Safety of ethyl acrylate to be used as flavouring

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Safety of ethyl acrylate to be used as flavouring

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), Vittorio Silano, Claudia Bolognesi, Laurence Castle, Kevin Chipman, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Trine Husøy, Sirpa Kärenlampi, Maria Rosaria Milana, Karla Pfaff, Gilles Riviere, Jannavi Srinivasan, Maria de Fátima Tavares Poças, Christina Tlustos, Detlef Wölfle, Holger Zorn, Romualdo Benigni, Mona-Lise Binderup, Leon Brimer, Francesca Marcon, Daniel Marzin, Pasquale Mosesso, Gerard Mulder, Agneta Oskarsson, Camilla Svendsen, Maria Anastassiadou, Maria Carfì, Siiri Saarma and Wim Mennes

Abstract

The EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was requested by the European Commission according to Art. 29 1(a) of the Regulation (EC) No 178/2002 to carry out a review of existing literature on the safety of ethyl acrylate [FL-no: 09.037] when used as a flavouring substance. Ethyl acrylate [FL-no: 09.037] was evaluated in 2010 by EFSA in FGE.71 as a flavouring substance, based on the 2006 JECFA evaluation. The Panel concluded that ethyl acrylate was of no safety concern at estimated level of intake as flavouring substance based on the Maximised Survey-Derived Daily Intake (MSDI) approach. The Panel has evaluated the new literature available and any previous assessments performed by JECFA (2006) and EFSA (2010). Moreover, new data on the use levels of ethyl acrylate as flavouring substance have been provided. For use as flavouring substance, the chronic dietary exposure estimated using the added portions exposure technique (APET), is calculated to be 3,545 μg/person per day for a 60-kg adult and 2,233 μg/person per day for a 15-kg 3-year-old child. Exposure from food contact materials may be up to 6,000 μg/person per day. The Panel considered that based on the available data, which covers all relevant genetic endpoints (i.e. gene mutations, structural and numerical chromosomal aberrations) there is no concern with respect to genotoxicity of ethyl acrylate. The Panel evaluated the available carcinogenicity studies conducted in rats and mice and agreed with the NTP evaluation (1998) concluding that the forestomach squamous cell papilloma and carcinoma observed in rodents were not relevant to humans. Additionally, there was no evidence of systemic toxicity in short-term and subchronic toxicity studies. Therefore, the Panel concluded that there is no safety concern for the use of ethyl acrylate as a flavouring substance, under the intended conditions of use.

Keywords: ethyl acrylate, [FL-no: 09.037], FGE.71, 140-88-5, genotoxicity, carcinogenicity, flavourings

Requestor: European Commission

Question number: EFSA-Q-2016-00426

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

The use of flavourings is regulated under Regulation (EC) No 1334/2008[1] of the European Parliament and Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[2].

The substance benzophenone, [FL no. 07.032] (CAS No. 119-61-9) is currently included in this Union List. It is a substance which is not currently under evaluation. This substance is also known as diphenyl ketone. This substance was included in the Union list on the basis of the EFSA evaluation in FGE 69 of 2008. FGE 69 includes this substance. Benzophenone was evaluated by JECFA as a flavouring substance, with JECFA no. 831. The studies evaluated by IARC were not considered in the EFSA opinion on FGE 69. There may be also additional studies on the safety of this substance.

The substance ethyl acrylate, [FL no. 09.037], (CAS No. 140-88-5) was included in the Union list on the basis of the EFSA evaluation in FGE 71 of 2010. FGE.71 includes this substance. Ethyl acrylate was evaluated by JECFA as a flavouring substance, with JECFA no. 1351. The studies evaluated by IARC were not considered in the EFSA opinion of FGE.71. There may be also additional studies on the safety of this substance.

1.1.2. Terms of Reference

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002[3], the European Commission requests the European Food Safety Authority (EFSA) to carry out a review of existing published literature on the safety of the flavouring substances benzophenone, [FL. No 07.032], (CAS No. 119-61-9) and ethyl acrylate, [FL 09.037], (CAS No. 140-88-5), and advise on their safety when used as flavouring substances.

1.2. Interpretation of the Terms of Reference

Since benzophenone [FL- no: 07.032] and ethyl acrylate [FL-no: 09.037] are not structurally related and were evaluated in different FGEs (FGE.69 and FGE.71), they will be evaluated in separate opinions. The present document will address the question of the Commission on ethyl acrylate. Meanwhile, EFSA has also received information on use levels which will be taken into consideration. Considering the Term of Reference, the Panel will not address in this assessment, the exposure that may result from the use as food contact material.

In respect to the approach to be followed for the assessment of ethyl acrylate used as a flavouring substance, the Panel was of the view that previous assessments should be used as starting points for this scientific opinion. The previous assessment will be updated with new information that is connected to the concerns identified in the background (Section 1.1).

In the background (Section 1.1), it is mentioned that the studies evaluated by IARC in the report from 1999 on carcinogenicity of ethyl acrylate were not considered in the EFSA evaluation from 2010, and that there may be additional studies on the safety of ethyl acrylate when used as a flavouring substance. Thus, the present evaluation is mainly focusing on the genotoxicity and carcinogenicity of ethyl acrylate, in addition to considering any new data on toxicity, which may have an impact on the

[1] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34–50.

[2] Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1–161.

[3] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

safety assessment of ethyl acrylate as flavouring. Moreover, previous assessments of ethyl acrylate by JECFA (2006) and EFSA (EFSA CEF Panel, 2010a) are briefly presented.

According to the request from the Commission (Section 1.1), the present evaluation will be based on available data on ethyl acrylate. Acrylic acid is a metabolite of ethyl acrylate and in that sense also the safety of acrylic acid is included in this evaluation. However, the safety of exposure directly to acrylic acid will not be evaluated in the present opinion, which according to the terms of reference will address the use of ethyl acrylate as a flavouring substance.

2. Data and methodologies

The search for literature was done by EFSA on ethyl acrylate [FL-no: 09.037] to review existing literature on the safety of this substance and to advise on its safety when used as flavouring substance. A literature search was carried out through Web of Science database until August 2017, using keywords "ethyl acrylate" or "140-88-5" and "genotox*", "canc*", "carc*", "tumor*", "toxicokin*", "metabol*", "absorb*", "distrib*", "excret*", and "reproduct*" while searching in "all databases". Additional searches were carried out through Scopus database, using the keyword combination of "ethyl acrylate" and "tox", and the database of Decernis, using the keyword "140-88-5".

3. Procedure of the safety assessment

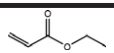
3.1. Assessment

The Panel considered studies already evaluated (JECFA, 2006; EFSA CEF Panel, 2010a) and additional studies from literature search. Except for genotoxicity, no new relevant studies were identified for short-term toxicity, chronic toxicity and carcinogenicity.

3.2. Technical data

Information on the identity of the substance and specifications are based on data already described in FGE.71 (EFSA CEF Panel, 2010a) and are presented in Table 1.

Table 1: Summary of specifications for the substance ethyl acrylate (EFSA CEF Panel, 2010a)

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. Form Mol. Formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index[d] Spec. gravity[e]
09.037 1351	Ethyl acrylate		2418 245 140-88-5	Liquid C5H8O2 100.12	Slightly Soluble Soluble	99–101 IR 97%	1.403–1.409 0.916–0.919

FL-no: FLAVIS number; JECFA: The Joint FAO/WHO Expert Committee on Food Additives; FEMA: Flavor and Extract Manufacturers Association; CoE: Council of Europe; CAS: Chemical Abstract Service; ID: Identity; IR: infrared spectroscopy.
(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95% ethanol, if not otherwise stated.
(c): At 1013.25 hPa, if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.

