Re-evaluation of glycerol (E 422) as a food additive

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Re-evaluation of glycerol (E 422) as a food additive

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Abstract

The ANS Panel provides a scientific opinion re-evaluating the safety of glycerol (E 422) used as a food additive. In 1981, the Scientific Committee on Food (SCF) endorsed the conclusion from the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1976 of 'acceptable daily intake (ADI) for man not specified'. The Panel concluded that glycerol has low acute toxicity and that local irritating effects of glycerol in the gastrointestinal tract reported in some gavage studies was likely due to hygroscopic and osmotic effects of glycerol. Glycerol did not raise concern with respect to genotoxicity and was of no concern with regard to carcinogenicity. Reproductive and prenatal developmental studies were limited to conclude on reproductive toxicity but no dose-related adverse effects were reported. None of the animal studies available identified an adverse effect for glycerol. The Panel conservatively estimated the lowest oral dose of glycerol required for therapeutic effect to be 125 mg/kg bw per hour and noted that infants and toddlers can be exposed to that dose by drinking less than the volume of one can (330 mL) of a flavoured drink. The Panel concluded that there is no need for a numerical ADI and no safety concern regarding the use of glycerol (E 422) as a food additive at the refined exposure assessment for the reported uses. The Panel also concluded that the manufacturing process of glycerol should not allow the production of a food additive, which contains genotoxic and carcinogenic residuals at a level which would result in a margin of exposure below 10,000. The Panel recommended modification of the EU specifications for E 422. The Panel also recommended that more information on uses and use levels and analytical data should be made available to the Panel.

Keywords: glycerol, glycerine, 1,2,3-propanetriol, trihydroxypropane, E 422, CAS Registry Number 56-81-5, food additive

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Question number: EFSA-Q-2011-00519

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Summary

Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to re-evaluate the safety of glycerol (E 422) when used as a food additive.

This re-evaluation refers exclusively to the uses and conditions of use of glycerol (E 422) as a food additive in food, and does not include a safety assessment of other uses of glycerol as described in Section 3.4.5.

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and reviews, additional literature that came available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

Glycerol (E 422) is authorised as a food additive in the European Union (EU) in accordance with Annex II and Annex III of Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012.

Glycerol was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1976. Based on the available toxicological studies and the fact that glycerol occurs naturally in fats and other substances consumed via food, JECFA allocated to glycerol an acceptable daily intake (ADI) 'not specified'.

The Scientific Committee for Food (SCF) evaluated glycerol as a food additive in 1981 and endorsed the conclusion from JECFA in 1976 of 'ADI for man not specified'. However, a rationale for the conclusion was not provided.

A request to use glycerol without restriction (*quantum satis* level) as a sweetener in concentrated juices especially intended for young children was rejected by the SCF in 1999.

Glycerol has also been reviewed by TemaNord in 2002. It was concluded that no new data were available indicating a need for a re-evaluation of glycerol. However, it was further concluded that the present permitted *quantum satis* use may lead to the conclusion that all levels are acceptable. In addition, it was concluded that it should be ensured that glycerol is not used as a sugar replacement.

The Panel noted that in addition to the information provided by industry on the manufacturing process, glycerol (E 422) can be produced by a variety of methods and that many of them lead to the presence or formation of contaminants, which are of toxicological concern.

Glycerol occurs naturally in several types of lipid and is an endogenous metabolite in mammals. Glycerol is rapidly and near completely absorbed from the gastrointestinal tract, it is then distributed into the total body water space and primarily metabolised in the liver. After phosphorylation and oxidation, glycerol is used as an energy substrate via glycolysis or participates in gluconeogenesis and lipogenesis. Glycerol is extensively oxidised and exhaled as carbon dioxide, with only minor amounts excreted via urine or faeces. The Panel noted that glycerol concentrations in blood higher than 92 mg/L would result in renal elimination of glycerol in rats and humans.

The Panel considered that glycerol has a low acute toxicity.

Short-term or subchronic studies were not performed according to current test guidelines. In a subchronic toxicity study (in drinking water) in rats, the effects reported were observed with doses in the range of the oral median lethal dose (LD_{50}) values for glycerol. The Panel considered that the local irritating effects of glycerol in the gastrointestinal tract reported in some gavage studies in rat (100% glycerol at 2,800 mg/kg body weight (bw) per day, the lowest dose tested), and dogs (100% glycerol at 5,600 mg/kg bw per day) was likely due to the hygroscopic and osmotic effects of large bolus doses of glycerol administered by gavage.

Glycerol did not show any genotoxic activity in a variety of *in vitro* assays. A lack of valid *in vivo* genotoxicity data was not of concern since clear negative findings were observed in *in vitro* assays. On this basis, the Panel considered that glycerol as a food additive did not raise concern with respect to genotoxicity.

From the available chronic toxicity and carcinogenicity studies, glycerol was not carcinogenic in mice and rats and did not show evidence of adverse effects in a 2-year chronic toxicity study. The Panel noted that no adverse effects were reported in rats receiving doses up to 10,000 mg/kg bw per day for 1 year, the highest dose tested. The Panel also noted that there was no increase in tumour incidences in rats receiving doses up to 5,000 mg/kg bw per day for 2 years, the highest dose tested.

The reports of the two and multigeneration reproductive toxicity studies have limitations but no adverse effects were reported. Prenatal developmental toxicity studies were also limited but showed

no dose-related developmental and maternal effects up to the highest dose tested (1,280, 1,600 or 1,180 mg glycerol/kg bw per day for mice, rats and rabbits, respectively).

The Panel considered that none of the animal studies available identified an adverse effect for glycerol.

The Panel considered that the therapeutic oral use of glycerol at 1,000–1,500 mg/kg bw given as bolus in patients with glaucoma triggered an increase in plasma osmolality and dehydration and resulted in some patients in side effects such as headache, nausea and vomiting in some individuals. Given the dose- and time period-range reported in a study, the Panel calculated that the minimum dose of glycerol required to induce a therapeutic reduction in intracranial pressures was within the range of 125–333 mg/kg bw per hour. The Panel considered that a conservative estimate of the lowest oral bolus dose of glycerol required for therapeutic effect was 125 mg/kg bw per hour. The Panel considered this dose would also be responsible for the side effects (nausea, headache and/or vomiting) observed in some patients.

The Panel considered that the exposure estimates in all exposure scenarios resulted in overestimates of the exposure to glycerol (E 422) from its use as a food additive according to Annex II to Regulation (EC) No 1333/2008. Although use levels and analytical data were only available for 28 out of the total of 68 food categories in which glycerol (E 422) is authorised for use according to Annex II, the Panel considered that this did not result in an underestimation of the exposure because:

- glycerol (E 422) belongs to Group I food additives, and therefore almost all the food categories for which glycerol (E 422) is authorised correspond to the general Group I food additives authorisation. Given the information on the uses provided by food industry and information from the Mintel's Global New Products Database (GNPD), it may be assumed that glycerol is not used in a number of these food categories.
- approximately 94% of the food products labelled with glycerol (E 422) in the Mintel GNPD belonged to food subcategories that were considered in the exposure assessment.

Furthermore, the Panel noted that the additional exposure to glycerol via natural sources, including wine and honey (the sources for which analytical data were available) did not significantly change the exposure to glycerol (E 422). The highest 95th percentile of exposure of glycerol (E 422) according to Annex II, carry-over (Annex III) and natural sources was estimated at 460 mg/kg bw per day in children in the refined non-*brand-loyal exposure* scenario.

The Panel considered that the most relevant situation where an acute bolus exposure to glycerol used as a food additive can be similar to the one occurring during therapeutic use is consumption of a beverage. Therefore, the Panel calculated the volume of flavoured drinks required to be consumed in order to exceed the acute bolus exposure (125 mg/kg bw per hour) calculated by the Panel to be the minimum dose required to have a therapeutic effect. When considering the available data, the Panel noted that infants and toddlers can be exposed to more than 125 mg glycerol/kg bw per hour by drinking less than the volume of one can (330 mL) of a flavoured drink.

According to the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA ANS Panel, 2014) and given that:

- the safety assessment carried out by the Panel is limited to the use and use levels received from industry and Member States in 28 food categories out of 68 food categories in which glycerol (E 422) is authorised;
- the highest 95th percentile of exposure of glycerol (E 422) according to Annex II, carry-over (Annex III) and natural sources was estimated at 460 mg/kg bw per day in children in the refined *non-brand-loyal exposure* scenario;
- glycerol (E 422) as a food additive is identical to a compound which is a normal constituent in the body (an endogenous compound) and is a regular component of the diet;
- sufficient toxicity data were available;
- the toxicological studies in animals did not provide any indication for adverse effects, including at the highest dose tested in a chronic toxicity study (10,000 mg/kg bw per day);

the Panel concluded that there is no need for a numerical ADI for glycerol (E 422).

The Panel concluded that there is no safety concern regarding the use of glycerol (E 422) as a food additive according to Annex II and III (Part 1, 2, 3, 4 and 5) for the general population at the refined exposure assessment for the reported uses of glycerol as a food additive.

However, the Panel identified that there remain uncertainties over the lack of identification and quantification of residuals, especially those that are genotoxic and carcinogenic. The Panel noted that

these residuals are mostly present when chemical synthesis is used to produce glycerol. The Panel concluded that the manufacturing process for glycerol should not allow the production of a food additive, which contains these residuals at a level, which would result in a margin of exposure (MOE) below 10,000.

The Panel concluded that if 3-monochloropropane-1,2-diol (3-MCPD) is present at its maximum authorised level of 0.1 mg 3-MCPD/kg glycerol, the maximum exposure to 3-MCPD was below the tolerable daily intake (TDI) of 0.8 μg/kg bw per day, and therefore exposure via glycerol (E 422) alone was of no concern.

The Panel could not calculate exposures to other genotoxic impurities or contaminants that may be present in glycerol (E 422) as a result of the manufacturing process, e.g. glycidol, due to the lack of data on their concentrations in the food additive.

The Panel concluded that infants and toddlers could be exposed to more than 125 mg/kg bw per hour by drinking less than the volume of one can (330 mL) of a flavoured drink. The Panel further concluded that the acute bolus exposure to glycerol (E 422) by its use as a food additive should stay below doses at which pharmacological or side effects could occur.

The Panel recommended that:

- given that during the manufacturing processes of glycerol, genotoxic impurities – e.g. glycidol, epichlorohydrin – could be formed, limits for such impurities should be included in the EU specifications for glycerol (E 422);
- given that during the manufacturing processes of glycerol, other potential impurities of toxicological concern (e.g. dichlorohydrin) could be formed, limits for such impurities should be included in the EU specifications for glycerol (E 422);
- more data should be generated to decrease uncertainty arising from the presence in the food additive (E 422) of compounds of toxicological concern (e.g. acrolein, 3-MCPD or 3-MCPD ester), which can be produced under some food processing conditions (e.g. use of glycerol (E 422) in parallel with lactic acid bacteria; use of glycerol (E 422) in food containing significant amounts of sodium chloride (more than 5%) and treated at temperatures above 160°C, etc.);
- a numerical limit for acrolein should be included in the EU specifications for glycerol (E 422);
- the maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for glycerol (E 422) should be revised in order to ensure that glycerol (E 422) as a food additive will not be a significant source of exposure to those toxic elements in food;
- more information on uses and use levels and analytical data should be made available to the Panel in order to perform an adequate exposure assessment, in particular in the case of estimate acute exposure, more data on flavoured drinks is needed.

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1. Introduction

The present opinion deals with the re-evaluation of glycerol (E 422) when used as a food additive.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background

Regulation (EC) No 1333/2008[1] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union (EU). In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the EU before 20 January 2009 has been set up under Regulation (EU) No 257/2010[2]. This Regulation also foresees that food additives are re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee for Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[3] of 2001. The report 'Food additives in Europe 2000[4]' submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with the highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

1.1.2. Terms of Reference

The Commission asks EFSA to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

1.2. Interpretation of the terms of references

This re-evaluation refers exclusively to the uses and conditions of use of glycerol (E 422) as a food additive in food, and does not include a safety assessment of other uses of glycerol as described in Section 3.4.5.

1.3. Information on existing authorisations and evaluations

Glycerol (E 422) is authorised as a food additive in the EU in accordance with Annex II and Annex III of Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012.[5]

[1] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 1–26.
[2] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.
[3] COM(2001) 542 final.
[4] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002, 560.
[5] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.

Glycerol was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1976 (JECFA, 1976). Based on the available toxicological studies and the fact that glycerol occurs naturally in fats and other substances consumed via food, JECFA allocated to glycerol an acceptable daily intake (ADI) 'not specified'.

The Scientific Committee for Food (SCF) evaluated glycerol as a food additive in 1981 and endorsed the conclusion from JECFA (1976) of 'ADI for man not specified' (SCF, 1981). However, a rationale for the conclusion was not provided. The SCF Opinion cited the JECFA evaluation (JECFA, 1976) but no further studies were described.

A request to use glycerol without restriction (*quantum satis* (QS) level) as a sweetener in concentrated juices especially intended for young children was rejected by the SCF in 1999 (SCF, 1999).

Glycerol has also been reviewed by TemaNord (2002). It was concluded that no new data were available indicating a need for a re-evaluation of glycerol. However, it was further concluded that the present permitted *QS* use may lead to the conclusion that all levels are acceptable. In addition, it was concluded that it should be ensured that glycerol is not used as a sugar replacement.

Glycerol has been registered under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation 1907/2006 (ECHA, online).

Glycerol is permitted in cosmetic products in the EU (European Commission database-CosIng[6]).

2. Data and methodologies

2.1. Data

The Panel was not provided with a newly submitted dossier. EFSA launched public calls for data[7,8,9] to collect information from interested parties.

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature up to January 2017. Attempts were made at retrieving relevant original study reports on which previous evaluations or reviews were based, however, not always these were available to the Panel.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database[10]) was used to estimate the dietary exposure.

The Mintel's Global New Products Database (GNPD) is an online resource listing food products and compulsory ingredient information that should be included in labelling. This database was used to verify the use of glycerol (E 422) in food products.

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee.

The ANS Panel assessed the safety of glycerol (E 422) as a food additive in line with the principles laid down in Regulation (EU) 257/2010 and in the relevant guidance documents: Guidance on submission for food additive evaluations by the Scientific Committee on Food (SCF, 2001).

When the test substance was administered in the feed or in the drinking water, but doses were not explicitly reported by the authors as mg/kg body weight (bw) per day based on actual feed or water consumption, the daily intake was calculated by the Panel using the relevant default values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012) for studies in rodents or, in the case of other animal species, by JECFA (2000). In these cases, the daily intake is expressed as 'equivalent'. When in human studies in adults (aged above 18 years), the dose of the test substance administered was reported in mg/person per day, the dose in mg/kg bw per day

[6] Available online: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.simple

[7] Call for scientific data on miscellaneous food additives permitted in the EU and belonging to several functional classes. Published: 9 June 2010. Available online: http://www.efsa.europa.eu/en/dataclosed/call/ans100609

[8] Call for food additives usages level and/or concentration data in food and beverages intended to human consumption. Published: 27 March 2013. Available online: http://www.efsa.europa.eu/en/dataclosed/call/130327

[9] Call for scientific data on selected food additives permitted in the EU- Extended deadline: 1 September 2014 (batch A), 1 November 2014 (batch B) Available online: http://www.efsa.europa.eu/en/dataclosed/call/140324.htm

[10] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

was calculated by the Panel using a body weight of 70 kg as default for the adult population as described in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012).

Dietary exposure to glycerol (E 422) from its use as a food additive, as well as due to natural occurrence, was estimated by combining food consumption data from the EFSA Comprehensive European Food Consumption Database with the maximum permitted levels and/or reported use levels and analytical data submitted to EFSA following a call for data. Different scenarios were used to calculate exposure (see Section 3.4.1). Uncertainties in the exposure assessment were identified and discussed. Acute dietary exposure to glycerol (E 422) per meal was estimated as the exposure via one food category (food categories as defined in legislation) consumed in one meal (acute exposure per food category).

In the context of this re-evaluation, the Panel followed the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EC) No 257/2010 (EFSA ANS Panel, 2014).

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

Glycerol (E 422) is a polyalcohol containing three hydroxyl (–OH) groups with the molecular formula $C_3H_8O_3$. It has a molecular weight of 92.1 g/mol. The CAS Registry Number is 56-81-5. The EINECS number is 200-289-5. Its chemical name is 1,2,3-propanetriol. The structural formula is presented in Figure 1.

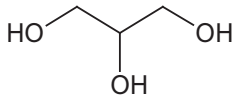

Figure 1: Structural formula of glycerol (E 422)

Physicochemical properties of glycerol are shown in Table 1 (OECD, 2002).

Table 1: Physicochemical properties of glycerol (OECD, 2002)

Physicochemical properties of glycerol	
Melting point (°C)	18
Boiling point (°C, 1,013 hPa)	290
Vapour pressure (hPa)	0.000106 at 25°C (calculated) and 0.0033 at 50°C (measured)
Solubility in water	Miscible
Relative density (20°C)	1.26
Log $P_{o/w}$	−1.76
Dissociation constant	7×10^{-15}
Flash point (°C)	160
Autoflammability (°C)	393
Dynamic viscosity (mPa s; 20°C)	1,410
Surface tension (mN/m; 20°C)	63.4

Glycerol is described as a clear, colourless, hygroscopic syrupy liquid, with not more than a slight characteristic odour, which is neither harsh nor disagreeable (Commission Regulation (EU) No 231/2 012). The substance is miscible in water and ethanol and immiscible in ether (JECFA, 2006).

Glycerol is hygroscopic and absorbs water from air. When contact with strong oxidising agents, such as chromium trioxide, potassium chlorate or potassium permanganate, it may be explosive (Merck Index, 1996).

The most common synonyms are 2-propanol, 1,3-dihydroxy-; 1,2,3-propanetriol; 1,2,3-trihydroxypropane, glycyl alcohol, glycerin or glycerine (SciFinder[11], software).

[11] SciFinder® the choice for chemistry researchTM.

3.1.2. Specifications

The specifications for glycerol (E 422) as defined in the Commission Regulation (EU) No 231/2012 and by JECFA (2006) are listed in Table 2.

