.*”

Halldorsson et al. (2010) investigated the link between sugar-sweetened and artificially sweetened soft drinks and preterm delivery. The study design, set up, conduct and quality are appropriate to meet the defined objectives. Their results consistently point towards an association between artificially sweetened soft drinks and preterm delivery in pregnant women. They presented primary and secondary analyses to show the robustness and consistency of their findings. They also accepted that they could not exclude that their findings could result from unidentified and unadjusted confounders and therefore recommended that their results were independently replicated using other data. EFSA agreed with this statement, and noted that, in view of the fact that the association identified by the authors was mainly driven by medically induced delivery, medical history and criteria on which the medical decisions to induce anticipated delivery should be further investigated.

Halldorsson et al. (2010) worked with the intention of hypothesis generation. Studies of this type are exploratory in nature and therefore need to be followed up with independent confirmatory studies that replicate any significant clinical findings as was suggested by the authors.

5. Discussion

The study by Soffritti et al. (2010) is a long-term carcinogenicity study starting with transplacental exposure of mice to the artificial sweetener aspartame, performed by the Cesare Maltoni Cancer Research Centre of the European Ramazzini Foundation (ERF). The authors report a significant increase in the incidence of hepatocellular and alveolar/bronchiolar carcinomas in male mice. No carcinogenic effect was observed by the authors in female mice. Based on these observations, the authors conclude that aspartame induces cancer in the livers and lungs of male Swiss mice.

EFSA noted that the descriptions of the study design and of the statistical analyses (e.g., outcome variable, covariates and assumptions, power calculation, blinding) are not sufficiently detailed, and until these issues are addressed, the relevance of the statistical analyses presented by Soffritti et al. (2010) cannot be assessed.

In relation to the design of the study, EFSA also noted that it is generally accepted that life time studies until or close to natural death can lead to erroneous conclusions because of the following limitations. Older animals are more susceptible to illness and have increased background pathology, which includes spontaneous tumours and have a higher probability of autolysis than younger animals. These attributes can differ between treated and control animals; thus, it is very difficult to causally link tumours in treated animals to treatment or some intervening factor (Goodman et al., 2009).

EFSA observed that the hepatic and pulmonary tumour incidences reported by the authors all fall within their own historical control ranges for spontaneous tumours. EFSA also noted that Swiss mice are known to have a high background incidence of spontaneous hepatic and pulmonary tumours and that induction of such tumours by a non-genotoxic compound should be interpreted with caution (Prejean et al., 1973; Fox et al., 2006). In particular, hepatic tumours in mice induced by non-genotoxic compounds are generally considered as irrelevant for human risk assessment (Gold and Slone, 1995; Carmichael et al., 1999; Boobis et al., 2006; Holsapple et al., 2006; Billington et al., 2010).

EFSA noted that Soffritti et al. (2010) suggested that the metabolism of aspartame leading to the formation of methanol might have played a role in the development of hepatocellular tumours. However, no evidence to support this hypothesis was provided in the publication.

The study by Halldorsson et al. (2010) reports an association between intake of artificially sweetened carbonated and noncarbonated soft drinks and an increased risk of preterm delivery in pregnancy. The association was observed for normal-weight and overweight pregnant women, and a stronger increase in risk was observed for early preterm and moderately preterm delivery than with late-preterm delivery. No association was found for sugar-sweetened carbonated soft drinks or sugar-sweetened noncarbonated soft drinks. The authors conclude that the daily intake of artificially sweetened soft drinks may increase the risk of preterm delivery in pregnant women.

EFSA considered that the study design, set up, conduct and quality of the Halldorsson et al. (2010) study were adequate to meet the defined objectives. However, as stated by the authors, due to the very nature of these types of studies, only an association between intake of artificially sweetened carbonated and noncarbonated soft drinks and an increased risk of preterm delivery can be identified. This is the first study on this subject, and EFSA noted that the study cannot be interpreted to support a causal relationship between the consumption of artificially sweetened soft drinks and preterm delivery in pregnant women. The authors put forward the hypothesis that the aspartame metabolite methanol might be associated with the observed preterm delivery but no direct supporting evidence was provided. In addition, EFSA observed that several sweeteners are used alone or in combination in soft drinks and that there is no justification to focus on any specific sweetener.

EFSA shared the view of the authors that further studies are needed to reject or confirm their findings. In particular, EFSA wishes to highlight that future studies should take into account potentially important confounders that could have affected the outcome of the study. For example, since the association identified by the authors was mainly driven by medically induced delivery, medical history and criteria on which the medical decisions to induce anticipated delivery are based are factors that should be investigated further. Also, differences in levels of the different artificial sweeteners between carbonated and non-carbonated drinks, and potential exposure to artificial sweeteners from sources other than soft drinks such as teas or dairy products should be taken into consideration in the analysis. Other potentially important confounding factors such as exposures from soft drinks or other dietary sources to substances that might have an effect on pregnancy (e.g. caffeine) should be investigated. Furthermore, in view of the fact that studies can contribute directly to the risk assessment of a specific

food additive only when they can ascribe the effects to this additive, EFSA advised that studies aiming at confirming an association should focus on a specific food additive.

6. Conclusions

EFSA has evaluated the carcinogenicity study with transplacental exposure to aspartame as reported by Soffritti et al. (2010). EFSA concluded that, on the basis of the information available in the publication, the validity of the study and its statistical approach cannot be assessed and its results cannot be interpreted. Furthermore, in view of the generally recognised lack of relevance for human risk assessment of the tumours observed in Swiss mice when they are induced by non-genotoxic compounds, EFSA concluded that the results presented in the publication by Soffritti et al. (2010) do not provide sufficient scientific evidence to reconsider the previous evaluations by EFSA on aspartame that concluded on the lack of genotoxicity and carcinogenicity of the sweetener.

EFSA also assessed the prospective cohort study on the association between intakes of sugar-sweetened and artificially sweetened soft drinks and preterm delivery by Halldorsson et al. (2010). Since the intention of this study has been to generate hypothesis, that such studies are exploratory in nature and given the fact that there is no evidence available to support a causal relationship between the consumption of artificially sweetened soft drinks and preterm delivery, additional studies are required to confirm or reject the association. For such studies, potential confounding factors should be investigated. Therefore, EFSA concluded that there is no evidence available to support a causal relationship between the consumption of artificially sweetened soft drinks and preterm delivery and that additional studies are required to reject or confirm an association.

Overall, EFSA concluded that the information available from the Soffritti et al. (2010) and Halldorsson et al. (2010) publications do not give reason to reconsider the previous evaluations of aspartame or of the other food additive sweeteners authorised in the European Union.

EFSA will continue monitoring the scientific literature in order to identify new scientific evidence on sweeteners that may indicate a possible risk for human health or which might otherwise affect the safety assessment of these food additives.

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GLOSSARY AND ABBREVIATIONS

ADI	Acceptable Daily Intake
AFC	Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
BMI	Body Mass Index
EC	European Commission
EFSA	European Food Safety Authority
ERF	European Ramazzini Foundation
FFQ	Food Frequency Questionnaire
SCF	Scientific Committee on Food