3.3. Information on existing evaluations from EFSA

Ethyl acrylate [FL-no: 09.037] has been evaluated by JECFA (2006), EFSA considered this evaluation and concluded, in FGE.71, that ethyl acrylate is of no safety concern at estimated level of intake as flavouring substance based on the Maximised Survey-Derived Daily Intake (MSDI) approach (EFSA CEF Panel, 2010a).

Ethyl acrylate was evaluated as a substance in food contact materials (FCM) by the Scientific Committee for food (SCF, 1999). They established a group temporary tolerable daily intake (TDI) of 0.1 mg/kg body weight (bw) for several acrylates, expressed as acrylic acid. The temporary TDI was based on data from experimental animals exposed to acrylic acid. Based on this TDI, a specific migration limit (SML) of 6 mg/kg of food was calculated for the group of substances that share acrylic acid as a common metabolite/degradation product.

In Regulation (EU) No 10/2011[4], ethyl acrylate is an approved monomer for use in FCM with a group SML for the sum of substances that have acrylic acid as a common metabolite/degradation product of 6 mg/kg of food.

3.4. Exposure

3.4.1. Concentration in processed and non-processed foods from natural sources

Ethyl acrylate [FL-no: 09.037] has been reported to occur in passion fruit, pineapple, Beaufort cheese and durian (IARC, 1999a, Triskelion, 2017). Quantitative data are reported for ethyl acrylate in pineapple (0.01 mg/kg) (Triskelion, 2017).

3.4.2. Chronic dietary exposure

The exposure assessment to be used in the Procedure for the safety evaluation of the candidate substance is the chronic added portions exposure technique (APET) estimate (EFSA CEF Panel, 2010b). The chronic APET for ethyl acrylate has been calculated for adults and children (see Table 2 and Appendix C). The chronic APET calculation is based on the combined normal occurrence level.

The Panel noted that the contribution from natural occurrence of ethyl acrylate to the APET is negligible compared to the added flavouring substance.

Table 2: APET – Chronic dietary exposure

Chronic APET	Added[a]		Other dietary sources[b]		Combined[c]	
	µg/kg bw per day	µg/person per day	µg/kg bw per day	µg/person per day	µg/kg bw per day	µg/person per day
Adults[d]	59.1	3,545	0.23	14.0	59.1	3,545
Children[e]	149	2,233	0.59	8.85	149	2,233

APET: added portions exposure technique; bw: body weight.
(a): APET Added is calculated on the basis of the normal amount of flavour added to a specific food category.
(b): APET Other Dietary Sources is calculated based on the natural occurrence of the flavouring in a specified food category.
(c): APET Combined is calculated based on the combined amount of added flavouring and naturally occurring flavouring in a specified food category.
(d): For the adult APET calculation, a 60-kg person is considered representative.
(e): For the child APET calculation, a 3-year old child with a 15 kg bw is considered representative.

The Panel noted that exposure to ethyl acrylate may also occur from FCM (SML 6 mg/kg food, as acrylic acid, corresponding to a maximum exposure of 6 mg/person per day; see Section 3.3).

3.4.3. Acute dietary exposure

Acute exposure is not evaluated, because this opinion addresses only chronic exposure.

[4] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 12, 15.1.2011, p. 1–89.

3.5. Biological and toxicological data

The toxicity of ethyl acrylate has been evaluated by JECFA in 2006 and concerning genotoxicity by EFSA (EFSA CEF Panel, 2010a). The carcinogenicity was evaluated by IARC in 1999a. Brief summaries of the previous evaluations will follow below and data not previously considered will be presented.

3.6. Absorption, distribution, metabolism and excretion

Ethyl acrylate is the ethyl ester of acrylic acid. Aliphatic esters formed from 2-alkenoic acids are generally rapidly hydrolysed to respective alcohols and carboxylic acids by carboxylesterases. Thus, ethyl acrylate is hydrolysed to acrylic acid and ethanol, which will participate in biochemical pathways, including the fatty acid pathway and tricarboxylic acid cycle (see review by JECFA, 2006).

In vitro studies

Miller et al. (1981) compared the rate of hydrolysis of ethyl acrylate in homogenates of different tissues and found the highest rate in liver, followed by whole blood, lung and kidney. In the liver homogenate, the hydrolysis rate of ethyl acrylate was 26.8 nmol/min. Potter and Tran (1992) demonstrated that ethyl acrylate reacts with both glutathione (GSH) and protein *in vitro*.

In vivo studies

^{14}C-Ethyl acrylate has been used for metabolic studies (DeBethizy et al., 1987). Rats were administered single oral doses of 2, 20 and 200 mg/kg bw and radioactivity was followed during 72 h. A rapid and high absorption was demonstrated and most of the radioactivity was exhaled as CO_2 (52–62% of dosed radioactivity) and almost all during the first 10 h after administration. The detection of 3-hydroxypropionic acid as a urinary metabolite led to the suggestion by the authors, that acrylic acid was incorporated into propionic acid metabolism, explaining the rapid formation of carbon dioxide. In urine two metabolites derived from GSH conjugation were also detected. At the two lower doses of ethyl acrylate, non-protein sulfhydryl (NPSH) content decreased significantly at the dosing site, forestomach and glandular stomach, but not in the liver. NPSH, mainly GSH, cysteine, coenzyme A and other thiols in the gastric mucosa, are suggested to play an important role in the protection of gastric mucosa against chemically-induced lesions (Nagy et al., 2007).

Oral dosing of rats with ethyl acrylate was performed in a study by Frederick et al. (1992) to validate a physiologically based pharmacokinetic and pharmacodynamics model for ethyl acrylate. The predicted very rapid metabolism was verified and consistent with the finding that no ethyl acrylate could be detected in tissues. The model also predicted glutathione depletion in forestomach.

Overall, ethyl acrylate is rapidly absorbed, metabolised, incorporated into endogenous biochemical pathways and excreted.

3.6.1. Short-term and subchronic toxicity

A single dose of 100, 200 or 400 mg/kg bw ethyl acrylate administered by gavage to F344 rats caused mucosal and submucosal oedema and vacuolisation of the tunica muscularis in the forestomach and mild submucosal oedema in the glandular stomach (Ghanayem et al., 1985). No gastric effects were observed after equivalent subcutaneous or intraperitoneal (i.p.) doses. After 2 or 4 consecutive daily doses of 200 mg/kg bw similar effects were observed, and in addition submucosal inflammation, mucosal erosions or ulcers in both portions of the stomach. There was no systemic toxicity. The same authors (Ghanayem et al., 1986) also studied the recovery of the lesions in rats after 14 daily gavage doses of 100 or 200 mg/kg bw ethyl acrylate. After 14 days of exposure, the glandular stomach was normal in all animals and appeared to have adapted to resist the toxicity of ethyl acrylate. The forestomach exhibited dose-dependent lesions, which were recovered in the low dose group at 14 days following the last dose, while in the high dose group mucosal hyperplasia was still present in the forestomach at 4 weeks after last exposure.

Male F344/N rats were dosed with ethyl acrylate either by gavage at doses from 2 to 200 mg/kg bw daily for 2 weeks or via drinking water at concentrations from 200 to 4,000 mg/L (corresponding to 23–369 mg/kg bw/day, according to the authors) (Frederick et al., 1990). A dose-dependent increase in irritation of the forestomach was observed in the animals dosed by gavage, while the animals dosed via drinking water had a much lower incidence and less severe lesions at the corresponding dose levels. There were no lesions in the glandular stomach. Severe depletion of NPSH

was observed in the forestomach at gavage doses ≥ 100 mg/kg bw, while no significant depletion was seen after administration via drinking water. The NPSH depletion in glandular stomach and liver were lower, and not at levels generally associated with toxicity. The authors suggested that the depletion of NPSH in the rat forestomach was causally related to the histopathology observed at relatively high doses. Thus, at doses of 100 mg/kg bw per day or greater by gavage, the NPSH content was severely depleted (below 25% of the initial concentration) and moderate to marked hyperplasia was observed together with ulceration and erosion of the forestomach epithelium. Minimal depletion of NPSH, producing minimal to mild hyperplasia, was reported at 20–50 mg/kg bw. At 10 mg/kg bw and less, no significant NPSH depletion and no histopathological findings were reported.