Table 2: Specifications for glycerol (E 422) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	**JECFA (2006)**
Assay	Content not less than 98% of glycerol on an anhydrous basis	Not less than 99% on an anhydrous basis
Description	Clear, colourless, hygroscopic syrupy liquid, with not more than a slight characteristic odour, which is neither harsh nor disagreeable	Clear, colourless, hygroscopic, syrupy liquid, having a not more than a slight characteristic odour, which is neither harsh nor disagreeable
Identification		
Acrolein formation on heating	Heat a few drops of the sample in a test tube with about 0.5 g of potassium bisulfate. The characteristic pungent vapours of acrolein are evolved	–
Specific gravity (25/25 °C)	Not less than 1.257	–
Refractive index $[n]_D^{20}$	Between 1.471 and 1.474	–
Solubility	–	Miscible with water and with ethanol; immiscible with ether
Test for glycerol	–	Passes test
Purity		
Water	Not more than 5% (Karl Fischer method)	Not more than 5% (Karl Fischer method)
Sulfated ash	Not more than 0.01% determined at 800 ± 25°C	Not more than 0.01%
Butanetriols	Not more than 0.2%	Not more than 0.2%
Acrolein, glucose and ammonium compounds	Heat a mixture of 5 mL of glycerol and 5 mL of potassium hydroxide solution (1 in 10) at 60°C for 5 min. It neither becomes yellow nor emits an odour of ammonia	–
Fatty acids and esters	Not more than 0.1% calculated as butyric acid	Not more than 30 mg/kg
Chlorinated compounds	Not more than 30 mg/kg (as chlorine)	Not more than 30 mg/kg (as chloride ion)
3-monochloropropane-1,2-diol (3-MCPD))	Not more than 0.1 mg/kg	
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–
Chlorides	–	Not more than 10 mg/kg
Colour	–	The colour of the sample, when viewed downward against a white surface in a 50-mL Nessler tube, is not darker than the colour of a standard made by diluting 0.4 mL of ferric chloride TSC with water to 50 mL and similarly viewed in a Nessler tube of approximately the same diameter and colour as that containing the sample

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Readily carbonisable substances	–	Rinse a glass-stoppered, 25-mL cylinder with sulfuric acid TS, and allow to drain for 10 min. Add 5 mL of the sample and 5 mL of sulfuric acid TS, shake vigorously for 1 min, and allow to stand for 1 h. The mixture is no darker than *Matching Fluid H*

The Panel noted that EU specification for glycerol content is not less than 98% on an anhydrous basis whereas JECFA specifies content not less than 99%.

The Panel noted that in the EU specifications for glycerol (E 422) there is no specific numerical limit for acrolein and that acrolein is classified as a substance of acute toxicity 2 when swallowed, according to Regulation (EC) No 1272/2008[12] (Index No. 605-008-00-3).

The Panel noted that, while the EU specifications have a limit for chlorinated compounds, the identities of specific chlorinated compounds are not indicated.

The Panel noted that, according to the EU specifications for glycerol (E 422), impurities of the toxic elements lead, mercury, arsenic and cadmium are accepted up to a concentration of 2, 1, 3 and 1 mg/kg, respectively. Contamination at those levels would have a significant impact on the exposure to these metals, for which the exposures are already close to the health-based guidance values established by EFSA (EFSA CONTAM Panel, 2009a,b, 2010a, 2012).

The Panel also noted that a limit of 0.1 mg/kg for 3-monochloropropane-1,2-diol (3-MCPD), a compound of toxicological concern, is defined in the EU specifications for glycerol (E 422). A tolerable daily intake (TDI) of 0.8 μg/kg bw per day for 3-MCPD has been derived by the EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel, 2016)[13].

3.1.3. Manufacturing process

3.1.3.1. Glycerol produced from fats and oils

According to Christoph et al. (2006), glycerol is mainly produced from fats and oils by high pressure splitting. It is obtained by feeding water and fat in counter current into a splitting column at 2–6 MPa and 220–260°C. This glycerol is marketed as 88% saponification- or hydrolysis-crude glycerol (Christoph et al., 2006).

The production of glycerol after saponification (treatment with caustic alkali or alkali carbonates) of neutral fats and oils is only partially applied. The final product is obtained from crude glycerol by purification via distillation or ion exchange chromatography (Christoph et al., 2006).

According to CEFIC (2013a [Documentation provided to EFSA n. 3]), 'vegetal' glycerol can be produced from vegetable oils after a treatment at a temperature higher than 100°C at low pressure, followed by distillation and further purification. The Panel noted that refined oils and fats may be contaminated with glycidol (EFSA CONTAM Panel, 2016). Glycidol is characterised as probably carcinogenic to humans 2A (IARC, 2000; BfR, 2009) and as a carcinogenic and genotoxic compound by the EFSA CONTAM Panel (EFSA CONTAM Panel, 2016).

3.1.3.2. Glycerol produced by chemical synthesis

Glycerol can be produced from propene (Christoph et al., 2006). Three pathways are known: (i) via the conversion of propene to acrolein and allyl alcohol; (ii) via the conversion of propene to propylene oxide and allyl alcohol; and (iii) via the conversion of propene to allyl chloride and epichlorohydrin.

In the pathway via acrolein, propene is oxidised to acrolein, which is then reduced to allyl alcohol. Allyl alcohol is then epoxidised with hydrogen peroxide to glycidol, which is further hydrolysed to glycerol.

[12] Regulation (EC) No 1972/2008 of the European Parliament and the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.

[13] The Panel is aware that the EFSA CONTAM Panel agreed at its 82nd plenary meeting to reopen its Scientific Opinion on the risks for human health related to the presence of 3- and 2-MCPD, and their fatty acid esters, and glycidyl fatty acid esters (EFSA-Q-2014-00535) to address the identified scientific divergence with the JECFA opinion. EFSA CONTAM Panel minutes available online: http://www.efsa.europa.eu/sites/default/files/event/170124-m_0.pdf

In the pathway via propylene oxide, propene is epoxidised to propylene oxide, which is then isomerised to allyl alcohol. In a second step, epoxidation is carried out to form glycidol, which is hydrolysed to glycerol.

In the pathway via allyl chloride, propene is oxidised with hypochlorite to dichlorohydrin, which, in the presence of calcium hydroxide or sodium hydroxide, is converted to epichlorohydrin. Epichlorohydrin is then hydrolysed to glycerol at 80–200°C with an aqueous solution of sodium hydroxide or sodium carbonate.

The Panel noted that in the synthesis pathway of glycerol via either acrolein or via propylene oxide, glycidol is produced as an intermediate, which may be present in the final product.

The Panel also noted that in the synthesis pathway of glycerol via allyl chloride, epichlorohydrin is formed as an intermediate, which may be present in the final product. Epichlorohydrin is classified as carcinogen 2A according to the International Agency for Research on Cancer (IARC, 1999).

The Panel further noted that there are no limits in the specifications for other starting or intermediate chemicals used in the synthesis of glycerol, including glycidol, propene, allyl alcohol, propylene oxide, allyl chloride and dichlorohydrin, and no numerical limit in the specifications for acrolein.

3.1.3.3. Glycerol produced by microbial fermentation

The Panel noted that processes are described for glycerol production via microbial fermentation using either yeasts (*Saccharomyces cerevisiae* or osmotolerant yeasts), bacteria (*Lactobacillus lycopersici* and *Bacillus subtilis*) or algae (*Dunaliella tertiolecta* and *Dunaliella bardawil*) (Wang et al., 2001). However, the Panel has no indication if these processes are used on an industrial level.

Schier et al. (2009) presented a literature survey of potential contaminants/intermediates resulting from the manufacturing or purification processes of glycerol. The authors indicated that when, e.g. glycerol is produced via hydrogenolysis of carbohydrates, ethylene glycol can be present as a contaminant. It is also indicated that when glycerol is produced via microbial fermentation, dioxane and aniline can be present as contaminants. All these contaminants are substances of toxicological concern.

Overall, the Panel noted that in addition to the information provided by CEFIC (2013), glycerol (E 422) can be produced by a variety of methods and that many of them lead to the presence or formation of contaminants, which are of toxicological concern. This should be considered in the EU specifications for glycerol (E 422). The Panel considered that the manufacturing process for glycerol (E 422) should not allow the production of a food additive which contains residuals of genotoxic or/and carcinogenic concern at a level which would result in a margin of exposure (MOE) below 10,000.

3.1.4. Methods of analysis in food

Different methods for the analysis of glycerol are available in the scientific literature.

A gas chromatography (GC) method for the determination of alcohols, phenols, glycols (including glycerol) and sugars has been published. Acetylation followed by GC/flame ionisation detection (FID) analysis is used (Wachowiak and Connors, 1979). No information is given on recovery rates or limit of detection (LOD).

Glycerol may be measured in wine vinegar, wine, and vinegar directly without any sample preparation by high-performance liquid chromatography (HPLC) using an anion exclusion column (Dewaele et al., 1991). There are two AOAC Official Methods (AOAC, 2000) for the qualitative and quantitative analysis of glycerol in eggs. Various procedures for the determination of glycerol in wine are based on enzymatic and analytical techniques, such as spectrophotometry, fluorimetry, amperometry, chemiluminescence and potentiometry, are cited by Fernandes et al. (2004), who have proposed an enzymatic method coupled with a colorimetric measurement which can be applied on flow during wine manufacturing. The Panel noted that glycerol (E 422) as a food additive is not authorised for those uses, but in wine it may be present as a result of the activity of the yeast.

Nuclear magnetic resonance (NMR) spectroscopy has been employed by Scano et al. (2012) to determine glycerol content in processed mullet (*Mugil cephalus*) roes but no information is given related to the LOD or recovery rates. According to de Souza et al. (2013), the glycerol content was analysed in various beverages: sugar cane liquor, grape juices, grape soda, cola soda and wine and three enzymatic kits combined with amperometric and/or colorimetric analysis were tested. The Panel noted that out of those samples tested glycerol (E 422) as a food additive is authorised only in sugar cane liquor and in soda drinks. Recovery rates were in accordance with internationally accepted performance criteria (Bravenboer et al., 1995) and the LOD was 1 μmol/L equal to 92.1 μg/L.

Amperometric polyenzymatic biosensors based on gold planar or nanocomposite electrode containing multiwalled carbon nanotubes for the determination of glycerol have been developed (Monosik et al., 2012). The biosensors were tested in wine samples only. Ion chromatography (IC) coupled with isotope ratio mass spectrometry (IRMS) using a liquid interface/chemical oxidation of organic matter has been used to quantify solutions containing glycerol in mixtures with carbohydrates and organic acids but the only real sample tested was red wine (Guyon et al., 2013). The Panel noted that glycerol (E 422) as a food additive is not authorised in wine.

Straadt et al. (2014) studied the levels of metabolites – including glycerol – in fresh porcine meat by using NMR spectroscopy. The Panel noted that the use of glycerol (E 422) as a food additive is not authorised in fresh meat.

An HPLC method for the analysis of glycerol in an alcoholic beverage (spirit) coming from ginger is described by Leonel et al. (2015). No information is given concerning performance criteria of the method.

A method for glycerol quantification in sugarcane spirits (cachaças) has been reported. The method is based on sample derivatisation using benzoyl chloride followed by solid-phase extraction and HPLC with diode array detection (Garcia et al., 2015). The reported LOD was 0.25 mg/L.

3.1.5. Stability of the substance and reaction and fate in food

Glycerol is highly stable under ordinary conditions of storage and use, remaining free from objectionable colour, odour or taste with the passage of time. Glycerol solutions subject to heat should not be processed or stored in iron- or copper-containing vessels since iron and copper salts will catalyse the oxidation of glycerol under such conditions (SDA, 1990).

According to industry, glycerol is stable at normal storage and use temperatures. However, temperatures above 54°C can result in decomposition. Decomposition products can include, but are not limited to, acrolein. Moisture and strong oxidisers should be avoided (Dow, online).

According to Leonel et al. (2015), lactic acid bacteria can metabolise glycerol to produce acrolein. Lactic acid bacteria can be used in manufacturing of yogurt, cheese, cultured butter, sour cream (with fat content above 20%), sausage, cucumber pickles, olives and sauerkraut; if glycerol (E 422) is added to those food items, acrolein may be formed. In the absence of data on the amount of acrolein formed, the Panel considered that more data should be generated to decrease uncertainty and allow for a risk assessment.

According to the EFSA CONTAM (2016) opinion, 'In model food heating systems containing water, sodium chloride and glycerol or lipid precursors 3-MCPD production increases with increasing temperature once above 160°C, and with NaCl concentration up to 10% with acylglycerol precursors but at about 5% NaCl with glycerol. The optimum water content is 15–20% for 3-MCPD' and 'Baked goods are the major source of 3- and 2-MCPD and the formation of these contaminants in model bakery systems has been studied in some detail in model systems (Hamlet et al., 2003, 2004a,b). Free glycerol produced by the action of yeast enzymes is the major precursor and the formation reactions of 3- and 2-MCPD follow zero-order kinetics. The levels of 3-MCPD and 2-MCPD formed increase exponentially with temperature up to the maximum (about 220°C) used in baking'. Therefore, the Panel considered that glycerol (E 422) should not be added in food containing significant amounts of salt (more than 5%) treated at temperatures above 160°C, as 3-MCPD or 3-MCPD esters can be formed.

3.2. Authorised uses and use levels

Maximum levels of glycerol (E 422) have been defined in Annex II to Regulation (EC) No 1333/2008[14] on food additives, as amended. In this opinion, these levels are named maximum permitted levels (MPLs).

Glycerol (E 422) is included in the Group I of food additives authorised at QS. Overall, glycerol (E 422) is an authorised food additive in the EU at QS in 68 food categories.

Table 3 summarises foods that are permitted to contain glycerol (E 422) at QS as set by Annex II to Regulation (EC) No 1333/2008.

[14] Commission Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.

Table 3: Foods in which glycerol (E 422) is authorised according to the Annex II to Regulation (EC) No 1333/2008

Food Category number	Food category name	E–Number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
01.3	Unflavoured fermented milk products, heat-treated after fermentation	Group I		QS
01.4	Flavoured fermented milk products including heat-treated products	Group I		QS
01.6.3	Other creams	Group I		QS
01.7.1	Unripened cheese excluding products falling in category 16	Group I	Except mozzarella	QS
01.7.5	Processed cheese	Group I		QS
01.7.6	Cheese products (excluding products falling in category 16)	Group I		QS
01.8	Dairy analogues including beverage whiteners	Group I		QS
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	Group I		QS
02.3	Vegetable oil pan spray	Group I		QS
03	Edible ices	Group I		QS
04.2.1	Dried fruit and vegetables	Group I		QS
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Group I		QS
04.2.4.1	Fruit and vegetable preparations excluding compote	Group I		QS
04.2.5.4	Nut butters and nut spreads	Group I		QS
04.2.6	Processed potato products	Group I		QS
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	E 422		QS
05.2	Other confectionery including breath refreshening microsweets	Group I		QS
05.3	Chewing gum	Group I		QS
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Group I		QS
06.2.2	Starches	Group I		QS
06.3	Breakfast cereals	Group I		QS
06.4.2	Dry pasta	Group I	Only gluten-free and/or pasta intended for hypoproteic diets in accordance with Directive 2009/39/EC	QS
06.4.4	Potato Gnocchi	Group I	Except fresh refrigerated potato gnocchi	QS
06.4.5	Fillings of stuffed pasta (ravioli and similar)	Group I		QS
06.5	Noodles	Group I		QS
06.6	Batters	Group I		QS
06.7	Pre-cooked or processed cereals	Group I		QS
07.1	Bread and rolls	Group I	Except products in 7.1.1 and 7.1.2	QS
07.2	Fine bakery wares	Group I		QS

Food Category number	Food category name	E–Number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
08.3.1	Non-heat-treated meat products	Group I		QS
08.3.2	Heat–treated meat products	Group I	Except *foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben*	QS
08.3.3	Casings and coatings and decorations for meat	Group I		QS
09.2	Processed fish and fishery products including molluscs and crustaceans	Group I		QS
09.3	Fish roe	Group I	Only processed fish roe	QS
10.2	Processed eggs and egg products	Group I		QS
11.2	Other sugars and syrups	Group I		QS
11.4.1	Table Top Sweeteners in liquid form	E 422		QS
12.1.2	Salt substitutes	Group I		QS
12.2.2	Seasonings and condiments	Group I		QS
12.3	Vinegars	Group I		QS
12.4	Mustard	Group I		QS
12.5	Soups and broth	Group I		QS
12.6	Sauces	Group I		QS
12.7	Salads and savoury based sandwich spreads	Group I		QS
12.8	Yeast and yeast products	Group I		QS
12.9	Protein products excluding products covered in category 1.8	Group I		QS
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/ EC (excluding products from food category 13.1.5)	Group I		QS
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	Group I		QS
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Group I	Including dry pasta	QS
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices	Group I	Only vegetable juices	QS
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	Group I	Only vegetable nectars	QS
14.1.4	Flavoured drinks	Group I		QS
14.1.5.2	Other	Group I	Excluding unflavoured leaf tea; including flavoured instant coffee	QS
14.2.3	Cider and perry	Group I		QS
14.2.4	Fruit wine and made wine	Group I		QS
14.2.5	Mead	Group I		QS
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Group I	Except whisky or whiskey	QS
14.2.7.1	Aromatised wines	Group I		QS
14.2.7.2	Aromatised wine-based drinks	Group I		QS
14.2.7.3	Aromatised wine-product cocktails	Group I		QS

Food Category number	Food category name	E–Number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	Group I		QS
15.1	Potato-, cereal-, flour- or starch-based snacks	Group I		QS
15.2	Processed nuts	Group I		QS
16	Desserts excluding products covered in category 1, 3 and 4	Group I		QS
17.1(a)	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	Group I		QS
17.2(a)	Food supplements supplied in a liquid form	Group I		QS
17.3(a)	Food supplements supplied in a syrup-type or chewable form	Group I		QS
18	Processed foods not covered by categories 1–17, excluding foods for infants and young children	Group I		QS

MPL: maximum permitted level; QS: *quantum satis*.
(a): FCS 17 refers to food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council excluding food supplements for infants and young children.

According to Annex III, Part 1 of Regulation (EC) No 1333/2008, glycerol (E 422) is also authorised as a carrier in all food additives at QS.

According to Annex III, Part 2 of Regulation (EC) No 1333/2008, glycerol (E 422) is also authorised as a food additive other than a carrier in all food additives at QS.

In addition, according to Annex III, Part 3 of Regulation (EC) No 1333/2008, glycerol (E 422) is authorised as a food additive including as a carrier in food enzymes with a maximum level in enzyme preparation and in final food (beverages or not) at QS.

According to Annex III, Part 4 of Regulation (EC) No 1333/2008, glycerol (E 422) is also authorised as a food additive including carriers in all food flavourings at QS.

Finally, according to Annex III, Part 5, Section A, of Regulation (EC) No 1333/2008, glycerol (E 422) is authorised as a food additive in all nutrients at QS.

3.3. Exposure data

3.3.1. Reported use levels or data on analytical levels of glycerol (E 422)

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment, especially for those food additives for which no MPL is set and which are authorised according to QS, as is the case for glycerol (E 422) (Table 3).

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued public calls[15,16] for occurrence data (usage level and/or concentration data) on glycerol (E 422). In response to these calls, both types of data were submitted to EFSA by industry and Member States, respectively.

[15] http://www.efsa.europa.eu/sites/default/files/consultation/ans15062010%2C0.pdf
[16] http://www.efsa.europa.eu/sites/default/files/consultation/130327%2C0.pdf

3.3.1.1. Summarised data on reported use levels in foods provided by industry

Industry provided EFSA with data on use levels (n = 91) of glycerol (E 422) in foods for 20 out of the 68 food categories in which glycerol (E 422) is authorised.

These data were provided by FoodDrinkEurope (FDE), the International Chewing Gum Association (ICGA), the Association of the European Self-Medication Industry (AESPG), Cutisin, Nutricia and Riemser Arzneimittel AG. Data were mainly reported for edible ices (FCS 03).

The Panel noted that the use levels of glycerol (E 422) reported by the food industry were high in some foods (> 50,000 mg/kg in dietary supplements, chewing gums and fine bakery wares) and varied largely between food categories: from < 0.01 mg/kg in breakfast cereals to 100,000 mg/kg in chewing gums.

Appendix A provides data on the use levels of glycerol (E 422) in foods as reported by industry.

3.3.1.2. Summarised data on concentration levels in food submitted by Member States

In total, 7,745 analytical results were reported to EFSA by one country, Germany. These data were mainly for wine and other products defined by the Council Regulation (EC) No 1234/2007[17] (FCS 14.2.2). Foods were sampled between 2000 and 2013, and all of them were analysed in the year of collection.

Almost 98% of the analytical results on glycerol (E 422) were quantified. In 72 samples, the results were not quantified (< limit of quantification (LOQ)) and in 89 samples, the results were reported as not detected (< LOD).

Complete information on the methods of analysis (e.g. validation) was not made available to EFSA, but all samples were derived from accredited laboratories.

In order to include only data over the last 10-year period, analytical results sampled before 2004 (n = 522) were excluded from further analyses.

In total, 1,133 analytical results reported for glycerol (E 422) in foods were taken into account by the Panel in the exposure assessment considering only direct addition of glycerol (E 422) to food according to Annex II of Regulation (EC) No 1333/2008. These data covered 14 out of 68 food categories in which glycerol (E 422) is authorised as a food additive according to Annex II to Regulation No 1333/2008.

The Panel noted that the remaining 6,090 analytical results were reported in food categories in which glycerol (E 422) is not authorised for direct addition according to Annex II of Regulation (EC) No 1333/2008, including: 'Honey as defined in Directive 2001/110/EC[18]' (FCS 11.3) and 'wine and other products defined by Regulation (EC) No 1234/2007 and alcohol-free counterparts' (FCS 14.2.2). It should be noted that glycerol (E 422) was quantified in almost all samples of these two food categories. The authorised use of glycerol (E 422) according to Annex III to Regulation (EC) No 1333/2008 (Parts 1, 2, 3, 4 and 5, Section A) may have resulted in carry-over and its detection in the wine food category. Natural fermentation could also result in the presence of glycerol in wine and other alcoholic beverages (VCF, 1963-2016), as well as in honey (Huidobro et al., 1993). Therefore, the analytical results in these food categories (honey (FCS 11.3), wine (FCS 14.2.2), other alcoholic beverages (FCS 14.2.3, 14.2.4, 14.2.5, 14.2.7.1, 14.2.7.2, 14.2.7.3, 14.2.8)) were not considered in the exposure assessment of glycerol (E 422) as a food additive according to Annex II, but included in the overall estimation of the exposure to glycerol (E 422) according to Annex II, via carry-over (Annex III) and natural sources.

Appendix B shows the analytical results of glycerol (E 422) in foods as reported by Member States.

When both concentration levels, i.e. reported use and analytical levels, were available (only for a few food categories), they were comparable (Appendices A and B).

3.3.2. Summarised data extracted from the Mintel's Global New Products Database

The Mintel GNPD is an online database which monitors product introductions in consumer packaged goods markets worldwide. It contains information of over 2 million food and beverage products of which more than 800,000 are or have been available on the EU food market. Mintel started covering

[17] Council Regulation (EC) No 1234/2007 of 22 October 2007 establishing a common organisation of agricultural markets and on specific provisions for certain agricultural products (Single CMO Regulation). OJ L 299, 16.11.2007, p. 1–149.

[18] Council Directive 2001/110/EC of 20 December 2001 relating to honey. OJ L 10, 12.1.2002, p. 1–6.

EU's food markets in 1996, currently having 20 out of its 28 member countries and Norway presented in the Mintel GNPD.[19]

For the purpose of this Scientific Opinion, the Mintel GNPD[20] was used for checking the labelling of products containing glycerol (E 422) within the European's food products as the Mintel GNPD shows the compulsory ingredient information presented on food product labels.

According to the Mintel GNPD, glycerol (E 422) was labelled on more than 10,000 food products between 2011 and 2016, with an increasing number of food items during the last 3 years.

Appendix C presents the percentage of the food products labelled with glycerol (E 422) between 2011 and 2016, out of the total number of food products per food subcategory according to the Mintel GNPD food classification.

3.3.3. Food consumption data used for exposure assessment

3.3.3.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a)). New consumption surveys recently[21] added in the Comprehensive database were also taken into account in this assessment.[10]

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 4).

Table 4: Population groups considered for the exposure estimates of glycerol (E 422)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks up to and including 11 month of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 month up to and including 35 month of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, the Netherlands, Spain, UK
Children[(a)]	From 36 month up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

[19] Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.
[20] http://www.gnpd.com/sinatra/home/ accessed on 26/10/2016.
[21] Available online: http://www.efsa.europa.eu/en/press/news/150428.htm

Consumption records in the EFSA Comprehensive Database were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the food categorisation system (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, FoodEx food codes were matched to the FCS food categories.

3.3.3.2. Food categories considered for the exposure assessment of glycerol (E 422)

The food categories in which the use of glycerol (E 422) is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

Some food categories or their restrictions/exceptions are not referenced in the EFSA Comprehensive Database and could therefore not be taken into account in the present estimate. This was the case for 10 food categories (Appendix D) and may have resulted in an underestimation of the exposure. The food categories which were not taken into account for this reason are described below (in ascending order of the FCS codes):

- 01.6.3 Other creams
- 02.3 Vegetable oil pan spray
- 05.4 Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4
- 06.6 Batters
- 06.7 Pre-cooked or processed cereals
- 08.3.3 Casings and coatings and decorations for meat
- 12.1.2 Salt substitutes
- 14.1.3 Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products, only vegetable nectars
- 14.2.4 Fruit wine and made wine
- 14.2.5 Mead

For the following food categories, the restrictions/exceptions which apply to the use of glycerol (E 422) could not be taken into account, and therefore, the whole food category was considered in the exposure assessment. This applies to 2 food categories and may have resulted in an overestimation of the exposure.

- 07.1 Bread and rolls, except products in 07.1.1 and 07.1.2
- 08.3.2 Heat-treated meat products, except *foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben*

In the EFSA Comprehensive database, no information is provided on the type of food supplements consumed by infants and young children. In the exposure assessment, even if this food category refers in the EU regulation to foods supplements excluding infants and young children, it was in this opinion therefore assumed that the food supplements consumed in these population groups were the same as those consumed in the older population groups for which concentration data were supplied, resulting in an overestimation of the exposure to glycerol (E 422) in these two population groups.

Furthermore, 30 food categories were not taken into account because no concentration data (reported use levels or analytical data) were provided to EFSA. For the remaining food categories, the refinements considering the restrictions/exceptions as set in Annex II to Regulation No 1333/2008 were applied.

Overall, 28 food categories out of 68 were included in the present exposure assessment to glycerol (E 422) (Appendix D).

3.4. Exposure estimate

3.4.1. Exposure to glycerol (E 422) from its use as a food additive

The Panel estimated chronic exposure to glycerol (E 422) for the following population groups: infants; toddlers, children, adolescents, adults and the elderly. Dietary exposure to glycerol (E 422) was calculated by multiplying glycerol (E 422) concentrations for each food category (Appendix D) with their respective consumption amount per kilogram body weight for each individual in the EFSA Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with

only 1 day per subject were excluded as they are considered as not adequate to assess repeated exposure.

The exposure was estimated for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 4). On the basis of these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. The 95th percentile of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a). Therefore, in the present assessment, the 95th percentile of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not estimated.

It should be noted that, in two dietary surveys from Finland, namely DIPP_2001_2009 and NWSSP07-08, the consumption of grain-based products including bread and fine bakery products was coded at the level of their ingredients (flour), which resulted in a very low exposure to glycerol in all Finnish populations compared with the other studies. Therefore, these two studies were excluded from the assessment.

Exposure assessment to glycerol (E 422) was carried out by the ANS Panel based on (1) maximum levels of data provided to EFSA (defined as the *maximum level exposure assessment scenario*) and (2) the reported use levels or analytical data (defined as the *refined exposure assessment scenario*). These two scenarios are discussed in detail below. Furthermore, considering that

- glycerol (E 422) is authorised in alcoholic beverages (several categories under FCS 14.2) but no use data were submitted by industry for this food category, and
- the presence of glycerol in alcoholic beverages can also be due to natural fermentation and carry-over,

the analytical data of glycerol in alcoholic beverages submitted to EFSA were considered as resulting from natural fermentation and carry-over and not from usage by food industry. The presence of glycerol in honey was considered to be solely derived from fermentation. Therefore, for glycerol (E 422), dietary exposure according to the *refined exposure assessment scenario* was assessed using two sets of concentration data:

1) Reported use levels and analytical data considering food categories for which direct addition of glycerol (E 422) is authorised according to Annex II to Regulation No 1333/2008, excluding alcoholic beverages (FCS 14.2) (data set 1).
2) Reported use levels and analytical data considering food categories for which direct addition of glycerol (E 422) is authorised according to Annex II to Regulation No 1333/2008, and analytical data for food categories which may contain glycerol due to carry-over (Annex III) and from natural sources (i.e. honey, wines and other alcoholic beverages) for which data were available (data set 2). Results using this data set are presented in Appendices F and G.

The exposure scenarios can consider only food categories for which concentration data were available to the Panel.

3.4.1.1. Maximum level exposure assessment scenario

The *regulatory maximum level exposure assessment scenario* is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008. As glycerol (E 422) is authorised according to QS in all food categories, a *maximum level exposure assessment scenario* was performed based on the maximum reported use levels provided by industry or high level of analytical data provided by MSs, as described in the EFSA Conceptual framework (EFSA ANS Panel, 2014), whichever was highest or available. The same food categories as included in data set 1 were considered for this scenario.

The Panel considers the exposure estimates derived following this scenario as the most conservative for the food categories taken into account, since it is assumed that the consumer will be continuously (over a long period of time) exposed to glycerol (E 422) in foods belonging to these food categories at maximum reported use levels/high level of analytical data.

3.4.1.2. Refined exposure assessment scenario

The *refined exposure assessment scenario* is based on use levels reported by industry and analytical results reported by Member States. This exposure scenario can consider only food categories for which these data were available to the Panel.

Appendix D summarises the concentration levels of glycerol (E 422) used in the refined exposure assessment scenario. For the two concentration data sets, the Panel calculated two refined exposure

estimates based on different model populations. In these two refined exposure assessment scenarios, the concentration levels considered were extracted from the whole data available to EFSA (i.e. reported use levels and analytical results):

- The *brand-loyal consumer scenario*: It was assumed that a consumer is exposed long-term to glycerol (E 422) present at the maximum reported use/analytical level for one food category. This exposure estimate is calculated as follows:
  - Combining food consumption with the maximum of the reported use levels or the 95th percentile of the analytical results, whichever was highest or available, for the main contributing food category at the individual level
  - Using the mean of the typical reported use levels or the mean of analytical results, whichever was highest or available, for the remaining food categories
- The *non-brand-loyal consumer scenario*: It was assumed that a consumer is exposed long-term to glycerol (E 422) present at the mean reported use/analytical levels in food. This exposure estimate is calculated using the mean of the typical reported use levels or the mean of analytical results, whichever was highest or available, for all food categories.

If both usage and analytical data were available for the same food category, the most reliable value under consideration was used. For glycerol (E 422), the Panel considered due to a low sample size of the analytical data, that the reported use levels were more reliable. However, the Panel also noted that the levels were comparable for both sources in the respective food categories.

To consider left-censored analytical data (i.e. analytical results < LOD or < LOQ), the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO, 2009) and the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010) was used. In the present opinion, analytical data below LOD or LOQ were assigned half of LOD or LOQ, respectively (medium bound (MB)). Subsequently, per food category, the mean or median, whichever was highest, MB concentration was calculated.

3.4.1.3. Dietary exposure to glycerol (E 422)

Table 5 summarises the exposure to glycerol (E 422) from its use as a food additive according to Annex II to Regulation (EC) No 1333/2008 in six population groups (Table 4) according to the different exposure scenarios. Detailed results per population group and survey are presented in Appendix E.

Table 5: Summary of exposure to glycerol (E 422) from its use as a food additive considering food categories for which direct addition of glycerol is authorised (from Annex II to Regulation No 1333/2008) excluding alcoholic beverages (under FCS 14.2) (data set 1) in the maximum level exposure assessment scenario and in the refined exposure scenarios, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks–11 month)	Toddlers (12–35 month)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Maximum level exposure assessment scenario						
Mean	10–138	158–583	239–480	128–326	68–185	76–138
95th percentile	269–457	484–938	489–910	283–629	161–404	168–268
Refined estimated exposure assessment scenario (data set 1)						
Brand-loyal scenario						
Mean	9–126	138–453	199–369	101–248	57–144	65–119
95th percentile	223–435	400–796	420–786	217–500	136–320	136–265
Non-brand-loyal scenario						
Mean	6–100	89–308	112–240	74–162	50–100	53–77
95th percentile	153–266	261–418	222–460	149–298	110–197	98–147

In the *maximum level exposure assessment scenario*, mean exposure to glycerol (E 422) ranged from 10 mg/kg bw per day in infants to 583 mg/kg bw per day in toddlers. The 95th percentile of exposure ranged from 161 mg/kg bw per day in adults to 938 mg/kg bw per day in toddlers.

In the *refined brand-loyal exposure scenario* considering data set 1, the mean exposure to glycerol (E 422) ranged from 9 mg/kg bw per day in infants to 453 mg/kg bw per day in toddlers, and the 95th percentile from 136 mg/kg bw per day in adults and the elderly to 796 mg/kg bw per day in toddlers. In the *non-brand-loyal scenario*, the mean exposure to glycerol (E 422) ranged from 6 mg/kg bw per day in infants to 308 mg/kg bw per day in toddlers, and the 95th percentile from 98 mg/kg bw per day in the elderly to 460 mg/kg bw per day in children.

The Panel noted that the exposure to glycerol (E 422) using data set 2 (Appendix F), both *brand-loyal* and *non-brand-loyal scenarios,* did not significantly change the exposure to glycerol (E 422) using data set 1 (Table 5).

The Panel also noted that only one, rather high, use level (30,000 mg/kg) was reported by industry for food category 07.1 'bread and rolls' (Appendix A) (no analytical data were reported for this food category). This level was for a niche product ('chapattis'), i.e. a not commonly eaten food item. This use level was applied to the whole food category, which will result in a significant increase in the exposure estimates (between 1.7 and up to 19 times more depending on the population groups at the mean for the *non-brand-loyal scenario*), especially since the consumption of food category 07.1 'bread and rolls' is fairly high in many surveys.

3.4.1.4. Main food categories contributing to exposure to glycerol (E 422)

Maximum level exposure assessment scenario (Table 6)

Table 6: Main food categories contributing to the exposure to glycerol (E 422) from its use as a food additive considering food categories for which direct addition of glycerol is authorised (from Annex II to Regulation No 1333/2008) excluding alcoholic beverages (under FCS 14.2) (data set 1) using maximum usage levels (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[(a)]					
03	Edible ices	–	14.7–14.7 (1)	5.6–13.2 (3)	5.1–5.1 (1)	–	–
05.2	Other confectionery including breath refreshening microsweets	–	–	34.6–34.6 (1)	5.9–39.6 (2)	8.3–8.3 (1)	–
07.1	Bread and rolls	27.7–85.1 (5)	9.4–68.7 (10)	17.7–51 (17)	22–44.8 (16)	27.1–64.1 (17)	31–74 (14)
07.2	Fine bakery wares	7.9–46.4 (4)	7.1–50.2 (10)	5.1–58.4 (17)	19.8–49.1 (15)	6.5–39.5 (17)	9.3–44 (14)
08.3	Meat products	7.2–7.2 (1)	–	–	–	–	–
12.6	Sauces	5.2–31.4 (4)	6.5–14.3 (7)	5.4–14 (12)	7.4–14.4 (10)	9–17.5 (10)	6.5–16.5 (10)
14.1.4	Flavoured drinks	10.2–26.9 (2)	7.1–35.7 (8)	5.7–36.4 (18)	11.8–38.3 (17)	6.3–30.6 (17)	6.5–15.6 (8)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	38.5–73.8 (2)	28.7–28.7 (1)	10.2–10.2 (1)	–	5.6–8.2 (3)	8–9.8 (2)

–: Food categories not contributing or contributing less than 5% to the total mean exposure.

(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific population.

Refined exposure assessment scenario using data set 1 (Tables 7 and 8)

Table 7: Main food categories contributing to the exposure to glycerol (E 422) from its use as a food additive considering food categories for which direct addition of glycerol is authorised (from Annex II to Regulation No 1333/2008) excluding alcoholic beverages (under FCS 14.2) (data set 1) using *the brand-loyal refined exposure scenario* (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
03	Edible ices	–	14.0 (1)	8.8 (1)	–	–	–
05.2	Other confectionery including breath refreshening microsweets	–	–	41.2 (1)	45.9 (1)	8.2 (1)	–
07.1	Bread and rolls	30.4–93.0 (5)	11.0–84.7 (10)	22.6–66.7 (17)	29.1–55.7 (16)	33.9–73.9 (17)	37.3–86.4 (14)
07.2	Fine bakery wares	19.4–42.8 (3)	14.1–51.5 (9)	16.9–62.2 (16)	14.8–53.6 (15)	5.5–39.2 (16)	10.7–43.4 (12)
08.3	Meat products	7.7 (1)	5.6 (1)	–	–	–	–
12.6	Sauces	14.4–30.9 (3)	5.9–11.7 (3)	5.0–11.6 (7)	5.2–10.6 (9)	6.8–14.9 (9)	5.8–13.2 (7)
14.1.4	Flavoured drinks	8.4–29.4 (2)	6.5–40.3 (7)	7.8–40.8 (16)	8.6–43.2 (17)	5.1–32.7 (16)	6.5–10.5 (7)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	36.2–76.1 (2)	27.6 (1)	9.2 (1)	–	5.3–10.1 (3)	6.0–9.7 (2)

–: Food categories not contributing or contributing less than 5% to the total mean exposure.
(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific population.