To design the 2-year carcinogenesis study on ethyl acrylate, the NTP performed several 14-day and 13-week gavage studies in B6C3F$_1$ mice and F344/N rats (NTP, 1986). The principal toxic effect in the 14-day studies was in the forestomach of both sexes in mice and rats. There was no evidence of systemic toxicity. The lowest dose where histological lesions in the forestomach were detected was 400 mg/kg bw in rats and 200 mg/kg bw in mice.

Sustainability of forestomach hyperplasia was studied in rats treated with ethyl acrylate for 13 weeks with gavage doses of 100 or 200 mg/kg bw per day (Ghanayem et al., 1991). Severe hyperplasia of the forestomach was observed after 13 weeks. No effects were detected in the glandular stomach or liver. A significant decline in incidence and severity of the hyperplasia was reported in rats after a recovery period of 8 weeks without treatment and a further decline in effects were seen after 13 weeks.

Overall, severe irritation of the forestomach and mild oedema of the glandular stomach resulted from a single dose of ethyl acrylate, administered by gavage. In short-term and subchronic gavage studies, histological lesions were observed in the forestomach but not in the glandular stomach. The histopathological lesions in the forestomach were associated to NPSH depletion. After cessation of exposure, there was a dose-dependent recovery of the forestomach lesions. Exposure of ethyl acrylate via drinking water caused lower incidence and less severe forestomach lesions. No gastric effects were observed after parenteral administrations. There was no evidence of systemic toxicity.

3.6.2. Genotoxicity

Summary of *in vitro* data assessed in FGE.71

In vitro genotoxicity studies described below were considered in FGE.71 (EFSA CEF Panel, 2010a), based on JECFA evaluation (2006) and are summarised in Appendix A, Table A.1.

Negative results were reported in Ames assays when *Salmonella* Typhimurium strains (TA97, TA98, TA100, TA1535, TA1537 and TA1538) were incubated with ethyl acrylate [FL-no: 09.037] at concentrations up to 10,000 μg/plate (Ishidate et al., 1981; Waegemaekers and Bensink, 1984; Tennant et al., 1987; Zeiger et al., 1992).

No evidence of mitotic chromosomal loss was obtained when ethyl acrylate at concentrations up to 1,095 μg/mL was incubated with *Saccharomyces cerevisiae* strain D61.M. Under a cold shock regimen, evidence of mitotic recombination was reported (Zimmermann and Mohr, 1992).

In the standard assay for forward mutation in mouse lymphoma cells, ethyl acrylate gave uniformly positive results in the absence of metabolic activation when tested with mouse lymphoma L5178Y Tk+ cells at cytotoxic concentrations (37.5–50 μg/mL) associated with a reduction of the relative total growth by 50–60% (Tennant et al., 1987; McGregor et al., 1988; Moore et al., 1988; Ciaccio et al., 1998). In assays for clastogenicity, ethyl acrylate elicited increases in sister chromatid exchanges (SCE) and chromosomal aberrations (CA) in Chinese hamster ovary (CHO) cells with metabolic activation, but showed no evidence of clastogenicity in the absence of metabolic activation (Loveday et al., 1990). The clastogenic potential was unaffected by changes in harvest time (Loveday et al., 1990). Ethyl acrylate induced an increase in SCE and CA in mouse lymphoma cells in a dose-dependent manner, beginning at 20 μg/mL, in the absence of metabolic activation (Moore et al., 1988). An increase in SCE and CA in CHO cells was also reported in another study with ethyl acrylate at concentrations of 150 and 299 μg/mL, respectively, with metabolic activation (Tennant et al., 1987). There was no evidence of clastogenicity in the absence of metabolic activation (Tennant et al., 1987). Increases in CA were reported at 9.8 μg/mL in Chinese hamster cells with or without metabolic activation (Ishidate et al., 1981).

***In vitro* data not previously considered**

In vitro genotoxicity studies, previously not considered in the EFSA evaluation are summarised in Appendix B, Table B.1.

Ethyl acrylate tested up to 5,000 μg/plate was negative in *S.* Typhimurium strain TA102 (Kirkland et al., 2016).

Ethyl acrylate tested up to 2,000 μg/plate in the *S.* Typhimurium strain YG7108 (*ogt*−, *ada*−) (mutant of TA1535 strain deficient in methyl transferase), transformed with the plasmid pin3ERb5, encoding for a complete electron transport chain, comprising P450 reductase, cytochrome b5 and cytochrome P450 2E1, did not induce any significant increase of the revertants (Emmert et al., 2006).

A number of studies are available on *in vitro* micronucleus (MN) assay in different rodent (V79, CHL, CHO and L5178Y) and human cell lines, p53 competent and p53 mutated, in the presence and absence of cytochalasin B (Fowler et al., 2012a,b; Whitwell et al., 2015). Ethyl acrylate was tested at different concentrations up to 55% of cytotoxicity for 3 h followed by recovery and for 24 h without recovery. The results showed increases of frequencies of micronucleated cells in rodent p53-deficient cell lines. Following treatment of human cell lines WIL2-NS (with mutated p53) and TK6 (with wild-type p53) using the cytochalasin-blocked cytokinesis assay, the majority of concentrations tested resulted in micronucleated cell frequencies similar to the control values, with single exceptions, characterised by a marginal increase at intermediate concentrations.

Using an alternative protocol in the absence of cytochalasin B following treatment of WIL2-NS and TK6 cells with ethyl acrylate, normal frequencies of mononucleated cells with MN were observed for all concentrations including those inducing 53% and 66% cytotoxicity, respectively, fulfilling the criteria for clear negative responses. In contrast, small but statistically significant increases in MN frequency were observed at the highest two concentrations analysed following treatment of L5178Y cells. Significant increases in caspase activity (greater than threefold over the vehicle control) associated with high level of toxicity were observed in TK6 cells after treatment with ethyl acrylate, suggesting apoptosis as a mechanism of induction of micronuclei frequencies (Fowler et al., 2012a,b; Whitwell et al., 2015).

Summary of *in vivo* data assessed in FGE.71

In vivo genotoxicity studies described below were considered in FGE.71 (EFSA CEF Panel, 2010a), based on JECFA evaluation (2006) and are summarised in Appendix A, Table A.1.

Single oral doses of ethyl acrylate at concentrations up to 4% were administered to male F344 rats (Morimoto et al., 1990). The forestomach exhibited oedema and inflammation, but no DNA damage was detected by alkaline elution.

In an *in vivo-in vitro* assay for clastogenicity, C57BL/6 male mice were given ethyl acrylate at doses of 0, 125, 250, 500 or 1,000 mg/kg bw by i.p. injection (Kligerman et al., 1991). Twenty-four hours later, animals were sacrificed, splenocytes were isolated and concanavalin A was added to stimulate cell division. In half cultures, bromodeoxyuridine was added for the analysis of CA in the first division cells, and SCE in the second division cells. In the remaining cultures, cytochalasin B was added for the scoring of MN in binucleated cells. No significant increase of CA and SCE was reported, while ethyl acrylate did induce a statistically significant increase of binucleated cells with MN (less than twofolds with respect to the control value) only at the highest dose (1,000 mg/kg bw); according to the study authors, this increase was apparently due to a relatively high response in one of five animals. The highest dose tested in this study is fivefold higher than the highest dose (200 mg/kg bw) used in the carcinogenicity study by the National Toxicology Program (1986) (Kligerman et al., 1991).