Table 8: Main food categories contributing to exposure to glycerol (E 422) from its use as food additive considering food categories for which direct addition of glycerol is authorised (from Annex II to Regulation No 1333/2008) excluding alcoholic beverages (under FCS 14.2)) (data set 1) using *the non-brand-loyal refined exposure scenario* (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
05.2	Other confectionery including breath refreshening microsweets	–	–	36.8 (1)	41.1 (1)	5.9 (1)	–
07.1	Bread and rolls	47.9–94.7 (5)	23.3–86.3 (10)	6.3–74.9 (18)	45.6–74.6 (16)	51.3–83.6 (17)	60.2–89.6 (14)
07.2	Fine bakery wares	8.7–16.7 (3)	8.9–20.3 (9)	8.4–29.9 (16)	7.5–23.8 (15)	8.5–15.8 (14)	7–18.4 (11)
08.3	Meat products	16.9 (1)	12.0 (1)	5.0 (1)	–	–	–
12.6	Sauces	13.5–22.5 (3)	6.1–10.3 (5)	5.0–15.0 (10)	6.6–10.6 (10)	6.6–13.7 (10)	5.4–13.2 (9)
14.1.4	Flavoured drinks	6.6–17.6 (2)	5.4–26.6 (7)	5.7–28.9 (17)	7.8–33.5 (17)	6.4–22.1 (15)	5.7–8 (7)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	26.0–65.2 (2)	27.8 (1)	10.3 (1)	–	6.1 (1)	5.2–6.5 (2)

–: Food categories not contributing or contributing less than 5% to the total mean exposure.
(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific population.

3.4.1.5. Uncertainty analysis

Uncertainties in the exposure assessment of glycerol (E 422) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the sources of uncertainties have been summarised and evaluated in Table 9.

Table 9: Qualitative evaluation of influence of uncertainties on the dietary exposure estimates of glycerol (E 422) from its use as food additive according to Annex II to Regulation (EC) No 1333/2008 (using data set 1)

Sources of uncertainties	Direction[a]
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels and analytical data to the food items in the EFSA Comprehensive Food Consumption Database:	
Uncertainties to which foods the levels refer to	+/–
Levels considered applicable to all foods within the food category	+/–
Food categories included in the maximum level exposure and refined exposure assessment scenarios:	
Exclusion of food categories due to missing FoodEx linkage (n = 10)	–
Concentration data not available for 30 food categories	–
Restrictions/exceptions not taken into account (e.g. bread and rolls)	+
Food supplements consumed by infants and young children	+
Reported use levels:	
Data not available from industry for 48 food categories	–
Use level of a niche product used for the whole food category bread and rolls	+/–
Analytical data:	
Not representative of foods on the EU market (coming from only one MS)	+/–
Food categories in which the presence of glycerol comes from natural sources	+
Treatment of left-censored data: use of MB scenario	+/–
Maximum level exposure assessment scenario:	
Food categories which may contain glycerol (E 422) due to carry-over not considered	–
Exposure calculations based on the maximum use levels (reported use from industries or analytical data)	+
Uncertainty on the actual use of glycerol (E 422) in food categories authorised for the Group I food additives	+
Refined exposure assessment scenarios:	
Food categories which may contain glycerol (E 422) due to carry-over was only considered for wine	–
Exposure calculations based on the maximum or mean levels (reported use from industries or analytical data)	+/–
Uncertainty on the actual use of glycerol (E 422) in food categories authorised for the Group I food additives	+
Uncertainty in possible national differences in use levels of food categories	+/–
Uncertainty in possible fermentation in other food products (fruit juice)	–

MB: middle bound.
(a):+, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Glycerol (E 422) is authorised as a Group I food additive in 66 food categories and has a specific authorised use in two food categories (Table 3). Since the majority of food categories correspond to the general Group I food additives authorisation, glycerol may not necessarily be used in some of these food categories. This may explain why reported use levels and analytical data of glycerol (E 422) were only available for 28 food categories, including one food category for which glycerol (E 422) has a specific use. The Panel calculated, based on the information in the Mintel GNPD, that out of the

foods authorised to contain glycerol (E 422) according to Annex II to Regulation (EC) No 1333/2008, 18–50% of the amount of food consumed per population group was reported to potentially contain glycerol (E 422) as a food additive. Based on this, the Panel noted that the information from the Mintel GNPD supported the observation that due to its Group I authorisation, glycerol may be used in less food categories than it is authorised.

Furthermore, the Panel noted that information from the Mintel GNPD (Appendix C) indicated that 54 out of 76 food subcategories, categorised according to the Mintel GNPD nomenclature, in which glycerol (E 422) was labelled were included in the current exposure assessment. These 54 food subcategories represented approximately 94% of the food products labelled with glycerol (E 422) in the database. In the remaining 22 food subcategories, in which glycerol (E 422) was labelled but which were not included in the exposure assessment, glycerol (E 422) was authorised in 18 food subcategories.

Given these observations, the Panel considered overall that the uncertainties identified would, in general, result in an overestimation of the exposure to glycerol (E 422) from its use as a food additive according to Annex II in both the maximum level and refined exposure scenario.

The Panel noted that food categories which may contain glycerol (E 422) due to carry-over (Annex III, Part 1, 2, 3, 4 and 5) were only partly considered (via wine and other alcoholic beverages) in the current exposure assessment using data set 2, and that the exposure to glycerol via natural sources included only wine, other alcoholic beverages and honey. Taking these sources also into consideration did, however, not significantly change the exposure to glycerol (E 422) (Appendix F).

3.4.2. Exposure to 3-MCPD from the use of glycerol (E 422) as a food additive

According to the EU specifications for glycerol (E 422), 3-MCPD may be present at its maximum level of 0.1 mg/kg glycerol. On this basis, the Panel calculated the exposure to 3-MCPD from the use of glycerol (E 422) as a food additive in six population groups according to the different exposure scenarios (Section 3.4.1) (Table 10).

Table 10: Summary of exposure to 3-MCPD from the use of glycerol (E 422) as a food additive in the *maximum level exposure assessment scenario* and in the refined exposure scenarios, in six population groups (minimum–maximum across the dietary surveys in ng/kg bw per day)

	Infants (12 weeks–11 month)	Toddlers (12–35 month)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Maximum level exposure assessment scenario						
Mean	1–14	16–58	24–48	13–33	7–19	8–14
95th percentile	27–46	48–94	49–91	28–63	16–41	17–27
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
Mean	1–13	14–45	20–37	10–25	6–14	6–12
95th percentile	22–44	40–80	42–79	22–50	14–32	14–26
Non-brand-loyal scenario						
Mean	0.6–10	9–31	11–24	7–16	5–10	5–8
95th percentile	15–27	26–42	22–46	15–30	11–20	10–15

In the *maximum level exposure assessment scenario*, the mean exposure to 3-MCPD ranged from 1 ng/kg bw per day in infants to 58 ng/kg bw per day in toddlers. The 95th percentile of exposure ranged from 16 ng/kg bw per day in adults to 94 ng/kg bw per day in toddlers. In the *refined brand-loyal exposure scenario*, mean exposure to 3-MCPD ranged from 1 ng/kg bw per day in infants to 45 ng/kg bw per day in toddlers, and the 95th percentile from 14 ng/kg bw per day in adults and the elderly to 80 ng/kg bw per day in toddlers. In the *non-brand-loyal scenario*, mean exposure to 3-MCPD ranged from 0.6 ng/kg bw per day in infants to 31 ng/kg bw per day in toddlers, and the 95th percentile from 10 ng/kg bw per day in the elderly to 46 ng/kg bw per day in children.

The Panel could not calculate exposure to any genotoxic impurities or other contaminants that may be present in glycerol (E 422), e.g. glycidol, due to the lack of data on their concentrations in the food additive.

3.4.3. Estimated acute exposure to glycerol (E 422)

The Panel noted that during therapeutic use of glycerol, some effects (nausea, headache and/or vomiting) were reported. Using a conservative approach (see Section 3.5.7), the Panel calculated that these effects may be observed after oral intake of a bolus dose of 125 mg glycerol/kg bw per hour.

The Panel considered that the most relevant situation where an acute bolus exposure to glycerol used as a food additive can be similar to the one occurring during therapeutic use is consumption of a beverage.

Therefore, the Panel calculated the volume of flavoured drinks that is required to be consumed in order to exceed the acute bolus exposure at which effects were reported during therapeutic use.

The calculation was done using either the highest value (12,700 mg/L) or the mean (rounded up to 5,000 mg/L) of the reported analytical data for glycerol in flavoured drinks and the mean body weight of each population, and considered the 95th percentile (Table 11).

Table 11: Volume (mL) required to be consumed to exceed the acute bolus exposure of 125 mg/kg bw per hour

Concentration of glycerol (E 422) in flavoured drinks (mg/L)	Volume (mL)				
	Infants	Toddlers	Children	Adolescents	Adults
12,700	70	130	230	500	700
5,000	175	300	600	1,300	1,800

From the results on Table 11, the Panel noted that for infants and toddlers, consumption of less than the volume of a can (330 mL) is sufficient to exceed the dose of 125 mg glycerol/kg bw per hour.

However, the Panel is aware that the approach used to assess the acute exposure has limitations. Thus, the Panel noted that the uncertainty about the exposure calculation is extremely high as:

- the calculation was done based on only 5 analytical data;
- there was a wide variation among the 5 values available (2 < LOD and the 3 others being 3,200, 7,700 and 12,700 mg/L).

In addition, acute dietary exposure to glycerol (E 422) per meal was estimated as the exposure via one food category (food categories as defined in legislation) consumed in one meal (acute exposure per food category). Calculations were based on food consumption data (EFSA Comprehensive Database) and using data set 1 (Section 3.4.1). Acute exposure per food category was estimated for each individual by multiplying the consumed amount of each food category per meal by its high occurrence level. Appendix H lists the mean and 95th percentile of the acute exposure per food category, meal and population group.

The Panel is aware that the approach used to assess the acute exposure has its limitations. Acute exposure is any possible exposure to a compound during a single meal or day. This exposure can be high (e.g. if someone consumes much of a food or the food contains a high level), but can also be lower (e.g. if the consumer consumes a food with a low occurrence level). The probability of being exposed to high levels depends on the amounts consumed and the chance that a consumer selects a food with a high occurrence level (EFSA PPR Panel, 2012). In the acute exposure calculations performed for glycerol (E 422), the Panel assumed that the probability of selecting a food with high levels of glycerol was equal to 1 for one food category, ignoring the presence of concentrations lower than this high value. This was done due to the limited occurrence data available for the exposure assessment and limitations in the models available to assess acute exposure. It is therefore estimated that acute exposure estimates reported here are likely overestimates of the exposure. The acute exposure estimates per food category should therefore be interpreted as upper 'worst case' acute exposure estimates via these food categories.

The acute exposure assessment was furthermore hampered by the same limitations regarding the analytical data (only provided by one country) and usage levels (very limited, except for edible ice) as the chronic exposure assessment. Considering that concentration data were not made available for all

authorised food categories, these categories could also not be considered in the acute exposure assessment.

3.4.4. Exposure via the regular diet

The Panel noted that glycerol is liberated from normal lipid constituents (e.g. triglycerides) but considered that this source of exposure to glycerol is not relevant to the exposure assessment since the glycerol is re-esterified at or soon after absorption (EFSA NDA Panel, 2010), and does not become systemically available as glycerol.

3.4.5. Exposure via other sources

Exposure to glycerol due to the following uses was not considered in this opinion.

3.4.5.1. Glycerol as an ingredient in food for sports people

The use of glycerol in beverages for athletes to enhance and maintain hydration status and to improve endurance exercise performance is described (Van Rosendal et al., 2010; Savoie et al., 2016). Glycerol when used as a component of a hyperhydration strategy is prohibited by the World Anti-Doping Agency (WADA) (Martindale, online; Savoie et al., 2016; WADA, 2016).

3.4.5.2. Pharmaceutical use

Glycerol is used in pharmaceutical products as an active ingredient as well as an excipient, e.g. solvent, plasticiser or lubricant (EMA, 2003; Martindale, online). When glycerol is used as an excipient in medicinal products for oral administration and the glycerol intake is equal or above 10 g per single dose of the medicinal product, the information 'May cause headache, stomach upset and diarrhea' is required for the package leaflet (EMA, 2003). As an active ingredient, glycerol is used as an osmotic dehydrating agent. It is given orally, e.g. for the reduction in intra-ocular pressure before and after ophthalmic surgery and as an adjunct in the management of acute glaucoma or to reduce intracranial pressure (Martindale, online) (see Section 3.5.7).

3.5. Biological and Toxicological studies

The Panel noted that not all the studies performed with glycerol have used glycerol concurring with the EU specifications for the food additive E 422.

3.5.1. Absorption, distribution, metabolism and excretion

3.5.1.1. Absorption

In animals

Höber and Höber (1937) examined the absorption of unlabelled glycerol from the ligated intestine in anaesthetised rats (no further details provided). After washing, the intestinal lumen was filled with 4 mL 100 mM glycerol solution. After an exposure period of 25 min, the lumen was emptied by rinsing with Ringer's solution and residual glycerol in the washing fluid was determined analytically. The absorption varied between 70 and 89%. Similar experiments with ligated rat stomach (Herting et al., 1956) revealed lower absorption (10–35%) after an exposure period of 6 h.

Rapid absorption of radiolabelled glycerol in rats after oral administration was demonstrated by Gidez and Karnovsky (1954). Experiments with ^{14}C-labelled glycerol were performed in 24 h fasted male Wistar rats (number of rats employed was not reported). Rats were administered 60 or 112 mg/rat by either gavage or intravenous (i.v.) injection and exhaled $^{14}CO_2$ was measured every 15 min for 60 min and then every 20–30 min for up to 240 min. No significant differences were observed between the two routes of administration in terms of exhaled $^{14}CO_2$. Peak levels of $^{14}CO_2$ were reached 50–80 min after administration, suggesting rapid absorption and metabolism after oral administration.

The mean endogenous glycerol level in whole blood of untreated rats (n = 200) was reported to be 0.254 mM (23 mg/L) (Ackerman et al., 1975).

Kato et al. (2005) examined the absorption of glycerol using a closed loop of the rat small intestine *in situ*. The absorption of glycerol was saturable and was inhibited by Na^+-free conditions, suggesting the involvement of a Na^+-dependent carrier-mediated transport system. Similar results were presented by Yuasa et al. (2003).

In humans

In a clinical study performed by McCurdy et al. (1966; see also section 3.2.7), five volunteers (no further details) consumed a 50% solution of unlabelled glycerol in water at a dose level of 1,000–1,270 mg/kg bw after overnight dehydration and administration of pitressin tannate (to further inhibit diuresis). The mean pretreatment glycerol concentration in serum was 0.51 mM (47 mg/L). The serum glycerol levels were measured every 20 min after ingestion for a total of 140 min. Twenty minutes after ingestion, the mean serum concentration increased to 7.7 mM (710 mg/L) and reached a maximum of 16.2 mM (1,490 mg/L) 80 min after the treatment. Thereafter, the serum level declined to 13.3 mM at 140 min. Glycerol appeared in urine and the amount accounted for 7.5–13.9% of the ingested dose (urine collected for 2.5 h after ingestion, from 6 volunteers). Although no conclusions can be drawn on the total absorption, the authors of this study stated that the data indicated rapid absorption of glycerol after ingestion.

Pelkonen et al. (1967) determined the glycerol serum level in 10 volunteers (pretreatment level 0.1 mM or 9.2 mg/L) every 15 min for 60 min after oral treatment with 70 mg glycerol/kg bw (as 5% aqueous solution of unlabelled glycerol). The maximum serum concentration of glycerol occurred 15 min after ingestion, 0.4 mM (37 mg/L). The serum level declined thereafter to pretreatment levels by 1 h.

3.5.1.2. Distribution

In a study in rats, it was shown that there was a net synthesis of blood glucose and liver glycogen as a result of ^{14}C-glycerol administration (88–89 mg/rat; intraperitoneal (i.p.)). Fifteen minutes after administration, 70% of newly formed blood glucose was derived directly from glycerol and 30 min after treatment this value increased to 100%. After 30 minutes, 39% of the newly formed liver glycogen was derived from the administered ^{14}C-glycerol. This value decreased during the following 1–6 h after application and fell to 15% at 6 h (Gidez and Karnovsky, 1954).

In further experiments, the incorporation of ^{14}C-glycerol into liver lipids was also reported (Gidez and Karnovsky, 1954). At a dose of 1 mM ^{14}C-labelled glycerol (92 mg/rats; i.p.), most radioactivity was found in liver lipids (5.9% of applied dose) 6 h after application with only minor amounts found in other organs (e.g. 0.26% in the brain and 0.16% in the kidney).

3.5.1.3. Metabolism

Glycerol occurs naturally in fats (e.g. triglycerides, phospholipids) and is also an endogenous metabolite (Tao et al., 1983). After ingestion of triglyceride, hydrolysis in the gastrointestinal tract yields glycerol and fatty acids which are then absorbed by the mucosa. Glycerol is also released by adipose tissue lipolysis; the catabolic process leading to the breakdown of triglycerides stored in fat cells. Glycerol may then be metabolised through a number of pathways (Lin, 1977; Brisson et al., 2001).

Glycerol may be phosphorylated by glycerol kinase to glycerol phosphate followed by oxidation to dihydroxyacetone phosphate via the enzyme glycerol phosphate dehydrogenase (Tao et al., 1983; Brisson et al., 2001). Dihydroxyacetone phosphate can participate in glycolysis and be further oxidised to carbon dioxide (oxidised as an energy substrate) or gluconeogenesis after formation of the intermediate glyceraldehyde 3-phosphate (Tao et al., 1983; Brisson et al., 2001). Exogenous glycerol also participates in lipogenesis (e.g. formation of triglycerides). When rats were i.p. injected with radiolabelled glycerol, radioactivity was found in lipids (Gidez and Karnovsky, 1954). With respect to glycerol utilisation, the liver accounts for up to at least 75% of the total body glycerol-utilising capacity and the kidney accounts for up to 25% (Lin, 1977; Tao et al., 1983). At physiological serum concentrations up to 1 mM glycerol (92 mg/L), uptake into hepatocytes follows first order kinetics (Tao et al., 1983).

Gidez and Karnovsky (1954) examined the relationship between the amount of glycerol oxidised compared to the amount administered. At dose levels above 30 mg/rat (about 150 mg/kg bw), an approximately constant proportion of the administered glycerol appeared to be oxidised and exhaled as $^{14}CO_2$. At lower concentrations, the proportion converted to CO_2 was higher.