Although an early report (Przybojewska et al., 1984) indicated that ethyl acrylate was genotoxic in a standard MN test in mice, subsequent studies (Ashby et al., 1989; Kligerman et al., 1991; Hara et al., 1994; Morita et al., 1997) confirmed that ethyl acrylate exhibits no genotoxic potential in this assay. An increase in the incidence of polychromatic erythrocytes (PCE) with MN was reported in bone marrow of BALB/C male mice treated with ethyl acrylate at doses of 225–1,800 mg/kg bw by i.p. injection in two doses separated by 24 h. A statistically significant decrease of the ratio between PCEs and normochromatic erythrocytes (NCEs) was observed in the same range of doses, indicating bone marrow toxicity (Przybojewska et al., 1984). There was no evidence of increase in MN cells collected 24 h after groups of six BDF1 male mice were given ethyl acrylate as a single or two doses at 0, 188, 375 or 750 mg/kg bw by oral gavage or when ethyl acrylate was administered as a single dose at 0, 375, 500 or 750 mg/kg bw by i.p. injection. A statistically significant reduction of reticulocyte percent after double oral gavage and i.p. administration at 750 mg/kg was reported (Hara et al., 1994). In another MN test, groups of five male and five female C57BL/6 mice were given ethyl acrylate as a single i.p. dose at 461 or 738 mg/kg bw and samples were collected at 24, 48 (738 mg/kg bw dose only) and 72 h (738 mg/kg bw dose only) (Ashby et al., 1989). In subsequent experiments, groups of 5–10 male C57BL/6 and BALB/c mice were given ethyl acrylate at a dose of 738 or 812 mg/kg bw in two doses administered by i.p. injection within 24 h, and erythrocytes were sampled at 30 h. In none of these

experiments, there was any evidence of increase in MN PCE in bone marrow of mice (Ashby et al., 1989). Negative results were obtained for MN induction when groups of six male BDF1 mice were given ethyl acrylate either as a single oral dose (188, 375 or 750 mg/kg bw) or a single intraperitoneal dose (188 or 375 mg/kg bw) and samples of bone marrow were collected after 24 h (Morita et al., 1997).

In an assay for mutagenicity *in vivo* in *Drosophila melanogaster*, there was no evidence of increase in sex-linked recessive lethals in three successive broods obtained from Basc virgin females mated with Canton-S wild-type males either injected with ethyl acrylate at a concentration of 20,000 mg/kg or fed a solution containing ethyl acrylate at a concentration of 40,000 mg/kg for 3 days. In a second experiment, there was no evidence of mutagenicity when *D. melanogaster* were fed a solution containing ethyl acrylate at a concentration of 18,000 or 20,000 mg/kg (Valencia et al., 1985).

In vivo data not previously considered

No new data are available.

Conclusions on genotoxicity

Overall, the Panel considered that based on the available data, which covers all relevant genetic endpoints (i.e. gene mutations, structural and numerical chromosomal aberrations), there is no concern with respect to genotoxicity of ethyl acrylate.

3.6.3. Chronic toxicity and carcinogenicity

Carcinogenesis studies of ethyl acrylate was performed by NTP in $B6C3F_1$ mice and F344/N rats given the test compound in corn oil by gavage at doses of 100 and 200 mg/kg bw per day, 5 days per week, for 103 weeks (NTP, 1986). There was no evidence of toxicity at any other sites than the forestomach. A dose-related increase in the incidences of hyperkeratosis, hyperplasia and inflammation of the forestomach were reported in both sexes and both species, in addition to a statistically significant positive trend in the incidence of squamous cell carcinoma and papilloma in the forestomach. The incidence of tumours in the forestomach was higher in rats than in mice, which was explained by a twofold higher concentration of ethyl acrylate in the gavage solution for rats than for mice, in the 2-year study. Statistically significant negative trends were reported for several tumours, such as hepatocellular carcinoma, follicular cell tumour of the thyroid and lymphocytic lymphoma in male mice. The conclusions from NTP (1986) were 'Under the conditions of these studies, ethyl acrylate was carcinogenic for the forestomach of F344/N rats and $B6C3F_1$ mice, causing squamous cell carcinomas in male rats and male mice, and squamous cell papillomas or carcinomas (combined) in male and female rats and mice. Evidence for carcinogenicity was greater in males than in females. Ethyl acrylate also caused irritation of the forestomach mucosa in male and female rats and mice'.

This conclusion was later modified (NTP, 1998), with the following recommendation 'It is recommended that ethyl acrylate be delisted from the Report on Carcinogens because the forestomach tumors, induced in animal studies, were seen only when the chemical was administered by gavage at high concentrations of ethyl acrylate, that induced marked local irritation and cellular proliferation and because significant chronic human exposure to high concentrations of ethyl acrylate monomer is unlikely'.

Ghanayem et al. (1993, 1994) studied the time required for sustained forestomach hyperplasia to produce neoplastic lesions in F344 rats administered ethyl acrylate at 200 mg/kg bw per day, 5 days per week, by gavage for 6 or 12 months. The increase in epithelial hyperplasia of the forestomach sustained as long as the exposure continued. However, the hyperplasia regressed and no neoplasms developed, when exposure was stopped after 6 months and the animals were allowed to recover until they were sacrificed at 24 months of age. In contrast, forestomach squamous cell carcinoma developed in rats treated for 12 months and allowed to recover for 9 months.

Acrylic acid, a metabolite of ethyl acrylate, was given to Wistar rats during 12 months in drinking water, containing 120, 800, 2,000 or 5,000 mg/L, providing doses of about 9, 61, 140 and 331 mg/kg bw per day, according to the authors (Hellwig et al., 1993). The two highest doses led to reduced consumption of drinking water, indicating palatability problems. There were no indications of systemic toxicity or carcinogenicity. Another study was performed in rats for 26 (males) and 29 (females) months with drinking water concentrations of 120, 400 or 1,200 mg/L, providing doses of 8, 27 or 78 mg/kg bw per day. There were no indications of toxic changes or carcinogenic potential.

Overall, increases in forestomach squamous cell papilloma and carcinoma were reported in rats and mice, when ethyl acrylate was administered via gavage. The neoplasms were preceded by extreme

sustained irritation of the epithelium, which was reversible when exposure was stopped after 6 months but not after 12 months. There was no evidence of systemic toxicity.

3.6.4. Discussion

The NTP carcinogenicity studies from 1986 concluded that ethyl acrylate administered by gavage was carcinogenic for the forestomach of mice and rats due to increases in forestomach squamous cell papilloma and carcinoma. The lesions were related to the concentration of ethyl acrylate in dosing solutions. Severe irritation of the forestomach was seen after single oral administration of ethyl acrylate. Hyperkeratosis, inflammation and hyperplasia of the forestomach were observed in subchronic and 2-year gavage studies. There were no gastric lesions after parenteral administration and no evidence of systemic toxicity in any of the studies. The Panel considered the observed local forestomach lesions to be a consequence of the known irritating properties of ethyl acrylate.

The conclusion on carcinogenicity of ethyl acrylate from the NTP studies was revised in 1998, when NTP recommended ethyl acrylate to be 'delisted from the Report on Carcinogens because the forestomach tumors, induced in animal studies, were seen only when the chemical was administered by gavage at high concentrations of ethyl acrylate, that induced marked local irritation and cellular proliferation and because significant chronic human exposure to high concentrations of ethyl acrylate monomer is unlikely'. This conclusion was supported by Williams and Iatropoulos (2009), who further emphasised that the route and rate of ethyl acrylate exposure in rodents for forestomach neoplasia are not relevant to humans, since humans do not have forestomach and are not exposed to ethyl acrylate by oral bolus.

Based on experimental data (publications up to 1993) in rodents showing lesions and carcinoma of forestomach, IARC (1999a) classified ethyl acrylate as possibly carcinogenic to humans (Group 2B). It should be noted, that IARC monographs are intended to be hazard evaluations, that is to identify whether a substance can be associated with the development of cancer, regardless of the dose or exposure conditions at which an increased risk occur (Goodman and Lynch, 2017). In the mechanistic considerations, IARC (1999a) noted that ethyl acrylate was clastogenic *in vitro*, but not *in vivo*.