3.5.1.4. Excretion

Rats

In male Sprague–Dawley rats (n = 4 per group) most of the absorbed glycerol was metabolised and exhaled as CO_2 (Michael and Coots, 1971). Within 51 h after gavage of 14–20 mg/kg bw ^{14}C-labelled glycerol, 73.3% of recovered radioactivity was exhaled as $^{14}CO_2$, 5.2% excreted in urine and 2% in faeces or gastrointestinal contents and 19.5% in the carcass.

At glycerol concentrations in the blood up to 1 mM, glycerol excretion in the urine was reported to be undetectable. At higher glycerol levels, a renal excretion of glycerol occurs (Ackerman et al., 1975; Lin, 1977). In male SIV rats, higher glycerol levels in blood were induced by i.v. infusion (Ackerman et al. (1975). Excretion of glycerol via the urine occurred but the interindividual variance of this effect was substantial. At a steady state blood concentration of 1.9 mM (infusion of glycerol at a rate of 500 mg/kg bw per hour; n = 20), an average and standard error of the mean of 3.7 ± 3.5%, respectively, of the applied glycerol was eliminated in urine. The infusion of 700 mg/kg bw per hour (n = 4) resulted in a range of steady state blood levels between 2 and 40 mM, and the range of renal excretion of glycerol varied between 3.5% and 23% without a clear relationship to the blood level.

Humans

The excretion of glycerol via urine was reported to occur in humans at high dose levels (McCurdy et al., 1966). In six volunteers (no further details), glycerol appeared in urine and accounted for 7.5–13.9% of applied dose (urine collected for 2.5 h after ingestion) after ingestion of 1,000–1,270 mg/kg bw unlabelled glycerol (no further data).

The disappearance of serum glycerol in humans was analysed in individuals that were injected i.v. with 2,400 mg unlabelled glycerol over a period of 2 min (Pelkonen et al., 1967). The serum levels of glycerol were determined over the subsequent 75–90 min. The removal rates of glycerol in four volunteers varied between 1.35 and 2.21 μmol/kg bw per minute (mean 1.7 μmol/kg bw per minute). The half-life (at equilibration; t_o = 10 min after injection) was 10.7–12.3 min (mean 11.7 min). A twofold increase in mean half-life and removal rate were measured in similar trials with 8 diabetics (Pelkonen et al., 1967).

It was shown in volunteers (9 lean and 13 obese subjects, no data about sex and age) that the removal of glycerol from the human blood after infusion varied in proportion to the glycerol plasma concentrations (Bortz et al., 1972). A basal turnover rate of approximately 100 μmol/minute was reported in adult volunteers. At plasma concentrations up to 0.45 mM (41.4 mg/L), the turnover rate increased to 700 μmol/minute in obese subjects, and to a maximum of 400 μmol/minute in lean subjects (0.22 mM glycerol level in plasma). In an intravenous glycerol tolerance test, the turnover rate was calculated as 1.5 μmol/kg bw per minute.

Overall, the Panel considered that glycerol occurs naturally in several types of lipid and is an endogenous metabolite in mammals. Glycerol is rapidly and near completely absorbed from the gastrointestinal tract; distributed into the total body water space and is primarily metabolised in the liver. After phosphorylation and oxidation, glycerol is used as an energy substrate via glycolysis or participates in gluconeogenesis and lipogenesis. Glycerol is extensively oxidised and exhaled as CO_2, with only minor amounts excreted via urine or faeces. The serum concentration of free glycerol in humans has been reported to be normally between 0.46 and 18.5 mg/L (Lin, 1977; Tao et al., 1983). The Panel noted that at glycerol concentrations in blood up to 92 mg/L, no glycerol was detected in the urine but higher levels result in renal elimination of glycerol in rats (Ackerman et al., 1975) and humans (McCurdy et al., 1966).

3.5.2. Acute toxicity

The oral median lethal dose (LD_{50}) values for glycerol were reported to be 12,600–28,800 mg/kg bw (rat); 15,000–38,000 mg/kg bw (mouse); 7,750–11,500 mg/kg bw (guinea pig) and 17,600–27,000 mg/kg bw in the rabbit (BIBRA, 1993 [Documentation provided to EFSA n. 2]).

Diuresis was reported to occur in dogs 15 min after gavage of 2,500 g/kg bw (Johnson et al., 1933) and in rats after 'toxic dose levels' (no further details; Suzuki et al., 1977).

No irritation was apparently seen when undiluted glycerol was applied to the lining of the oral cavities of rats, rabbits and dogs (Informatics Inc, 1973, as referred to by BIBRA, 1993 [Documentation provided to EFSA n. 2]).

The Panel considered that glycerol has a low acute toxicity.

3.5.3. Short-term and subchronic toxicity

3.5.3.1. Rats

In a study by Staples et al. (1967), female rats (10 animals/group; strain not reported) were treated three times daily for 3 days with 0, 0.75, 1.5 or 3 mL/kg bw undiluted glycerol by gavage (equivalent to 0, 2,800, 5,600 and 11,200 mg/kg bw per day), control animals received 3 mL water by

gavage. The study was restricted to examining irritant effects in the stomach and duodenum. At 0.75 mL/kg bw (2,800 mg/kg bw per day) and higher, the authors reported dose-dependent local irritant effects including slight to severe hyperaemia, petechial haemorrhages and erosions but no systemic toxicity. Administration of 3,800 mg glycerol/kg bw (thrice daily) as either a 20, 40, 60, 80 or 100% aqueous solution (dose volume varied between 3 (for 100%) and 15 mL (for 20%)) resulted in local irritant effects at all dilutions but with severity reduced by dilution such that 1 animal in 10 showed slight effects in the 20% group.

Thirty adult male Wistar rats (6 animals/group) were treated by gavage with 0 (control/saline), 200, 400, 800 and 1,600 mg glycerol/kg bw per day (bidistilled glycerine, 99.85% glycerol) for 28 days (Lisenko et al., 2015). Glycerol did not induce any statistically significant change in average daily weight gain or water consumption or excretion levels. Glycerol supplementation did induce a significant decrease in the average daily feed intake levels. Supplementation with glycerol did not affect plasma triglyceride, glucose, total cholesterol, lipoprotein concentrations, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Plasma glucose and total cholesterol levels demonstrated a significant increase in values up to a dose of 800 mg/kg, followed by a decrease at 1,600 mg/kg bw per day. In terms of high-density lipoprotein levels, there was an increase up to a dose of 800 mg/kg bw per day, followed by a decrease at 1,600 mg/kg bw per day, but levels were still within the normal range for the species. For very low-density lipoprotein and low-density lipoprotein levels, there was a decrease with increasing glycerol doses, which remained within the standards. The liver enzymes AST and ALT showed a decrease trend up to the dose of 800 mg/kg bw per day, with an increase at 1,600 mg/kg bw per day. Glycerol supplementation did not result in any change in relative organ weights. Leucocyte levels differed between the groups, although all were within the normal range. The differential count for lymphocytes and neutrophils did not change between the experimental groups. No lesions were observed in any tissue (pancreas, kidney, liver) from animals receiving different levels of glycerol.

In a limited study by Fischer et al. (1949), male rats (20 animals/group; strain not reported) were treated for 44 days with 0, 100, 500, 1,000 or 2,000 mg glycerol/kg bw per day by gavage. The authors reported no clinically adverse signs at any dose; no effects on mortality or body weight gain. For the haematological parameters examined, there were no effects reported on haemoglobin, erythrocyte, leucocyte and lymphocyte counts and differential blood counts. Histopathological examination was restricted to liver, kidney and urinary bladder, and no effects were observed.

Adult male Wistar rats were subjected to 6 weeks of aerobic training (after an acclimatisation to swimming, the animals swam for 60 min daily, at a frequency of 5 times per week, with relative overload of 5% of their body mass) (Andrade et al., 2014). In the last 4 weeks, the animals were treated with saline, glucose or glycerol (800 mg/kg bw per day) by gavage (it is not stated how frequently animals were dosed). Treatment with glycerol did not induce statistically significant differences in body mass, water consumption, urinary production or relative weight of liver or kidney compared to negative control. Glycerol supplementation did induce a significant reduction in food consumption compared to negative control. In terms of blood biochemical parameters, no difference was observed in glycaemic level or lipoprotein concentrations in plasma compared to negative control. However, a statistically significant increase in both plasma triacylglyceride and total cholesterol levels was observed after supplementation with glycerol. Statistically significant increases in adipocyte diameter and area, as well as villous:crypt ratios of the duodenum and ileum were observed following glycerol supplementation. No differences were observed in adipocyte density or the jejunum villous: crypt ratio in glycerol-supplemented animals compared to negative control.

In a study by Anderson et al. (1950), female rats (5 animals/group; strain not reported) were treated for 26 weeks with 0 or 5% glycerol by volume in the drinking water (either derived from natural sources or synthetically synthesised), equivalent to 0 and 4,500 mg glycerol/kg bw per day). Haematology was performed monthly and was composed of erythrocyte and leucocyte counts, haemoglobin and differential blood counts. At 26 weeks, necropsy and histopathology of heart, lungs, liver, spleen, stomach, intestines, kidneys, thymus, thyroid and adrenals was performed, and no effects were observed by the authors except mineralisation in renal tubules. Glycerol treatment had no effects on haematological parameters. The Panel considered mineralisation in renal tubules to be due to the high dose of glycerol used in the study.

In a 3-month study by Löser et al. (1954), male and female rats (strain unspecified, 6 animals/group) were treated with glycerol as a 0, 1%, 2%, 5%, 10% or 20% solution in drinking water (equivalent to 0, 800, 1,600, 4,000, 8,100 and 16,200 mg/kg bw per day and 0, 900, 1,800, 4,500, 9,100 and 18,200 mg/kg bw per day for males and female, respectively). No effects were noted in the

animals receiving 1%, 2%, 55 or 10% glycerol. In the group receiving 20% glycerol, 2 animals out of 12 animals died. The remaining 18 exhibited some retardation in weight gain and development. However, they recovered while under treatment and had no abnormalities at the end of the study. The authors reported that some organs were investigated (liver, spleen, kidney, endocrine organs) and that no effects of glycerol were found. Identical results for glycerol were reported in a shorter version in a publication, by Bornmann (1954). The Panel considered it probable that this paper reported the same study.

3.5.3.2. Dogs

In a study by Staples et al. (1967), mongrel dogs (1–2 animals/group) were treated three times daily for 3 days with 0, 0.75, 1.5 or 3 mL/kg bw undiluted glycerol (equivalent to 0, 2,800, 5,600 and 11,200 mg/kg bw per day) by gavage, control animals received 3 mL water by gavage. The study was restricted to examining irritant effects in the stomach and duodenum. At 5,600 mg/kg bw per day and higher, the authors reported dose-dependent local irritating effects including hyperaemia, petichial haemorrhages and erosions.

Overall, the Panel noted that no studies were performed according to current test guidelines. The Panel also noted that in a subchronic toxicity study (in drinking water) in rats (Löser et al., 1954) the effects reported were observed with doses in the range of the LD_{50} for glycerol. The Panel considered that the local irritating effects of glycerol in the gastrointestinal tract reported in some gavage studies in rat (100% glycerol at 2,800 mg/kg bw per day, the lowest dose tested (Staples et al., 1967)), and dogs (100% glycerol at 5,600 mg/kg bw per day (Staples et al., 1967)) was likely due to the hygroscopic and osmotic effects of large doses of glycerol administered by gavage.

3.5.4. Genotoxicity

3.5.4.1. *In vitro*

Numerous studies have been published for glycerol on reverse gene mutation in bacteria, chromosome aberrations and gene mutations in mammalian cells. A summary of available data is presented in Table 12.

Table 12: Summary of studies on genotoxicity of glycerol *in vitro*

Test system	Tested organisms	Tested concentrations vehicle[(a)]	Cytotoxic concentration	Results –MA	Results +MA	Reference
Gene mutation in bacteria						
Ames test	*Salmonella* Typhimurium TA100, TA1535, TA1537, TA98, TA1538; *Escherichia coli* WP2uvrA	0.005–5 mg/plate (7 dose levels) water	Not cytotoxic but tested up to 5 mg/plate	Negative	Negative	Shimizu et al. (1985)
Ames test	*S.* Typhimurium TA100, TA1535, TA1537, TA98	0.01–10 mg/plate water	Tested up to 10 mg/plate	Negative	Negative	Haworth et al. (1983)
Ames test	*S.* Typhimurium TA100, TA1535, TA1537, TA98, TA1538	0.001–10 mg/plate DMSO	Tested up to 10 mg/plate	Negative	Negative	Clark et al. (1979)
Ames test	*S.* Typhimurium TA100, TA1535, TA1537, TA98, TA1538	0.2–1 mg/plate n.d.	Not cytotoxic	Negative	Negative	Doolittle et al. (1988)
Ames test	*S.* Typhimurium TA102 and TA97	0.1–10 mg/plate water	Tested up to 10 mg/plate	Negative	Negative	Fujita et al. (1994)
Ames test	*S.* Typhimurium TA100, TA1535, TA1537, TA98, TA92, TA94	Up to 50 mg/plate BP	Tested up to 50 mg/plate	Negative	Negative	Ishidate et al. (1984)
Ames test	*S.* Typhimurium TA100	Up to 92 mg/plate n.d.	No data	Negative	Negative	Stolzenberg and Hine (1979)
Ames test	*S.* Typhimurium TA100	0.5 mg/plate n.d.	No data	Negative	n.d.	Yamaguchi (1982)

Test system	Tested organisms	Tested concentrations vehicle[a]	Cytotoxic concentration	Results –MA	Results +MA	Reference
Ames test	*S.* Typhimurium TA100	n.d. n.d.	No data	Negative	n.d.	Higashimoto et al. (1991)
Chromosomal aberrations						
Chromosome aberration test	Chinese hamster lung fibroblast cells	Up to 1 mg/mL (3 dose levels) PS	Not cytotoxic	Negative	n.d.	Ishidate et al. (1984)
Chromosome aberration test	Chinese hamster ovary (CHO) cells	0, 69, or 92 mg/mL water	Not cytotoxic[g]	Negative	n.d.	Galloway et al. (1987)
Chromosome aberration test	CHO cells	0.1–1 mg/mL (6 dose levels) water	Not cytotoxic	Negative	Negative	Doolittle et al. (1988)
Gene mutation in mammalian cells						
HGPRT gene mutation assay	CHO cells	0.1–1 mg/mL (6 dose levels) water	Not cytotoxic	Questionable	Negative	Doolittle et al. (1988)
DNA damage						
Unscheduled DNA synthesis	Isolated male rat hepatocytes	0.1–1 mg/mL (5 dose levels) water	n.d.	Negative	Not applicable	Doolittle et al. (1988)
Sister chromatid exchange assay	CHO cells	0.2–1 mg/mL (5 dose levels) water	Not cytotoxic	Negative	Negative	Doolittle et al. (1988)
rec assay	*Bacillus subtilis* H17 or M45	n.d. n.d.	n.d.	Positive	n.d.	Nonaka (1982)

DMSO: dimethylsulfoxide; CHO: Chinese hamster ovary; HGPRT: hypoxanthine-guanine phosphoribosyltransferase; MA: metabolic activation system; n.d.: no data available; PB: phosphate buffer; PS: physiological saline.

The most reliable data for the bacterial reverse mutation assay in *Salmonella* Typhimurium strains TA100, TA1535, TA1537, TA98, TA1538 and *Escherichia coli* WP2uvrA were presented by Shimizu et al. (1985). The study is comparable to current standards. Glycerol concentrations up to 5 mg/plate were tested, the maximal dose level recommended in OECD Guideline 471. Negative results were obtained with and without addition of S9 mix (prepared from livers of male rats pre-treated with polychlorinated biphenyl). Vehicle and positive controls were performed and showed the expected results. No mutagenic activity was detected in other studies (Clark et al., 1979; Stolzenberg and Hine, 1979; Yamaguchi, 1982; Haworth et al., 1983; Ishidate et al., 1984; Doolittle et al., 1988; Higashimoto et al., 1991; Fujita et al., 1994).

Ishidate et al. (1984) published a chromosome aberration test in Chinese hamster lung fibroblast cells (see Table 12). The cells were exposed to three different doses for 24 and 48 h without addition of a metabolic activation system. The maximum dose tested was 1 mg/mL. This concentration represented the highest non-cytotoxic dose level tested in preliminary tests (50% cell-growth inhibition). One hundred metaphases per concentration were analysed for polyploid cells and structural chromosomal aberrations (no further details). Chromosome and chromatid gaps were included in the evaluation of clastogenic effects. No data were presented about concurrent positive controls but clastogenic effects were reported in parallel experiments with other test substances. The exposure to glycerol resulted in no increase in polyploidy or chromosome aberration. The authors considered glycerol to be non-clastogenic and non-mutagenic in this test system, but concentrations up to the cytotoxic threshold were not tested. However, no cytotoxic effects, nor clastogenic activity, was reported by Galloway et al. (1987) at higher concentrations (92 mg/mL), a dose level 18 times greater than the maximum dose level recommended *in vitro* by OECD Guideline no. 473. In this study, no concurrent positive control was tested for verification of the results. The concentrations used by Doolittle et al. (1988, see Table 12) also gave negative results but did not induce cytotoxic effects nor reach the recommended maximum concentration. Overall, however, the available studies are sufficient

for evaluation of this endpoint and were considered by the Panel to be sufficiently robust to support the results reported, indicating no clastogenic activity of glycerol in *in vitro* studies.

Equivocal results were reported in a mammalian cell gene mutation assay in trials without addition of a metabolic activation system (Doolittle et al., 1988). An increase in the mutation frequency compared to negative (4.8-fold increase) and solvent control (12-fold increase) was found at a concentration of 0.8 mg/mL but no clear increase at the highest dose level of 1 mg/mL. The effect was not dose-dependent and therefore considered by the authors to be without biological relevance. The Panel noted that mutation frequencies in the concurrent untreated and solvent treated controls were unusually low. No independent second trial was performed. Negative results were obtained with addition of a metabolic activation system. Other studies on this endpoint were not available.

No genotoxic activity was reported in limited studies on DNA damage in mammalian cells (Doolittle et al., 1988). There were only limited details reported for the rec assay in *B. subtilis* (Nonaka, 1982).

3.5.4.2. *In vivo*

Barilyak and Kozachuk (1985) reported a bone marrow chromosome aberration assay and a dominant lethal assay, both performed in male rats. No other *in vivo* genotoxicity data were available for glycerol.