The predictive value of rodent forestomach tumours in evaluating carcinogenic risks to humans was discussed by IARC (1999b), which considered in particular exposure conditions and tissue specificity. Exposure conditions should be considered, especially if oral gavage is used, because it can result in high local concentration of the test substance in the forestomach and prolonged exposure of the epithelial tissue. 'Agents that produce tumors in rodent forestomach only after prolonged exposure through non-DNA reactive mechanisms, may be less relevant to humans because human exposure would have to overcome time-integrated dose thresholds to result in a carcinogenic response' (IARC, 1999b).

In the present safety evaluation, genotoxicity of ethyl acrylate was assessed from all available data, both previously assessed and previously not considered. Ethyl acrylate was negative in bacterial reverse mutation assays. Ethyl acrylate resulted positive when tested in some *in vitro* assays for gene mutation and CA in mammalian cells, however, in *in vivo* studies no genotoxicity effects were observed as also reported for other acrylates (Johannsen et al., 2008). Therefore, based on the available data, which covers all relevant genetic endpoints (i.e. gene mutations, structural and numerical chromosomal aberrations), the Panel considers that there is no concern with respect to genotoxicity of ethyl acrylate.

The Panel agrees with the NTP re-evaluation (1998) and considers that the forestomach squamous cell papilloma and carcinoma are preceded by extreme sustained irritation of the epithelium, which is reversible when exposure is stopped after 6 months but not after 12 months. No lesions are observed in the glandular stomach. Moreover, in short-term, subchronic and chronic toxicity studies, there was no evidence of systemic toxicity.

The initiating events for the effects in the forestomach involve depletion of NPSH, which was observed in the forestomach at gavage doses >100 mg/kg bw per day, while no significant depletion was seen after administration via drinking water at 200–4,000 mg/L (Frederick et al., 1990). The depletion of NPSH in the rat forestomach was related to the histopathology observed at relatively high doses. At 10 mg/kg bw and less, no significant NPSH depletion and no histopathological findings were reported.

The estimated chronic dietary exposure of ethyl acrylate in adults and children were 60 and 150 μg/kg bw per day, respectively, calculated by the APET approach, with minimal contribution from natural occurring flavour in food. Maximum exposure from FCM may be up to 100 μg/kg bw per day.

The Panel considers that the forestomach tumours, observed in rodents, are not a relevant toxicological endpoint to humans since they are not exposed to ethyl acrylate by oral bolus. Furthermore, there is an absence of correlation between forestomach in rats and oesophageal lesions

in humans, as reported in the reviews by Wester and Kroes (1988) and Proctor et al. (2007). The Panel considers the effects of ethyl acrylate in the forestomach to be local and dependent on the concentration of ethyl acrylate. The concentration and exposure time, which induce the irritating effect in the forestomach would not be reached by exposure to ethyl acrylate via the use as a flavouring. Consequently, the Panel considers that the calculated chronic dietary exposure in adults and children would not be of safety concern.

4. Conclusions

The Panel concluded that ethyl acrylate is not genotoxic *in vivo* and that carcinogenic effects observed in rodent studies are not relevant to humans. Therefore, the Panel concluded that there is no safety concern for the use of ethyl acrylate as a flavouring substance in food, under the intended conditions of use.

5. Recommendations

The Panel takes note of other potential sources of dietary exposure such as from FCM. Exposure from plastic FCM could be up to the temporary TDI of 100 μg/kg bw per day, bringing the potential exposure from both plastic and flavouring above this TDI. The Commission may wish to take this into account.

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Abbreviations

APET	added portions exposure technique
bw	body weight
CA	chromosomal aberrations
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHL	Chinese hamster lung (cells)
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
FAO	Food and Agriculture Organization of the United Nations
FCM	food contact materials
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GSH	glutathione
IARC	International Agency for Research on Cancer
ID	Identity
IR	infrared spectroscopy
i.p.	intraperitoneal
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MN	micronuclei
MSDI	Maximised Survey-derived Daily Intake
NCE	normochromatic erythrocyte
NOAEL	no observed adverse effect level
NPSH	non-protein sulfhydryl
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development

PCE	polychromatic erythrocytes
SCE	sister chromatid exchanges
SCF	Scientific Committee on Food
SML	specific migration limit
SPET	single portion exposure technique
TDI	tolerable daily intake
WHO	World Health Organization

Appendix A – Genotoxicity data evaluated in FGE.71

Table A.1: Genotoxicity data on ethyl acrylate [FL-no: 09.037] evaluated by JECFA (2006) and considered by EFSA in FGE.71 (2010)

Chemical name FL-no JECFA-no	End-point	Test system	Concentration	Results	Reference	Comments
In vitro						
Ethyl acrylate 09.037 1351	Reverse mutation	*Salmonella* Typhimurium TA98, TA100, TA1535, TA1537 and TA1538	30–2,000 μg/plate	Negative[(a)]	Waegemaekers and Bensink (1984)	Reliable with the following restriction: the study complied with current recommendations with the exception that tester strains TA102 or *E. coli* WP2uvrA were not used
		S. Typhimurium TA97, TA98, TA100 and TA1535	33–3,333 μg/plate	Negative[(a),(b)]	Zeiger et al. (1992)	Reliable with the following restriction: the study complied with current recommendations with the exception that tester strains TA102 or *E. coli* WP2uvrA were not used
		S. Typhimurium TA98, TA100, TA1535 and TA1537	100–10,000 μg/plate	Equivocal[(a),(b),(c)]	Haworth et al. (1983)	Reliable with the following restriction: the study complied with current recommendations with the exception that tester strains TA102 or E. coli WP2*uvrA* were not used
		S. Typhimurium TA98, TA100 and TA1537	NR	Negative[(a),(b)]	Ishidate et al. (1981)	No data reported
		S. Typhimurium	≤ 10,000 μg/plate	Negative[(a)]	Tennant et al. (1987)	Reliable with restrictions (review paper without detailed results)
	Mitotic chromosomal recombination	*Saccharomyces cerevisiae* D61.M	≤ 1,095 μg/mL	Negative[(d),(e)]	Zimmermann and Mohr (1992)	Reliable. Endpoint of low relevance, no OECD guideline available
		S. cerevisiae D61.M	≤ 1,095 μg/mL	Positive[(d)]		
		S. cerevisiae D61.M	≤ 914 μg/mL	Negative[(e)]		
	Forward mutation	Mouse lymphoma L5178Y Tk+/− cells	20 μg/mL	Positive[(f)]	Tennant et al. (1987)	Reliable with restrictions (review paper without detailed results)
		Mouse lymphoma L5178Y Tk+/− cells	10, 15, 20, 25, 27.5, 30, 32.5, 35, 40 or 50 μg/mL	Positive[(f),(g)]	Ciaccio et al. (1998)	Reliable without restrictions. Study in line with OECD guideline No. 490