A single gavage dose of 1 g/kg bw glycerol (n = 10, controls n = 11) induced an increase in chromosomal aberrations (2.2 ± 0.6% vs 0% in control; without gaps) and polyploidy cells (3.2 ± 0.8% vs 0.5 ± 0.2% in control) (Barilyak and Kozachuk, 1985). The bone marrow was processed for cytogenetic analysis 50 h after treatment and at least 50 metaphases per animal were analysed. However, the Panel noted that incidence of chromosomal aberrations observed was low and fell within the range of values often observed in untreated or solvent control animals. Moreover, the Panel also noted methodological shortcomings (e.g. collection of bone marrow outside the recommended range of 12–36 h, no positive control; no aberrations in 1,000 control metaphases; no statistical analysis and one dose level only) and limited information (e.g. no data on toxic effects). On this basis, the Panel considered this study not reliable. In the same publication, a rat dominant lethal assay was documented. Male rats were exposed to 0, 10, 100 or 1,000 mg/kg bw glycerol 'at the stage of late spermatids' (2 weeks before fertilisation) and mated with untreated females (n = 11–12 per dose). A dose-dependent and significant increase in post-implantation loss was reported with a statistically significant increase at ≥ 100 mg/kg bw. The Panel noted that the reported positive results were based on insufficient number of female animals to assess dominant lethal mutations and the number of treated male animals was not indicated. On this basis, the Panel considered this study not reliable.

Overall, glycerol did not show any genotoxic activity in different *in vitro* assays, which include negative results in the bacterial reverse mutation assay (Ames test), in chromosome aberration assays and in studies on DNA damage in mammalian cells. Questionable results obtained in a hypoxanthine-guanine phosphoribosyltransferase (HGPRT) gene mutation assay did not show a dose–response effect and were therefore judged of no biological significance. A lack of valid *in vivo* genotoxicity data is not of concern since clear negative findings were observed in *in vitro* assays. On this basis, the Panel considered that glycerol as a food additive did not raise concern with respect to genotoxicity.

3.5.5. Chronic toxicity and carcinogenicity

3.5.5.1. Mice

Witschi et al. (1989) investigated the effects on lung and liver tumour incidences in male and female CH3 mice (between 31 and 51 mice/group) after administration for 1 year of drinking water containing 0% or 5% glycerol (equivalent to 0 and 6,500 mg glycerol/kg bw per day for males and 0 and 8,300 mg/kg bw per day for female). Although the reporting of this study was limited, the Panel noted that lung and liver tumour incidences were not significantly elevated in male or female mice (no further parameters examined).

3.5.5.2. Rats

As reported by JECFA (1976), male and female Sprague–Dawley rats (9 animals/group) were fed a diet supplemented with 0%, 5%, 10% or 20% natural or synthetic glycerol (equivalent to 0, 2,200, 4,500 and 9,000 mg/kg bw per day for male and 0, 2,900, 5,800 and 11,600 mg/kg bw per day for female) for 50 weeks (Atlas Chemical Industries Co. Ltd., 1969, as referred to by JECFA, 1976). At necropsy, growths in the pituitary and histopathological effects were observed in the kidney in females

treated with either test compounds. The Panel noted limited methodological detail, effects only in females and data lacking any dose–response effects.

Hine et al. (1953) examined the effects of supplementing the diet with glycerol (natural and synthetic, no further details on test substances) for up to 2 years on Long-Evans male and female rats (between 10 and 14 animals per group) at 0, 2,500, 5,000 or 10,000 mg/kg bw per day. The Panel noted that the study has a number of limitations – all rats in the high-dose group were culled after 1 year, with no concurrent control group; no clinical chemistry; limited parameters in haematology and urinalysis and limited histopathology. Body weight and food consumption were measured weekly and body weight gain calculated at 4 week intervals. Clinical signs were recorded daily and careful inspections for health and activity were made once a week. Urinalysis and blood sampling for haematology were performed at 3, 6, 12, 18 and 24 month (n = 5 per dose, no data about sex). Urinalysis was limited to microscopical examination and determination of glucose and albumin concentrations. Haematology was limited to erythrocyte counts, leucocyte counts and haemoglobin levels. High-dose rats were sacrificed after 1 year and liver glycogen and lipids determined (no control values were available). For the other groups, including the control group, the study was terminated after 105 weeks. In all groups, complete necropsy was performed and weights for the liver, lung, kidney, heart and spleen determined. Histopathology was limited to the liver, spleen, adrenals, kidney, small intestine, reproductive organs and urinary bladder. Lipid stains in the liver were prepared for all groups sacrificed after 105 weeks. The results were similar for the groups treated with natural or synthetic glycerol. No data were presented on food consumption, clinical signs or mortality. No significant effects were found on the body weight gain (statistical analysis restricted to mean values at week 13, 52 and 105); data on absolute body weight were not given. No consistent treatment-related effects were found in haematology, urinalysis, evaluation of organ weights and pathology. Liver glycogen and lipids did not differ between the two high-dose groups, a comparison with the control group was not possible. Similarly, data on organ weights and pathological effects in the high-dose group (10,000 mg/kg bw per day) were compared with a control group sacrificed at 105 weeks, limiting the validity of the results in the high-dose group. No no observed adverse effect level (NOAEL) was identified by the authors of the study. Although this study had minor limitations (e.g. lacking concurrent control in the 1-year study group), the Panel noted that no adverse effects were reported in animals receiving doses up to 10,000 mg/kg bw per day for 1 year, the highest dose tested. The Panel also noted that there was no increase in the tumours incidences up to doses of 5,000 mg/kg bw per day for 2 years, the highest dose tested.

JECFA reported a study in rat (Atlas Chemical Industries Co. Ltd., 1969, as referred to by JECFA, 1976) in which male and female Sprague–Dawley rats (24 animals/group) were fed a diet supplemented with 0%, 5%, 10% or 20% glycerol (equivalent to 0, 2,200, 4,500 and 9,000 mg/kg bw per day for male and 0, 2,900, 5,800 and 11,600 mg/kg bw per day for female) for 2 years. Animals were treated with natural glycerol (99.8% glycerol, similar caloric density for test and control diets) or synthetic glycerol (99.76% glycerol and 1,2,3-butanetriol and 1,2,4-butanetriol, 0.09%, similar caloric density for test and control diets). For both natural and synthetic glycerol, at all dose levels, glycerol treatment resulted in an increase in body weight gain (no further details). At the 10% dose level, there was an increase in relative heart weights (no data regarding other dose levels). At necropsy, the 20% glycerol group was examined for mortality, body weight, efficiency of caloric utilisation, water consumption, urinalysis (volume, specific gravity, sediment, acetone, albumin, sugar and oxalate), haematology (white blood cells and differential count, red blood cells, haematocrit, and haemoglobin) and clinical chemistry (blood glucose, urea, nitrogen, plasma cholesterol, serum alkaline phosphatase and transaminases, and bromosulfophthalein retention). In addition, organ weights were determined and complete histopathology performed (no further details). The only reported change noted was an increase in relative kidney weight at 20% glycerol dose level with both natural and synthetic glycerol. The Panel considered that the increase in relative heart weights did not show a dose–response relationship and that the increase in relative kidney weight at 20% glycerol dose level in both studies as adaptive since no histopathological changes were reported.

Overall, the Panel considered glycerol to be of no concern with regard to carcinogenicity.

3.5.5.3. Promoting and/or co-carcinogenic effects of glycerol

The Panel noted a series of studies, which had investigated potential modulating effects of glycerol administration on the development of cancer in animal models treated with an initiating agent (Inayama, 1986; Inayama et al., 1986; Nagahara, 1987; Witschi et al., 1989; Nagahara et al., 1990;

Yano et al., 1993, 1994; Itoh et al., 2002; Kanojia and Vaidya, 2006). The Panel considered these studies not relevant for the risk assessment of glycerol as a food additive.

3.5.6. Reproductive and developmental toxicity

3.5.6.1. Reproductive toxicity

In a study reported by Johnson et al. (1933), rats were kept on diets containing 61% starch and no glycerol (control), or 20% starch plus 41% glycerol (equivalent to 20,500 mg/kg bw per day), or no starch plus 61% glycerol (equivalent to 30,500 mg/kg bw per day), limited information, e.g. no data about number of animals per group. Reproduction was impaired by diets containing 41% glycerol and no pregnancies occurred in rats that were on diets containing 61% glycerol (undernourished conditions). However, this was an old study and the documentation of the results is not sufficient for evaluation of this endpoint.

In a study performed by Guerrant et al. (1947), groups of male and female rats (3 males and 6 females per group) received a synthetic diet containing 0 or 30% glycerol (equivalent to 15,000 mg/kg bw per day) and were carried through seven successive generations. Young rats from treated females weighed on average 20% less than controls. The Panel noted this study was limited: the number of animals per group was low and the diet changed during the generations.

Ten male and 10 female rats were orally gavaged daily either with glycerol (20% solution in water) at a dose level of 2,000 mg/kg bw per day or with distilled water (control) for 8 weeks before mating (Wegener, 1953). After mating, one subgroup of females (n = 5) received glycerol throughout pregnancy and up to parturition. The other subgroup (n = 5) was treated until weaning of the F_1 generation. Body weights of F_1 rats were measured every second day during the post-natal period from day 15–60. At post-natal day 100, F_1 rats were sacrificed except for 10/sex which were used to produce the F_2-generation. The authors reported that there were no effects of glycerol on fertility of parental (P) rats, F_1 litter size or F_1 weight gain (however, data were not presented). In total, 12 F_1 rats died during post-natal day 1–50 in the treatment group and three rats in the control; this mortality was not further discussed and could not be judged by the Panel as the total number of pups/dam was not presented. The onset of oestrus cycle in F_1 females was comparable in both groups and histopathology of F_1 rats revealed no effects on testes, ovaries, thyroid glands, pituitary and adrenal glands (no other organs were examined). F_2 litter size was comparable to control. No further parameters were investigated. The Panel noted that the study has limitations due to some methodological weaknesses (e.g. low number of pregnant P rats, no F_1 pup weights, no data about external malformations or toxic effects in P generation) but there is no indication for adverse effects on reproductive performance of P rats at a dose of 2,000 mg/kg bw per day, the highest dose level tested. The Panel, therefore, considered that this study was limited as the number of animals per (sub) group was very low and the reporting was limited.

3.5.6.2. Developmental toxicity

Several developmental toxicity studies of glycerol were conducted in CD-1 mice, Wistar rats golden hamsters and Dutch-belted rabbits (FDRL, 1974a,b,c). Animals were administered different doses of glycerol (not specified) in water by gavage; the control groups were vehicle-treated (dose volume is not reported). Body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weight of live fetuses were recorded. All fetuses were examined grossly for external abnormalities, one-third underwent detailed visceral examinations and two-thirds were stained and examined for skeletal defects.

Groups of 25 adult female albino CD-l outbred mice were dosed by gavage from gestation day (GD) 6 to 15 with 0, 12.8, 59.4, 276 or 1,280 mg glycerol/kg bw per day (dose volume 10 mL/kg bw per day) (FDRL 1974a). At necropsy on GD 17, the surviving dams appeared to be completely normal and the number of implantations, and live fetuses was comparable to the control group. Doses up to 1,280 mg glycerol/kg bw per day had no effects on implantation nor on maternal and fetal survival. The numbers of live or dead fetuses, resorptions and average implant sites and also fetal weights did not differ among the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the glycerol-treated groups, did not differ from the number in vehicle-treated dams of the control group.

Groups of 25–29 virgin adult female Wistar rats were dosed by gavage from GD 6 and to 15 with 0, 13.1, 60.8, 282 or 1,310 mg glycerol/kg bw per day (dose volume 1, 1, 1, 2, or 5 mL/kg bw per day)

(FDRL, 1974b). At necropsy on GD 20, doses up to 1,600 mg glycerol/kg bw per day appeared to be completely normal and had no effects on implantation or on maternal and fetal survival. The numbers of live or dead fetuses, resorptions, average implantations and fetal weights did not differ among the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the glycerol-treated groups, did not differ from the number in vehicle-treated dams of the control group.

Groups of 15–20 virgin adult female Dutch-belted rabbits were treated by gavage once daily from GD 6 to 18 with doses of 0, 11.8, 54.8, 254.5 or 1,180 mg glycerol/kg bw per day (dose volume 1, 1, 1, 2 or 5 mL/kg bw per day) (FDRL, 1974c). Only 10–14 rabbits became pregnant and were evaluated at termination. At necropsy on GD 29, the surviving dams appeared normal throughout the observation period and had normal fetuses. No effect was observed on the number of implantations. The numbers of live or dead fetuses, resorptions, average implant sites or fetal weights did not differ among the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the glycerol-treated groups, did not differ from the number in vehicle-treated dams of the control group.

The Panel noted that the identically performed tests with glycerol in mice, rats and rabbits by the FDRL (1974a,b,c) were reported in a limited way but cover the organogenesis period of the reproductive cycle. These prenatal developmental toxicity studies showed no dose-related developmental and maternal effects up to the highest dose tested 1,280, 1,600 or 1,180 mg glycerol/kg bw per day for mice, rats and rabbits.

3.5.7. Other studies

3.5.7.1. Observations in humans

Glycerol has been used therapeutically by intravenously or oral administration to mobilise oedema and to induce osmotic diuresis. Clinical experience and data from clinical studies are found in the literature indicating that doses up to 60,000 mg intravenously, infused over 1–4 h, for a treatment duration of 1 week and in some studies, several weeks (Frank et al., 1981), and bolus oral doses (initial dose 1,500 mg/kg bw, followed by 500–700 mg/kg bw every 3 h) (Cantore et al., 1964) or 500–1,000 mg/kg bw (Wald and McLaurin, 1982).

The Panel considered that the therapeutic administration of glycerol by oral administration to patients reported to be suffering disease that could impact significantly on their physiological functionality was not relevant in the safety assessment of glycerol (E 422) as a food additive. However, glycerol has been prescribed for reduction in intraocular pressure in patients with glaucoma and may be prescribed prior to intraocular surgery (Bartlett, 1991). For this indication, glycerol was reported to be administered orally as a bolus dose of 1,000–1,500 mg/kg bw (as a 50% solution) with a daily total oral dose not higher than 120,000 mg (about 1,700 mg/kg bw) (Gilman, 1990). The Panel considered that the therapeutic administration of glycerol by oral administration to patients reported to be suffering ocular disease – such as glaucoma and administration of glycerol to control participants in clinical studies – were relevant for the safety assessment of glycerol (E 422) as a food additive.

The Panel noted that for oral therapies to patients with ocular disease or healthy controls, glycerol was administered as a bolus at 1,350 mg/kg bw (Buckell and Walsh, 1964); 1,500 mg/kg bw (Drance, 1964); 1,500 mg/kg bw (Consul and Kulsretha, 1965); 1,000 mg/kg bw (Kornblueth et al., 1967); 1,000–1,270 mg/kg bw (McCurdy et al., 1966); 1,350 mg/kg bw (Charan and Sarda, 1967); 1,200 mg/kg bw (Krupin et al., 1970) and 1,260 mg/kg bw (Friedman et al., 1980). The only side effects observed in these oral therapies to patients with ocular disease or healthy controls were either none or one or more of nausea, headache and/or vomiting.

Guidelines for glycerol use in hyperhydration and rehydration associated with exercise report 28 studies in which oral doses between 500 mg/kg bw and 1,500 mg/kg bw were given in the total of 238 subjects (Van Rosendal et al., 2010). Three studies reported side effects after rapidly administering the glycerol as a concentrated bolus followed by fluid ingestion. Four subjects in two of the trials were nauseous after glycerol ingestion. In another study, two subjects developed diarrhoea in the 24 h after the trial. In a further three studies, a low incidence of gastrointestinal distress (bloatedness) or light-headedness were reported.

Using the studies of Wald and McLaurin (1982), the Panel noted that patients were administered glycerol orally as bolus doses of 500–1,000 mg/kg bw every 3–4 h, dependent on the patients' intracranial pressures (ICPs). Specific individual dosages ranged from 4,000 mg to 70,000 mg (average 54,000 mg) and it was administered via a nasogastric tube as a 50% solution (by mixing a 100%

glycerol solution with an equal volume of either 5% dextrose in water or 5% dextrose in 0.4% normal saline, depending upon the systemic electrolyte status). If no change in ICP was achieved or a significant volume of solution was aspirated from the stomach, intravenous mannitol was administered and another trial of the drug was initiated 4–24 h later. For the six patients in which data were reported in this study, maximum ICP reduction and maximum serum glycerol concentration occurred around 60–90 min after oral bolus ingestion. In most cases, the serum glycerol concentration returned to pre-treatment levels around 3 h after oral administration.

Given the dose- and time period-range reported in this study, the Panel calculated that the dose of glycerol required to induce a therapeutic reduction in ICP was within the range of 125–333 mg/kg bw per hour.

The Panel therefore considered that a conservative estimate of the lowest oral bolus dose of glycerol required for therapeutic effect was 125 mg/kg bw per hour. The Panel considered this dose would also be responsible for the side effects (nausea, headache and/or vomiting) observed in some patients.

Glycerol therapy in diabetics

Hyperosmolar non-ketotic coma occurred in diabetic patients after repeated use of oral and intravenous glycerol (e.g. Oakley and Ellis, 1976; Sear, 1976). According to Sear (1976), the non-ketotic hyperosmolar hyperglycaemic state usually occurs in cases of maturity onset diabetes or pre-diabetes and not in non-diabetic subjects.

3.6. Discussion

This re-evaluation refers exclusively to the uses and conditions of use of glycerol (E 422) as a food additive in food, and does not include a safety assessment of other uses of glycerol as described in Section 3.4.5.

The EU specification for glycerol content is not less than 98% on an anhydrous basis, whereas JECFA specifies a content not less than 99%. The Panel noted that in the EU specifications for glycerol (E 422) there is no specific limit for acrolein and while the EU specifications has a limit for chlorinated compounds, the identities of specific chlorinated compounds are not identified. The Panel considered that the identification of possible chlorinated impurities would be needed for their evaluation of toxicological significance.

According to the EU specifications for glycerol (E 422), impurities of the toxic elements lead, mercury, arsenic and cadmium are accepted up to a concentration of 2, 1, 3 and 1 mg/kg, respectively. The Panel considered that contamination at those levels would have a significant impact on the exposure to these metals for which the exposures are already close to the health-based guidance values established by EFSA (EFSA CONTAM Panel, 2009a,b, 2010a,b, 2012).

The Panel noted that glycidol may be present in glycerol from its manufacture from either refined oils and fats or when it is chemically synthesised. Glycidol is characterised as probably carcinogenic to humans 2A (IARC 2000) and as a carcinogenic and genotoxic compound by the EFSA CONTAM Panel (EFSA CONTAM Panel, 2016). The Panel considered that its presence in glycerol (E 422) would raise a safety concern.