Chemical name FL-no JECFA-no	End-point	Test system	Concentration	Results	Reference	Comments
		Mouse lymphoma L5178Y Tk+/− cells	20, 25, 30 or 37.5 μg/mL	Positive[(f),(h)]	Moore et al. (1988)	Reliable without restrictions. Study in line with OECD guideline No. 490 Large majority of mutants are small-colony mutants
		Mouse lymphoma L5178Y Tk+/− cells	2.5–40 μg/mL	Positive[(f),(i)]	McGregor et al. (1988)	Reliable without restrictions. Study in line with OECD guideline No. 490 Positive only at highest dose. Equivocal using the 'global evaluation factor approach'
	Chromosomal aberration	Chinese hamster ovary cells	299 μg/mL <29.9 μg/mL	Positive[(j)] Negative[(f),(h)]	Loveday et al. (1990)	Reliable without restrictions Study in line with OECD guideline No. 473
		Mouse lymphoma L5178Y Tk+/− cells	20, 25, 30 or 37.5 μg/mL	Positive[(f),(h)]	Moore et al. (1988)	Reliable without restrictions Study in line with OECD guideline No. 473
		Chinese hamster lung cells	0.0098 mg/mL[(k)] (9.8 μg/mL)	Positive[(a)]	Ishidate et al. (1981)	Reliable with restrictions (review paper without detailed results)
		Chinese hamster ovary cells	299 μg/mL	Positive[(j)] Negative[(f)]	Tennant et al. (1987)	Reliable with restrictions (review paper without detailed results)
	Sister chromatid exchange	Chinese hamster ovary cells	150 μg/mL	Positive[(j)] Negative[(f)]		Reliable with restrictions (review paper without detailed results) OECD guideline was withdrawn
		Chinese hamster ovary cells	150 μg/mL <5 μg/mL	Weak positive[(j)] Negative[(f),(h)]	Loveday et al. (1990)	Reliable without restrictions. The endpoint has low relevance (OECD guideline was withdrawn)
		Mouse lymphoma L5178Y Tk+/− cells	20.0–37.5 μg/mL	Positive[(f),(h)]	Moore et al. (1988)	Reliable without restrictions. The endpoint has low relevance (OECD guideline was withdrawn)
		Mouse splenocytes	10–80 μg/mL	Negative[(l)]	Kligerman et al. (1991)	Reliable without restrictions. The endpoint has low relevance (OECD guideline was withdrawn)
			1–20 μg/mL	Negative[(m)]		

Chemical name FL-no JECFA-no	End-point	Test system	Concentration	Results	Reference	Comments
In vivo						
Ethyl acrylate 09.037 1351	Sister chromatid exchange	Male C57BL/6 mice	125, 250, 500 or 1,000 mg/kg[(n)]	Negative	Kligerman et al. (1991)	*In vivo-vitro* study (animals were exposed, splenocytes were isolated and cultured *in vitro*) (OECD guideline was withdrawn)
	Chromosomal aberration	Male C57BL/6 mice	125, 250, 500 or 1,000 mg/kg[(n)]	Negative		*In vivo-vitro* study (animals were exposed, splenocytes were isolated and cultured *in vitro*) this experimental protocol is not described in OECD guideline No. 475
	Micronucleus formation	Male C57BL/6 mice	125, 250, 500 or 1,000 mg/kg[(n)]	Negative[(q)]	Kligerman et al. (1991)	*In vivo-vitro* study (animals were exposed, splenocytes were isolated and cultured *in vitro*) This experimental protocol is not described in OECD guideline No. 474
		Male BALB/c mice	225–1,800 mg/kg[(r)]	Positive	Przybojewska et al. (1984)	Reliable with restrictions: 4 animals/ group were treated. Highest dose (1,800 mg/kg) was toxic. 2/4 animals died
		Male and female C57BL/6 mice	461[(n),(s)] or 738 mg/kg[(n),(t)]	Negative	Ashby et al. (1989)	Reliable with restrictions (review paper)
		Male BALB/c mice	812 mg/kg[(u)]	Negative		
		Male C57BL/6 mice	738 mg/kg[(u)]	Negative		
		Male BDF_1 mice	188, 375 or 750 mg/kg[(s),(v)]	Negative	Hara et al. (1994)	Reliable with restrictions (poor reporting)
			188, 375 or 750 mg/kg[(w)]	Negative[(x)]		
			375, 500 or 750 mg/kg[(n),(s)]	Negative[(x)]		
		Male BDF_1 mice	188, 375, 750 or 1,000 mg/kg[(n),(s),(v)]	Negative[(y)]	Morita et al. (1997)	Reliable with restrictions Basically following the OECD guidelines. Except that no data were reported on PCE/NCE ratio

Chemical name FL-no JECFA-no	End-point	Test system	Concentration	Results	Reference	Comments
	DNA alkaline elution	Male F344 rat forestomach squamous epithelium	0.1–4.0% (50–2,000 mg/kg)(v)	Negative	Morimoto et al. (1990)	Reliable without restrictions. No OECD guidelines are available
	Sex-linked recessive lethal mutations	*Drosophila melanogaster*	20,000 mg/kg	Negative(o)	Valencia et al. (1985)	Reliable without restrictions. Low relevance of target organism. OECD guideline withdrawn
	Micronucleus formation	*D. melanogaster*	18,000; 20,000 or 40,000 mg/kg	Negative(p)	Valencia et al. (1985)	Reliable without restrictions. Low relevance of target organism. No OECD guideline available

OECD: Organisation for Economic Cooperation and Development; PCE: polychromatic erythrocyte; NCE: normochromatic erythrocyte.
(a): With and without metabolic activation.
(b): Pre-incubation method.
(c): The Ames tests were performed by two different laboratories, resulting in positive and negative results in the first and second experiments, respectively. The authors considered the results from the confirmation (second) experiment to be more definitive.
(d): Cold shock regimen (i.e. 4-h incubation at 28°C, followed by a 16-h storage in an ice bath and a 4-h incubation at 28°C).
(e): Uninterrupted 16-h incubation at 28°C.
(f): Without metabolic activation.
(g): Relative cell growth was 20% and 13%, respectively, at the two highest doses tested (40 and 50 μg/mL, respectively).
(h): Cytotoxicity was observed at the highest dose tested.
(i): Statistically significant increases in mutant fraction were observed at doses of 20 and 40 μg/mL.
(j): With metabolic activation.
(k): Dose at which chromosomal aberrations were detected in 20% of metaphase cells.
(l): Exposure during the Go phase of the cell cycle. Cytotoxicity was observed at doses of >30 μg/mL.
(m): Exposure during the G-S phase of the cell cycle. Cytotoxicity was observed at doses of >10 μg/mL.
(n): Administered intraperitoneally.
(o): Injection experiment.
(p): Feeding experiment.
(q): A slight but significant increases in the frequency of micronucleus formation was observed at the highest dose tested (1000 mg/kg), which was thought to be due to an elevated frequency in one of the four treated mice.
(r): Administered intraperitoneally in two doses within 24 h.
(s): Assessment of bone marrow for formation of micronuclei 24 h after dosing.
(t): Assessment of bone marrow for formation of micronuclei 24, 48 or 72 h after dosing.
(u): Administered intraperitoneally at 0 and 24 h, followed by assessment of bone marrow for formation of micronuclei 6 h later.
(v): Administered orally.
(w): Administered orally twice within 24-h.
(x): A significant decrease in the reticulocyte ratio was observed at the highest dose tested compared with vehicle controls.
(y): Mortality was observed at the highest dose tested (1,000 mg/kg).

Appendix B – Previously not considered genotoxicity data

Table B.1: *In vitro* genotoxicity studies on ethyl acrylate [FL-no: 09.037]