The Panel also noted that in the synthesis pathway of glycerol via allyl chloride, epichlorohydrin is formed as an intermediate, which may be present in the final product. Epichlorohydrin is classified as carcinogen 2A according to IARC (1999) and the Panel considered that its presence in glycerol (E 422) would raise a safety concern.

Furthermore, some chemically synthesised preparations of glycerol, starting or intermediate chemicals used in the synthesis, including propene, allyl alcohol, propylene oxide, allyl chloride and dichlorohydrin may be present and there are no maximum limits for these substances in the EU specifications for glycerol (E 422).

The Panel considered that lactic acid bacteria may be used in manufacturing of some foods (e.g. yogurt, cheese, cultured butter, sour cream (with fat content above 20%), sausage, cucumber pickles, olives and sauerkraut) and that if glycerol (E 422) is added to those food items, acrolein may be formed.

The Panel noted that in addition to the information provided by CEFIC (2013a [Documentation provided to EFSA n. 3]), glycerol (E 422) can be produced by a variety of methods and that many of them lead to the presence or formation of contaminants in glycerol (E 422), which are of toxicological concern. This should be considered in the EU specifications for glycerol (E 422). The Panel considered

that the manufacturing process for glycerol (E 422) should not allow the production of a food additive which contains residuals of genotoxic or/and carcinogenic concern at a level which would result in a MOE below 10,000.

Glycerol occurs naturally in several types of lipid and is an endogenous metabolite in mammals. Glycerol is rapidly and near completely absorbed from the gastrointestinal tract; distributed into the total body water space and is primarily metabolised in the liver. After phosphorylation and oxidation, glycerol is used as an energy substrate via glycolysis or participates in gluconeogenesis and lipogenesis. Glycerol is extensively oxidised and exhaled as CO_2, with only minor amounts excreted via urine or faeces. The Panel noted that glycerol concentrations in blood higher than 92 mg/L would result in renal elimination of glycerol in rats (Ackerman et al., 1975) and humans (McCurdy et al., 1966).

Glycerol has a low acute toxicity.

Short-term or subchronic studies were not performed according to current test guidelines. In a subchronic toxicity study (in drinking water) in rats, the effects reported were observed with doses in the range of the LD_{50} for glycerol. The Panel considered that the local irritating effects of glycerol in the gastrointestinal tract reported in some gavage studies in rat (100% glycerol at 2,800 mg/kg bw per day, the lowest dose tested (Staples et al., 1967)), and dogs (100% glycerol at 5,600 mg/kg bw per day (Staples et al., 1967)) was likely due to the hygroscopic and osmotic effects of glycerol.

Glycerol did not show any genotoxic activity in different *in vitro* assays, which include negative results in the bacterial reverse mutation assay (Ames test), in chromosome aberration assays and in studies on DNA damage in mammalian cells. Questionable results obtained in a HGPRT gene mutation assay did not show a dose–response effect and were therefore judged of no biological significance. A lack of valid *in vivo* genotoxicity data was not of concern since clear negative findings were observed in *in vitro* assays. On this basis, the Panel considered that glycerol as a food additive did not raise concern with respect to genotoxicity.

From the available chronic toxicity and carcinogenicity studies, glycerol was not carcinogenic in mice and rats and did not show evidence of adverse effects in a 2-year chronic toxicity study. The Panel noted that no adverse effects were reported in rats receiving doses up to 10,000 mg/kg bw per day for 1 year, the highest dose tested. The Panel also noted that there was no increase in the tumours incidences in rats receiving doses up to 5,000 mg/kg bw per day for 2 years, the highest dose tested.

The reports of the two- and multigeneration reproductive toxicity studies have limitations but no adverse effects were reported. Prenatal developmental toxicity studies were also limited but showed no dose-related developmental and maternal effects up to the highest dose tested (1,280, 1,600 or 1,180 mg glycerol/kg bw per day for mice, rats and rabbits, respectively).

The Panel considered that none of the animal studies available identified an adverse effect for glycerol.

The Panel considered that the therapeutic oral use of glycerol at 1,000–1,500 mg/kg bw given as bolus in patients with glaucoma triggered an increase in plasma osmolality and dehydration, and resulted in side effects such as headache, nausea and/or vomiting in some individuals. Given the dose- and time period-range reported in the Wald and McLaurin (1982) study, the Panel calculated that the dose of glycerol required to induce a therapeutic reduction in ICP was within the range of 125–333 mg/kg bw per hour. The Panel considered that a conservative estimate of the lowest oral bolus dose of glycerol required for therapeutic effect was 125 mg/kg bw per hour. The Panel considered this dose would also be responsible for the side effects (nausea, headache and/or vomiting) observed in some patients.

The exposure estimates in all exposure scenarios resulted in overestimates of the exposure to glycerol (E 422) from its use as a food additive according to Annex II to Regulation (EC) No 1333/2008 (Section 3.4.1). Although, use levels and analytical data were only available for 28 out of the total of 68 food categories in which glycerol (E 422) is authorised for use according to Annex II, the Panel considered that this did not result in an underestimation of the exposure because:

- glycerol (E 422) belongs to Group I food additives, and therefore almost all the food categories for which glycerol (E 422) is authorised correspond to the general Group I food additives authorisation (Table 3). Given the information on the uses provided by food industry and information from the Mintel GNPD, it may be assumed that glycerol (E 422) is not used in some of these food categories.

- approximately 94% of the food products labelled with glycerol (E 422) in the Mintel GNPD belonged to food subcategories that were considered in the exposure assessment (Appendix C).

Furthermore, the Panel noted that the additional exposure to glycerol via natural sources, including wine, other alcoholic beverages and honey (the sources for which analytical data were available) did not significantly change the exposure to glycerol (E 422) (Appendix F). The Panel further noted that the exposure to glycerol from its use according the Annex III (Parts 1, 2, 3, 4 and 5) was only partly considered in the exposure assessment (via wine and other alcoholic beverages). The highest 95th percentile of exposure of glycerol (E 422) according to Annex II, carry-over (Annex III) and natural sources was, however, estimated at 460 mg/kg bw per day in children in the refined *non-brand-loyal exposure* scenario. The food categories contributing most to the mean exposure to glycerol (E 422) were bread and rolls and fine bakery wares for which brand-loyalty was not assumed relevant.

The Panel considered that the most relevant situation where an acute bolus exposure to glycerol used as a food additive can be similar to the one occurring during therapeutic use, is consumption of a beverage. Therefore, the Panel calculated the volume of flavoured drinks required to be consumed in order to exceed the acute bolus exposure (125 mg/kg bw per hour), calculated by the Panel, to be the minimum dose required to have a therapeutic effect. When considering the available data, the Panel noted that infants and toddlers can be exposed to more than 125 mg glycerol/kg bw per hour by drinking less than the volume of one can (330 mL) of a flavoured drink.

The Panel considered that the exposure to 3-MCPD coming from the use of glycerol (E 422) as a food additive at a maximum level of 0.1 mg 3-MCPD/kg glycerol, did not exceed the TDI of 0.8 μg/kg bw per day for 3-MCPD (EFSA CONTAM Panel, 2016) in any of the exposure assessment scenarios (maximum being up to 6% in the children population at the 95th percentile in the non-brand-loyal scenario; Section 3.4.3).

4. Conclusions

According to the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA ANS Panel, 2014) and given that:

- the safety assessment carried out by the Panel is limited to the use and use levels received from industry and Member States in 28 food categories out of 68 food categories in which glycerol (E 422) is authorised;
- the highest 95th percentile of exposure of glycerol (E 422) according to Annex II, carry-over (Annex III) and natural sources was estimated at 460 mg/kg bw per day in children in the refined *non-brand-loyal exposure* scenario;
- glycerol (E 422) as a food additive is identical to a compound which is a normal constituent in the body (an endogenous compound) and is a regular component of the diet;
- sufficient toxicity data were available;
- the toxicological studies in animals did not provide any indication for adverse effects, including at the highest dose tested in a chronic toxicity study (10,000 mg/kg bw per day);

the Panel concluded that there is no need for a numerical ADI for glycerol (E 422).

The Panel concluded that there is no safety concern regarding the use of glycerol (E 422) as a food additive according to Annex II and III (Part 1, 2, 3, 4 and 5) for the general population at the refined exposure assessment for the reported uses of glycerol as food additive.

However, the Panel identified that there remain uncertainties over the lack of identification and quantification of residuals especially those that are genotoxic and carcinogenic. The Panel noted that these residuals are mostly present when chemical synthesis is used to produce glycerol. The Panel concluded that the manufacturing process for glycerol should not allow the production of a food additive, which contains these residuals at a level which would result in a MOE below 10,000.

The Panel concluded that if 3-MCPD is present at its maximum authorised level of 0.1 mg 3-MCPD/kg glycerol, the maximum exposure to 3-MCPD was below the TDI of 0.8 μg/kg bw per day, and therefore exposure via glycerol (E 422) alone was of no concern.

The Panel could not calculate exposures to other genotoxic impurities or contaminants that may be present in glycerol (E 422) as a result of the manufacturing process, e.g. glycidol, due to the lack of data on their concentrations in the food additive.

The Panel concluded that infants and toddlers could be exposed to more than 125 mg/kg bw per hour by drinking less than the volume of one can (330 mL) of a flavoured drink. The Panel further concluded that the acute bolus exposure to glycerol (E 422) by its use as a food additive should stay below doses at which pharmacological or side effects could occur.

5. Recommendations

The Panel recommended that:

- given that during the manufacturing processes of glycerol, genotoxic impurities – e.g. glycidol, epichlorohydrin – could be formed, limits for such impurities should be included in the EU specifications for glycerol (E 422);
- given that during the manufacturing processes of glycerol, other potential impurities of toxicological concern (e.g. dichlorohydrin) could be formed, limits for such impurities should be included in the EU specifications for glycerol (E 422);
- more data should be generated to decrease uncertainty arising from the presence in the food additive (E 422) of compounds of toxicological concern (e.g. acrolein, 3-MCPD or 3-MCPD ester), which can be produced under some food processing conditions (e.g. use of glycerol (E 422) in parallel with lactic acid bacteria; use of glycerol (E 422) in food containing significant amounts of sodium chloride (more than 5%) and treated at temperatures above 160°C, etc.).
- a numerical limit for acrolein should be included in the EU specifications for glycerol (E 422);
- the maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for glycerol (E 422) should be revised in order to ensure that glycerol (E 422) as a food additive will not be a significant source of exposure to those toxic elements in food;
- more information on uses and use levels and analytical data should be made available to the Panel in order to perform an adequate exposure assessment, in particular in the case of estimate acute exposure, more data on flavoured drinks is needed.

Documentation provided to EFSA

1) AESGP (Association of the European Self-Medication Industry), 2013. Data on usage levels of glycerol (E 422) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted to EFSA on 29 November 2013.
2) BIBRA, 1993. Toxicity profile: glycerol. Submitted by CEFIC, February 2013.
3) CEFIC, 2013a. Flow chart manufacturing process vegetal glycerine and complementary documents. Submitted by CEFIC, February 2013.
4) CEFIC, 2013b. SIDS Initial Assessment Report for SIAM 14 (OECD SIDS, 2002). Submitted by CEFIC, February 2013.
5) Cutisin, 2010. Usages data provided to EFSA following a call for scientific data on miscellaneous food additives permitted in the EU and belonging to several functional classes. Submitted by Cutisin, sro Jilemnice, December 2010.
6) FDE (FoodDrinkEurope), 2013. Data on usage levels of glycerol (E 422) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted to EFSA on 29 November 2013.
7) ICGA (International Chewing Gum Association), 2013. Data on usage levels of glycerol (E 422) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted to EFSA on 29 November 2013.
8) Nutricia, 2011. Usages data provided to EFSA following a call for scientific data on miscellaneous food additives permitted in the EU and belonging to several functional classes. Submitted by Nutricia, June 2011.
9) Pre-evaluation document prepared by Fraunhofer, March 2012.

10) Riemser Arzneimittel AG, 2010. Usages data provided to EFSA following a call for scientific data on miscellaneous food additives permitted in the EU and belonging to several functional classes. Submitted by Riemser Arzneimittel AG, May 2010.

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Abbreviations

3-MCPD	3-monochloropropane-1,2-diol
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AESPG	Association of the European Self-Medication Industry
ALT	alanine aminotransferase
ANS	EFSA Scientific Panel on Food Additives and Nutrient Sources added to Food
AOAC	Association of Official Analytical Chemists
AST	aspartate aminotransferase
BfR	Bundesinstitut für Risikobewert
bw	body weight
CAS	Chemical Abstracts Service
CHO	Chinese hamster ovary
CONTAM	EFSA Panel on Contaminants in Food Chain
DMSO	dimethylsulfoxide
EMA	European Medicines Agency
FAO	Food and Agriculture Organization of the United Nations
FCS	food categorisation system
FDE	FoodDrinkEurope
FDRL	Food and Drug Research Laboratories
FID	flame ionisation detection
GC	gas chromatography
GD	gestation day
GNPD	Global New Products Database
HGPRT	hypoxanthine-guanine phosphoribosyltransferase
HPLC	high-performance liquid chromatography
IARC	International Agency for Research on Cancer
IC	ion chromatography
ICGA	International Chewing Gum Association
ICP	intracranial pressure
i.p.	intraperitoneal
IRMS	isotope ratio mass spectrometry
i.v.	intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives

LD_{50}	median lethal dose
LOD	limit of detection
LOQ	limit of quantification
MB	medium bound
MPL	maximum permitted level
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PS	physiololical saline
QS	*quantum satis*
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SCF	Scientific Committee on Food
SDA	The Soap and Detergent Association
TemaNord	is a publishing series for results of the often research-based work that working groups or projects under Nordic Council of Ministers have put in motion
TDI	tolerable daily intake
VCF	Volatile Compounds in Food
WADA	World Anti-Doping Agency
WHO	World Health Organization

Appendix A – Summary of the reported use levels (mg/kg or mg/L as appropriate) of glycerol (E 422) provided by industry

Food category number	Food category name	MPL	Number of samples	Usage level mean		Information provided by	Comments
				Typical mean (range)	Mean max (range)		
01.6.3	Other creams	QS	3	17,181.8 (50–50,000)	20,515.2 (50–60,000)	FDE	
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	QS	1	2.5	2.5	FDE	
03	Edible ices	QS	16	187.5 (8–930)	929.8	FDE	Non-milk-based ice-cream
			36	2,312.7 (0.6–20016.3)	20,016.3	FDE	Milk-based ice-cream
05.2	Other confectionery including breath freshening microsweets	QS	3	11,945.7 (5,837–15,000)	21,945.7 (5,837–30,000)	FDE	
05.3	Chewing gum	QS	1	17,100	100,000	ICGA	Used in most of chewing gum recipes (from 50% up to 90% of recipes depending on producers). Highly representative
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	QS	3 (1 NP)	67,723.3 (50,870–77,300)	90,766.7 (77,300–100,000)	FDE	*Without taking into account NP*
06.3	Breakfast cereals	QS	1	0.0017	0.0017	FDE	
07.1	Bread and rolls – except products in 7.1.1 and 7.1.2	QS	1 NP	30,000	31,000	FDE	Refers to 'chapattis' bread
07.2	Fine bakery wares	QS	6 (2 NP)	21,631 (0–56,000)	30,464.3 (0–63,000)	FDE	
08.3.1	Non-heat-treated meat products	QS	1	1,500	1,500	FDE	

Food category number	Food category name	MPL	Number of samples	Usage level mean		Information provided by	Comments
				Typical mean (range)	Mean max (range)		
08.3.3	Casings and coatings and decorations for meat	QS	1	250	250	FDE	
			–	1.1	1.9	Cutisin[(a)]	(20–30%) glycerol/dry solid of casing. Dry solid of casing approx 75%. Casing = (0.5–0.85%) by weight of foods (i.e. sausages)
12.2.2	Seasonings and condiments	QS	1	48,800	48,800	FDE	
12.5	Soups and broth	QS	5	2.9 (0.8–10)	3 (0.8–10)	FDE	
12.6	Sauces	QS	5	20,013.1 (4–50,000)	20,015.9 (4–50,000)	FDE	
12.8	Yeast and yeast products	QS	1	43.5	43.6	FDE	
13.2	Dietary food for special medical purposes (FSMP) regulated according to Directive 1999/21/EC	QS	–	390.0	1,180.0	Nutricia[(a)]	Typical level = 0.66 g/L, Maximum level = 2 g/L. Glycerol is used as a carrier in a nutrient preparation where it is present at a level of 59%. Levels in final foods: typical = 660 mg/ L x 59% = 390 mg/L; maximum: 2,000 mg/ L x 59% = 1,180 mg/L
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	QS	1	34,967	34,967	FDE	
16	Desserts excluding products covered in category 1, 3 and 4	QS	1	6	6	FDE	
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	QS	3	138,333.3 (2,000–330,000)	143,400 (2,200–330,000)	AESGP	
			3	4,682.8 (0.2–9,269.8)	9,269.8	Riemser Arzneimittel AG[(a)]	
17.3	Food supplements supplied in a syrup-type or chewable form	QS	1	85,000	94,000	AESGP	

MPL: maximum permitted level; QS: *quantum satis*; NP: Niche product.
(a): Call for data from 2010.

Appendix B – Summary of analytical results (mg/kg) of glycerol (E 422) provided by Members States

Food category number	Food category name	Restrictions	MPL	Number of samples	% LC	LOD		LOQ		All data – medium-bound				
						Min	Max	Min	Max	Min	Median	Mean	p95	Max
04.2.1	Dried fruit and vegetables		QS	11	100	0.4	0.4	2.0	500.0	0.2	250.0	227.3	250.0	250.0
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC		QS	31	0					40.0	83.0	116.1	278.0	442.0
07.2	Fine bakery wares		QS	1	0					19900.0	19900.0	19900.0	19900.0	19900.0
11.3	Honey as defined in Directive 2001/110/EC		–	289	6	5.0	5.0	10.0	15.0	7.5	40.0	77.7	265.1	1080.0
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		QS	1	0	0.4	0.4	2.0	2.0	390.0	390.0	390.0	390.0	390.0
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices		QS	167	31	0.4	500.0	1.0	2000.0	0.2	350.0	419.4	880.0	1470.0
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products		QS	3	100			500.0	500.0	250.0	250.0	250.0	250.0	250.0
14.1.4	Flavoured drinks		QS	5	40	500.0	500.0	1500.0	1500.0	250.0	3200.0	4820.0	12700.0	12700.0
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts		–	5,792	1	40.0	150.0	40.0	700.0	75.0	6575.0	6490.8	9300.0	61000.0
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts	Only alcohol-free	–	9	0	40.0	150.0	40.0	150.0	1300.0	7200.0	5460.0	8200.0	8200.0
14.2.3	Cider and perry		QS	252	0	40.0	500.0	40.0	1500.0	1100.0	3000.0	3068.1	4580.0	11110.0
14.2.4	Fruit wine and made wine		QS	117	0	80.0	166.7	200.0	500.0	700.0	4300.0	4237.5	6600.0	8700.0
14.2.5	Mead		QS	72	0	80.0	166.7	200.0	500.0	3300.0	5895.0	6445.4	9200.0	14200.0
14.2.7.1	Aromatised wines		QS	64	2	40.0	150.0	40.0	700.0	35.0	4655.0	4835.5	7440.0	16800.0
14.2.7.2	Aromatised wine-based drinks		QS	259	0	40.0	150.0	40.0	150.0	1800.0	7400.0	9328.8	23200.0	48800.0
14.2.7.3	Aromatised wine-product cocktails		QS	72	0	40.0	150.0	40.0	150.0	1600.0	3900.0	6386.1	23600.0	34900.0

Food category number	Food category name	Restrictions	MPL	Number of samples	% LC	LOD		LOQ		All data – medium-bound				
						Min	Max	Min	Max	Min	Median	Mean	p95	Max
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol		QS	78	0	40.0	166.7	40.0	700.0	900.0	3525.0	3889.5	6600.0	9600.0

% LC: % of left-censored data; p95: 95th percentile; LOD: limit of detection; LOQ: limit of quantification.