Chemical name FL-no JECFA-no	End-point	Test system	Concentration	Results	Reference	Comments
Ethyl acrylate 09.037 1351	Ames test	*Salmonella* Typhimurium TA102	Up to 5,000 μg/plate	Negative	Kirkland et al. (2016)	Reliable without restrictions. The study complies with OECD Test Guideline 471
		S. Typhimurium strain YG7108, transformed with the plasmid pin3ERb5	Up to 2,000 μg/plate	Negative	Emmert et al. (2006)	Reliable without restrictions. The study was designed to test the compound in a metabolically competent *S.* Typhimurium strain
	Micronucleus Assay	p53-deficient rodent cell lines: V79	0–20[(b),(c)]; 0–8[(b),(d)] μg/mL	Positive[(b),(c),(d)]	Fowler et al. (2012a)	Reliable without restrictions In the short-term treatment, MN were induced at concentrations of ethyl acrylate resulting in cytotoxicity of 50–60% The study was designed to investigate the different results in the MN assay depending on the cell line
		CHO	0–32[(b),(c)]; 0–12[(b),(d)] μg/mL	Positive[(b),(c)]; Negative[(b),(d)]		
		CHL	0–40[(b),(c)]; 0–14[(b),(d)] μg/mL	Positive[(b),(c) and (b),(d)]		
		p53-competent human cells: peripheral blood lymphocytes	0–50[(b),(c)]; 0–10.4[(b),(d)] μg/mL	Positive[(b),(c)]; Negative[(b),(d)]		
		TK6 human lymphoblastoid cells	0–28[(b),(c)]; 0–12[(b),(d)] μg/mL	Positive[(b),(c)]; Negative[(b),(d)]		
		HepG2	0–96[(b),(c)]; 0–150[(b),(d)] μg/mL	Equivocal[(b),(c)] Negative[(b),(d)]		
		p53-mutant mouse lymphoma L5178Y	0–18 μg/mL	Equivocal in the absence of cytochalasin B	Whitwell et al. (2015)	Reliable without restrictions. The study was designed to investigate the possible role of p53 and apoptosis in the micronuclei induction
		p53-competent human lymphoblastoid TK6	0–10 μg/mL	Equivocal with both human cell linesin the presence of cytochalasin B		

Chemical name FL-no JECFA-no	End-point	Test system	Concentration	Results	Reference	Comments
		p53-mutant human WIL2-NS cells	0–9 μg/mL	Negative with both human cell lines in the absence of cytochalasin B		

OECD: Organisation for Economic Cooperation and Development.
(a): With metabolic activation.
(b): Without metabolic activation.
(c): 3 h treatment + 21 h recovery.
(d): 24 h treatment + 0 h recovery.

Appendix C – Exposure

Calculation of the Dietary Exposure - APET

Chronic Dietary Exposure – 'Added Portions Exposure Technique' (APET)[5]

The chronic APET calculations are based on the normal combined occurrence level by adding the highest contributing portion of food and highest contributing portion of beverages (either among soft drinks or alcoholic beverages). APET for children is calculated by adding the highest contributing portion of food and highest contributing portion of beverages (among soft drinks). Furthermore, in the APET calculation for children, the portion sizes listed in Table 8 is adjusted by a factor 0.63 to take into account the smaller portion sizes consumed by the child.

Adults ('Added Portions Exposure Technique' [APET])

On the basis of normal occurrence level from the added flavouring only

Solid food: The maximum intake will be from category 1.0 (Dairy products) with the normal combined occurrence level of 2,120 μg/adult per day.

Beverage: The category 14.1 (Non-alcoholic ('soft') beverages, excl. dairy products) to which the candidate substance is added have the same normal combined occurrence level of 1,425 μg/adult per day.

The total APET will be 3,545 μg/adult per day corresponding to 59.1 μg/kg bw per day for a 60-kg person.

Children (3-year-old child of 15 kg body weight)

Solid food: The maximum intake will be from category 1.0 (Dairy products) with the normal combined occurrence level of 2,120 x 0.63 = 1,335 μg/child per day.

Beverage: The category 14.1 (Non-alcoholic ('soft') beverages, excl. dairy products) to which the candidate substance is added have the same normal combined occurrence level of 1,425 × 0.63 = 898 μg/child per day.

The total APET will be 2,233 μg/child per day corresponding to 149 μg/kg bw per day for a 15-kg child.

Conclusion

The higher of the two values among adults and children, expressed per kg/bw per day, should be used as the basis for the safety evaluation of the candidate substance, i.e. the value of 150 μg/kg bw per day for a 15-kg child should be compared to the appropriate no observed adverse effect level (NOAEL) for the candidate substance.

Combined Dietary Exposure

This is an estimate of total dietary exposure deriving from both the addition of the flavouring substance to foods and beverages and other dietary sources. To estimate the APET for combined dietary exposure, the occurrence of the substance in grapes and vanilla was also taken into account in the estimation.

Adults ('Added Portions Exposure Technique' [APET])

Solid Food: The maximum intake will be from category 1.0 (Dairy products) with the normal combined occurrence level of 2,120 μg/adult per day.

Beverage: The category 14.1 (Non-alcoholic ('soft') beverages, excl. dairy products) to which the candidate substance is added have the same normal combined occurrence level of 1,425 μg/adult per day.

The total APET will be 3,545 μg/adult per day corresponding to 59.1 μg/kg bw per day for a 60-kg person.

Children (3-year-old child of 15 kg body weight)

Solid food: The maximum intake will be from category 1.0 (Dairy products) with the normal combined occurrence level of 2,120 × 0.63 = 1,335 μg/child per day.

[5] The APET has been calculated based on the occurrence levels in the food subcategories reported in the above table, with the exclusion of categories 13.2 (complementary foods for infants and young children).

Beverage: The category 14.1 (Non-alcoholic ('soft') beverages, excl. dairy products) to which the candidate substance is added have the same normal combined occurrence level of 1,425 × 0.63 = 898 μg/child per day.

The total APET will be 2,233 μg/child per day corresponding to 149 μg/kg bw per day for a 15-kg child.

Table C.1: Normal and maximum occurrence levels for refined categories of foods and beverages

Food categories[a]		Standard portions[b] (g)	Occurrence level as added flavouring substance (mg/kg)		Occurrence level from other sources[c] (mg/kg)		Combined occurrence level from all sources[e] (mg/kg)	
			Normal	Maximum	Average[d]	Maximum	Normal	Maximum
01.1	Milk and dairy-based drinks	200	10.6	15.2			10.6	15.2
01.2	Fermented and renneted milk products (plain), excluding food category 01.1.2 (dairy-based drinks)	200	10.6	15.2			10.6	15.2
01.3	Condensed milk and analogues (plain)	70	10.6	15.2			10.6	15.2
01.4	Cream (plain) and the like	15	10.6	15.2			10.6	15.2
01.5	Milk powder and cream powder and powder analogues (plain)	30	10.6	15.2			10.6	15.2
01.6	Cheese and analogues	40	10.6	15.2			10.6	15.2
01.7	Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)	125	10.6	15.2			10.6	15.2
01.8	Whey and whey products, excluding whey cheeses	200	10.6	15.2			10.6	15.2
02.1	Fats and oils essentially free from water	15						
02.2	Fat emulsions mainly of type water-in-oil	15						
02.3	Fat emulsions mainly of type water-in-oil, including mixed and/or flavoured products based on fat emulsions	15						
02.4	Fat-based desserts excluding dairy-based dessert products of category 1.7	50						
03.0	Edible ices, including sherbet and sorbet	50						
04.1.1	Fresh fruit	140			0.1	0.1	0.1	0.1
04.1.2	Processed fruit	125			0.1	0.1	0.1	0.1
04.1.2.5	Jams, jellies, marmalades	30						
04.2.1	Fresh vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed	200						
04.2.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g. peanut butter) and nuts and seeds	200						
04.2.2.5	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g. peanut butter)	30						
05.1	Cocoa products and chocolate products, including imitations and chocolate substitutes	40	10.14	13.3			10.14	13.3

Food categories[a]		Standard portions[b] (g)	Occurrence level as added flavouring substance (mg/kg)		Occurrence level from other sources[c] (mg/kg)		Combined occurrence level from all sources[e] (mg/kg)	
			Normal	Maximum	Average[d]	Maximum	Normal	Maximum
05.1.3	Cocoa-based spreads, including fillings	30	10.14	13.3			10.14	13.3
05.2	Confectionery, including hard and soft candy, nougats, etc., other than 05.1, 05.3 and 05.4	30	10.14	13.3			10.14	13.3
05.3	Chewing gum	3	0.07	0.11			0.07	0.11
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	35	10.14	13.3			10.14	13.3
06.1	Whole, broken or flaked grain, including rice	200	6.77	10.84			6.77	10.84
06.2	Flours and starches (including soya bean powder)	30	6.77	10.84			6.77	10.84
06.3	Breakfast cereals, including rolled oats	30	6.77	10.84			6.77	10.84
06.4	Pastas and noodles and like products (e.g. rice paper, rice vermicelli, soya bean pastas and noodles)	200	6.77	10.84			6.77	10.84
06.5	Cereal- and starch-based desserts (e.g. rice pudding, tapioca pudding)	200	6.77	10.84			6.77	10.84
06.6	Batters (e.g. for breading or batters for fish or poultry)	30	6.77	10.84			6.77	10.84
06.7	Pre-cooked or processed rice products, including rice cakes (Oriental type only)	200	6.77	10.84			6.77	10.84
06.8	Soya bean products (excluding soya bean products of food category 12.9 and fermented soya bean products of food category 12.10)	100	6.77	10.84			6.77	10.84
07.1	Bread and ordinary bakery wares	50	11.75	13.76			11.75	13.76
07.2	Fine bakery wares (sweet, salty, savoury) and mixes	80	11.75	13.76			11.75	13.76
08.1	Fresh meat, poultry and game	200	0.1	0.1			0.1	0.1
08.2	Processed meat, poultry and game products in whole pieces or cuts	100	0.1	0.1			0.1	0.1
08.3	Processed comminute meat, poultry and game products	100	0.1	0.1			0.1	0.1
08.4	Edible casings (e.g. sausage casings)	1	0.1	0.1			0.1	0.1
09.1.1	Fresh fish	200						
09.1.2	Fresh molluscs, crustaceans and echinoderms	200						
09.2	Processed fish and fish products, including molluscs, crustaceans and echinoderms	100						

Food categories[a]		Standard portions[b] (g)	Occurrence level as added flavouring substance (mg/kg)		Occurrence level from other sources[c] (mg/kg)		Combined occurrence level from all sources[e] (mg/kg)	
			Normal	Maximum	Average[d]	Maximum	Normal	Maximum
09.3	Semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms	100						
09.4	Fully preserved, including canned or fermented, fish and fish products, including molluscs, crustaceans and echinoderms	100						
10.1	Fresh eggs	100						
10.2	Egg products	100						
10.3	Preserved eggs, including alkaline. salted and canned eggs	100						
10.4	Egg-based desserts (e.g. custard)	125						
11.1	Refined and raw sugar	10						
11.2	Brown sugar excluding products of food category 11.1	10						
11.3	Sugar solutions and syrups, and (partially) inverted sugars, including molasses and treacle, excluding products of food category 11.1.3 (soft white sugar, soft brown sugar, glucose syrup, dried glucose syrup, raw cane sugar)	30						
11.4	Other sugars and syrups (e.g. xylose, maple syrup, sugar toppings)	30						
11.5	Honey	15						
11.6	Table-top sweeteners, including those containing high-intensity sweeteners	1						
12.1	Salt and salt substitutes	1						
12.10	Protein products other than from soybeans	15						
12.2	Herbs, spices, seasonings and condiments (e.g. seasoning for instant noodles)	1						
12.3	Vinegars	15						
12.4	Mustards	15						
12.5	Soups and broths	200						
12.6	Sauces and like products	30						
12.7.a	Salads 120 g (e.g. macaroni salad, potato salad) excluding cocoa- and nut-based spreads of food categories	120						
12.7.b	Sandwich spreads (20 g), excluding cocoa- and nut-based spreads of food categories	20						

Food categories[a]		Standard portions[b] (g)	Occurrence level as added flavouring substance (mg/kg)		Occurrence level from other sources[c] (mg/kg)		Combined occurrence level from all sources[e] (mg/kg)	
			Normal	Maximum	Average[d]	Maximum	Normal	Maximum
12.8	Yeast and like products	1						
12.9	Soybean-based seasonings and condiments	15						
12.9.1	Fermented soya bean products (e.g. miso)	40						
12.9.2	Soybean sauce	15						
12.9.3	Fermented soybean sauce	15						
13.2.a	Complementary foods for infants and young children: Dry instant cereals (with or without milk), including pasta	110						
13.2.b	Complementary foods for infants and young children: Meat-based or fish-based dinner	170						
13.2.c	Complementary foods for infants and young children: Dairy-based dessert	110						
13.2.d	Complementary foods for infants and young children: Vegetables, potatoes, broth, soups, pulses	170						
13.2.e	Complementary foods for infants and young children: Biscuits and cookies	20						
13.2.f	Complementary foods for infants and young children: Fruit purée	110						
13.2.g	Complementary foods for infants and young children: Fruit juice	120						
13.2.h	Milk for young children	200						
13.3	Dietetic foods intended for special medical purposes (excluding food products of category 13.1 'Infant formulae, follow-up formulae and other formulae for special medical purposes for infants')	200						
13.4	Dietetic formulae for slimming purposes and weight reduction	200						
13.5	Dietetic foods (e.g. supplementary foods for dietary use), excluding products of food categories 13.1 (Infant formulae, follow-up formulae and other formulae for special medical purposes for infants), 13.2–13.4 and 13.6	200						
13.6	Food supplements	5						
14.1	Other non-alcoholic ('soft') beverages (expressed as liquid)	300	4.75	8.75			4.75	8.75
14.2.1	Beer and malt beverages	300	0.38	1			0.38	1
14.2.2	Cider and perry	300	0.38	1			0.38	1

Food categories[a]		Standard portions[b] (g)	Occurrence level as added flavouring substance (mg/kg)		Occurrence level from other sources[c] (mg/kg)		Combined occurrence level from all sources[e] (mg/kg)	
			Normal	Maximum	Average[d]	Maximum	Normal	Maximum
14.2.3	Grape wines	150	0.38	1			0.38	1
14.2.4	Wines (other than grape)	150	0.38	1			0.38	1
14.2.5	Mead	150	0.38	1			0.38	1
14.2.6	Distilled spirituous beverages containing more than 15% alcohol	30	0.38	1			0.38	1
14.2.7	Aromatised alcoholic beverages (e.g. beer, wine and spirituous cooler-type beverages, low alcoholic refreshers)	300	0.38	1			0.38	1
15.1	Snacks, potato-, cereal-, flour- or starch-based (from roots and tubers, pulses and legumes)	30						
15.2	Processed nuts, including coated nuts and nut mixtures (with e.g. dried fruit)	30						
15.3	Snacks – fish-based	30						
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) – foods that could not be placed in categories 01–15	300						

(a): Most of the categories reported are the sub-categories of Codex GSFA (General Standard for Food Additives, available at http://www.codexalimentarius.net/gsfaonline/CXS_192e.pdf), used by the JECFA in the SPET technique (FAO/WHO, 2008). In the case of category 13.2 (complementary foods for infants and young children), further refined categories have been created so that a specific assessment of dietary exposure can be performed in young children.

(b): For Adults. In case of foods marketed as powder or as concentrates, occurrence levels must be reported for the reconstituted product, considering the instructions reported on the product label or one of the standard dilution factors established by the JECFA (FAO/WHO, 2008):

- 1/25 for powder used to prepare water-based drinks such as coffee, containing no additional ingredients,
- 1/10 for powder used to prepare water-based drinks containing additional ingredients such as sugars (ice tea, squashes, etc.),
- 1/7 for powder used to prepare milk, soups and puddings,
- 1/3 for condensed milk.

(c): As natural constituent and/or developed during the processing and/or as carry over resulting from their use in animal feed.

(d): In order to estimate normal values in each category, only foods and beverages in which the substance is present in significant amount will be considered (e.g. for the category 'Fresh fruit' 04.1.1., the normal concentration will be the median concentration observed in all kinds of fruit where the flavouring substance is known to occur).

(e): As added flavouring or from other sources. The normal and maximum combined occurrence levels of the substance will be assessed by the applicant either by adding up occurrence levels from added use to that from other sources or by expert judgement based on the likelihood of their concomitant presence. This will be done both for normal use levels and for maximum use levels.