Appendix C – Number and percentage of food products labelled with glycerol (E 422) out of the total number of food products present in the Mintel GNPD per food subcategory between 2011 and 2016

Food subcategory[a]	Total number of products	Food products labelled with glycerol (E 422)	
		Number	%
Gum	1,423	942	66.2
Snack/cereal/energy bars	5,386	1,415	26.3
Cakes, pastries & sweet goods	13,966	3,498	25.0
Mixed assortments	332	63	19.0
Liquorice	819	103	12.6
Sticks, liquids & sprays	92	11	12.0
Meal kits	2,200	218	9.9
Sandwiches/wraps	2,765	197	7.1
Pastilles, gums, jellies & chews	4,018	239	5.9
Bread & bread products	10,721	612	5.7
Chocolate countlines	2,473	131	5.3
Lollipops	419	22	5.3
Sweet biscuits/cookies	18,562	909	4.9
Baking ingredients & mixes	9,565	387	4.0
Medicated confectionery	1,092	36	3.3
Non-individually wrapped chocolate pieces	5,531	178	3.2
Individually wrapped chocolate pieces	2,683	81	3.0
Other frozen desserts	1,690	48	2.8
Snack mixes	1,566	42	2.7
Chocolate tablets	8,871	212	2.4
Seasonal chocolate	5,765	130	2.3
Shelf-stable desserts	3,203	68	2.1
Boiled sweets	1,008	21	2.1
Toffees, caramels & nougat	1,990	39	2.0
Cold cereals	6,699	129	1.9
Dairy-based frozen products	8,342	151	1.8
Other natural sweeteners	723	12	1.7
Standard & power mints	898	13	1.4
Meat snacks	1,072	13	1.2
Marshmallows	522	6	1.1
Chilled desserts	6,588	68	1.0
Sports drinks	848	8	0.9
Dessert toppings	669	6	0.9
Other sugar confectionery	1,159	10	0.9
Water-based frozen desserts	1,282	10	0.8
Hot cereals	1,306	10	0.8
Meal replacements & other drinks	1,370	10	0.7
Flavoured alcoholic beverages	2,102	15	0.7
Caramel & cream spreads	310	2	0.6
Baby snacks	340	2	0.6
Vodka	620	3	0.5
Rice snacks	425	2	0.5
Hors d'oeuvres/canapes	4,262	20	0.5
Meat pastes & pates	3,183	12	0.4

Food subcategory[a]	Total number of products	Food products labelled with glycerol (E 422)	
		Number	%
Fruit snacks	3,759	14	0.4
Beverage concentrates	2,449	8	0.3
Pizzas	4,618	15	0.3
Energy drinks	1,772	5	0.3
Seasonings	10,041	26	0.3
Spoonable yogurt	10,427	26	0.2
Wheat & other grain-based snacks	2,076	5	0.2
Meat substitutes	2,540	6	0.2
Savoury biscuits/crackers	5,047	10	0.2
Prepared meals	11,608	20	0.2
Sucrose	1,172	2	0.2
Fish products	13,353	22	0.2
Chocolate spreads	1,223	2	0.2
Soft cheese desserts	1,610	2	0.1
Meat products	17,204	18	0.1
Other sauces & seasonings	984	1	0.1
Carbonated soft drinks	5,991	6	0.1
Pastry dishes	2,024	2	0.1
Wine	4,164	4	0.1
Malt & other hot beverages	1,072	1	0.1
Rice/nut/grain & seed based drinks	1,296	1	0.1
Poultry products	6,944	5	0.1
Fruit/flavoured still drinks	3,043	2	0.1
Liqueur	1,811	1	0.1
Table sauces	6,326	3	0.0
Processed cheese	2,229	1	0.0
Fruit	2,949	1	0.0
Soft cheese & semi-soft cheese	6,051	2	0.0
Pasta sauces	4,017	1	0.0
Confiture & fruit spreads	5,004	1	0.0
Pickled condiments	5,869	1	0.0
Beer	8,696	1	0.0
Total sample	**461,729**	**10,319**	**2.2[b]**

(a): According to Mintel food categorisation.
(b): In total, around 2.2% of the foods available on the Mintel GNPD are labelled with glycerol (E 422) between 2011 and 2016.[19]

Appendix D – Concentration levels of glycerol (E 422) used in the refined and acute exposure scenarios (mg/kg or mL/kg as appropriate)

Food category number	Food category name	MPL	Concentration levels used in the refined exposure assessment scenario		Comments
			Mean	High level[(b)]	
01.3	Unflavoured fermented milk products, heat-treated after fermentation	QS	–	–	Not taken into account (no concentration data)
01.4	Flavoured fermented milk products including heat-treated products	QS	–	–	Not taken into account (no concentration data)
01.6.3	Other creams	QS	–	–	Not taken into account (no corresponding FoodEx code)
01.7.1	Unripened cheese excluding products falling in category 16 – except mozzarella	QS	–	–	Not taken into account (no concentration data)
01.7.5	Processed cheese	QS	–	–	Not taken into account (no concentration data)
01.7.6	Cheese products (excluding products falling in category 16)	QS	–	–	Not taken into account (no concentration data)
01.8	Dairy analogues, including beverage whiteners	QS	–	–	Not taken into account (no concentration data)
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	QS	2.5	2.5	Reported use levels
02.3	Vegetable oil pan spray	QS	–	–	Not taken into account (no corresponding FoodEx code and no concentration data)
03	Edible ices	QS	1,659	20,016	Reported use levels
04.2.1	Dried fruit and vegetables	QS	227	250	Analytical data
04.2.2	Fruit and vegetables in vinegar, oil, or brine	QS	–	–	Not taken into account (no concentration data)
04.2.4.1	Fruit and vegetable preparations excluding compote	QS	–	–	Not taken into account (no concentration data)
04.2.5.4	Nut butters and nut spreads	QS	–	–	Not taken into account (no concentration data)
04.2.6	Processed potato products	QS	–	–	Not taken into account (no concentration data)
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	QS	116	278	Analytical data
05.2	Other confectionery including breath freshening microsweets	QS	11,946	30,000	Reported use levels
05.3	Chewing gum	QS	17,100	100,000	Reported use levels
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	QS	–	–	Not taken into account (no corresponding FoodEx code)
06.2.2	Starches	QS	–	–	Not taken into account (no concentration data)
06.3	Breakfast cereals	QS	0.0017	0.0017	Reported use levels

Food category number	Food category name	MPL	Concentration levels used in the refined exposure assessment scenario		Comments
			Mean	High level[b]	
06.4.2	Dry pasta – only gluten-free and/or pasta intended for hypoproteic diets in accordance with Directive 2009/39/EC	QS	–	–	Not taken into account (no concentration data)
06.4.4	Potato Gnocchi – except fresh refrigerated potato gnocchi	QS	–	–	Not taken into account (no concentration data)
06.4.5	Fillings of stuffed pasta (ravioli and similar)	QS	–	–	Not taken into account (no concentration data)
06.5	Noodles	QS	–	–	Not taken into account (no concentration data)
06.6	Batters	QS	–	–	Not taken into account (no corresponding FoodEx code and no concentration data)
06.7	Pre-cooked or processed cereals	QS	–	–	Not taken into account (no corresponding FoodEx code and no concentration data)
07.1	Bread and rolls – except products in 7.1.1 and 7.1.2	QS	30,000	31,000	Reported use levels
07.2	Fine bakery wares	QS	9,446	58,000	Reported use levels
08.3.1	Non-heat-treated meat products	QS	1,500	1,500	Reported use levels
08.3.2	Heat–treated meat products – except foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben	QS			
08.3.3	Casings and coatings and decorations for meat	QS	–	–	Not taken into account (no corresponding FoodEx code)
09.2	Processed fish and fishery products including molluscs and crustaceans	QS	–	–	Not taken into account (no concentration data)
09.3	Fish roe – only processed fish roe	QS	–	–	Not taken into account (no concentration data)
10.2	Processed eggs and egg products	QS	–	–	Not taken into account (no concentration data)
11.2	Other sugars and syrups	QS	–	–	Not taken into account (no concentration data)
11.3	Honey as defined in Directive 2001/110/EC	NA	78	265	Analytical data[a]
11.4.1	Table Top Sweeteners in liquid form	QS	–	–	Not taken into account (no Concentration data)
12.1.2	Salt substitutes	QS	–	–	Not taken into account (no corresponding FoodEx code and no concentration data)
12.2.2	Seasonings and condiments	QS	48,800	48,800	Reported use levels
12.3	Vinegars	QS	–	–	Not taken into account (no concentration data)
12.4	Mustard	QS	–	–	not taken into account (no concentration data)
12.5	Soups and broth	QS	3	10	Reported use levels
12.6	Sauces	QS	20,013	50,000	Reported use levels

Food category number	Food category name	MPL	Concentration levels used in the refined exposure assessment scenario		Comments
			Mean	High level[b]	
12.7	Salads and savoury based sandwich spreads	QS	–	–	not taken into account (no concentration data)
12.8	Yeast and yeast products	QS	44	44	Reported use levels
12.9	Protein products, excluding products covered in category 1.8	QS	–	–	Not taken into account (no concentration data)
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	QS	390	1180	Reported use levels
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	QS	34,967	34,967	Reported use levels
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009 – including dry pasta	QS	–	–	Not taken into account (no concentration data)
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices – only vegetable juices	QS	420	880	Analytical data
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products – only vegetable nectars	QS	–	–	Not taken into account (no corresponding FoodEx code)
14.1.4	Flavoured drinks	QS	4,820	12,700	Analytical data
14.1.5.2	Other – excluding unflavoured leaf tea; including flavoured instant coffee	QS	–	–	Not taken into account (no concentration data)
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts	NA	6,491	9,300	Analytical data[a]
14.2.3	Cider and perry	QS	3,068	4,580	Analytical data[a]
14.2.4	Fruit wine and made wine	QS	–	–	Not taken into account (no corresponding FoodEx code)
14.2.5	Mead	QS	–	–	Not taken into account (no corresponding FoodEx code)
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008 – except whisky or whiskey	QS	–	–	Not taken into account (no concentration data)
14.2.7.1	Aromatised wines	QS	8,065	23,600	Analytical data[a]
14.2.7.2	Aromatised wine-based drinks	QS			
14.2.7.3	Aromatised wine-product cocktails	QS			
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	QS	3,890	660	Analytical data[a]
15.1	Potato-, cereal-, flour- or starch-based snacks	QS	–	–	Not taken into account (no concentration data)
15.2	Processed nuts	QS	–	–	Not taken into account (no concentration data)

Food category number	Food category name	MPL	Concentration levels used in the refined exposure assessment scenario		Comments
			Mean	High level[(b)]	
16	Desserts excluding products covered in category 1, 3 and 4	QS	6	6	Reported use levels
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	QS	125,000	330,000	Reported use levels
17.2	Food supplements supplied in a liquid form	QS			
17.3	Food supplements supplied in a syrup-type or chewable form	QS			
18	Processed foods not covered by categories 1–17, excluding foods for infants and young children	QS	–	–	Not taken into account (no concentration data)

MPL: maximum permitted level; QS: *quantum satis*.
NA: not authorised according to Annex II to Regulation No 1333/2008.
(a): Levels taken into account when estimating exposure from glycerol (E 422) as a food additive and due to carry-over (Annex III) and from natural sources (i.e. honey, wines and other alcoholic beverages) *(data set 2).
(b): Maximum of reported use levels or p95 of analytical data.

Appendix E – Summary of total estimated exposure of glycerol (E 422) from its use as a food additive for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)

	No of subjects	Max scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	95th percentile	Mean	95th percentile	Mean	95th percentile
Infants							
Bulgaria (NUTRICHILD)	659	138	457	126	435	83	266
Germany (VELS)	159	128	442	109	392	74	257
Denmark (IAT 2006_07)	826	115	302	105	271	100	259
United Kingdom (DNSIYC_2011)	1,366	78	269	66	223	46	153
Italy (INRAN_SCAI_2005_06)	12	10	–	9	–	6	–
Toddlers							
Belgium (Regional_Flanders)	36	583	–	453	–	308	–
Bulgaria (NUTRICHILD)	428	390	769	330	701	220	415
Germany (VELS)	348	453	938	356	796	222	418
Denmark (IAT 2006_07)	917	290	484	235	400	223	368
Spain (enKid)	17	158	–	138	–	89	–
United Kingdom (NDNS-RollingProgrammeYears1-3)	185	300	601	229	451	153	307
United Kingdom (DNSIYC_2011)	1,314	221	497	175	406	123	261
Italy (INRAN_SCAI_2005_06)	36	178	–	161	–	95	–
Netherlands (VCP_kids)	322	425	785	324	674	236	413
Children							
Austria (ASNS_Children)	128	349	689	278	535	190	326
Belgium (Regional_Flanders)	625	463	856	360	674	240	402
Bulgaria (NUTRICHILD)	433	453	910	369	786	238	460
Czech Republic (SISP04)	389	351	727	277	594	176	349
Germany (EsKiMo)	835	269	510	210	420	155	285
Germany (VELS)	293	462	877	353	654	220	372
Denmark (DANSDA 2005-08)	298	318	552	242	421	212	351
Spain (enKid)	156	268	550	215	487	147	274
Spain (NUT_INK05)	399	278	489	216	421	157	269
France (INCA2)	482	326	632	270	528	131	246
United Kingdom (NDNS-RollingProgrammeYears1-3)	651	331	596	242	449	162	282
Greece (Regional_Crete)	838	251	518	220	472	112	222
Italy (INRAN_SCAI_2005_06)	193	239	518	199	449	131	276
Latvia (EFSA_TEST)	187	281	688	225	554	131	318
Netherlands (VCP_kids)	957	402	740	305	620	214	357
Netherlands (VCPBasis_AVL2007_2010)	447	480	852	358	652	239	384
Sweden (NFA)	1,473	424	783	303	561	183	316
Adolescents							
Austria (ASNS_Children)	237	217	444	175	361	122	232
Belgium (Diet_National_2004)	576	259	515	201	399	125	227
Cyprus (Childhealth)	303	128	283	101	217	74	149
Czech Republic (SISP04)	298	283	629	224	500	144	298

	No of subjects	Max scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	95th percentile	Mean	95th percentile	Mean	95th percentile
Germany (National_Nutrition_ Survey_II)	1,011	199	470	161	398	108	227
Germany (EsKiMo)	393	221	458	178	387	127	228
Denmark (DANSDA 2005-08)	377	186	339	148	287	118	210
Spain (AESAN_FIAB)	86	147	311	125	260	78	149
Spain (enKid)	209	212	481	165	370	121	265
Spain (NUT_INK05)	651	200	364	153	288	114	203
France (INCA2)	973	194	402	158	337	87	168
United Kingdom (NDNS-RollingProgrammeYears1-3)	666	207	384	157	303	100	175
Italy (INRAN_SCAI_2005_06)	247	154	326	121	289	88	200
Latvia (EFSA_TEST)	453	210	455	165	369	110	238
Netherlands (VCPBasis_AVL2007_2010)	1,142	327	608	248	473	162	290
Sweden (NFA)	1,018	263	500	195	369	123	222
Adults							
Austria (ASNS_Adults)	308	154	335	129	302	82	152
Belgium (Diet_National_2004)	1,292	181	411	145	325	95	198
Czech Republic (SISP04)	1,666	142	315	120	263	85	166
Germany (National_Nutrition_ Survey_II)	10,419	154	344	126	286	84	166
Denmark (DANSDA 2005-08)	1,739	117	229	94	197	79	143
Spain (AESAN)	410	111	249	93	208	61	127
Spain (AESAN_FIAB)	981	109	241	90	206	59	126
Finland (FINDIET2012)	1,295	129	274	102	225	73	149
France (INCA2)	2,276	130	263	105	218	70	142
United Kingdom (NDNS-RollingProgrammeYears1-3)	1,266	121	258	93	203	63	125
Hungary (National_Repr_Surv)	1,074	103	218	89	186	78	150
Ireland (NANS_2012)	1,274	147	289	111	225	82	154
Italy (INRAN_SCAI_2005_06)	2,313	89	186	73	153	59	125
Latvia (EFSA_TEST)	1,271	126	288	105	242	77	168
Netherlands (VCPBasis_AVL2007_2010)	2,057	186	379	143	312	100	189
Romania (Dieta_Pilot_Adults)	1,254	68	161	57	136	50	110
Sweden (Riksmaten 2010)	1,430	135	287	106	229	69	138
The elderly							
Austria (ASNS_Adults)	92	139	268	119	265	78	135
Belgium (Diet_National_2004)	1,215	121	247	97	208	71	130
Germany (National_Nutrition_ Survey_II)	2,496	129	269	109	242	76	139
Denmark (DANSDA 2005-08)	286	89	168	75	137	68	123
Finland (FINDIET2012)	413	111	246	90	210	64	133
France (INCA2)	348	111	225	91	202	71	148
United Kingdom (NDNS-RollingProgrammeYears1-3)	305	106	204	83	166	54	99
Hungary (National_Repr_Surv)	286	93	186	81	154	71	124
Ireland (NANS_2012)	226	120	239	99	209	68	130

	No of subjects	Max scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	95th percentile	Mean	95th percentile	Mean	95th percentile
Italy (INRAN_SCAI_2005_06)	518	84	169	72	151	62	133
Netherlands (VCPBasis_AVL2007_2010)	173	132	244	101	196	75	120
Netherlands (VCP-Elderly)	739	124	211	93	165	73	119
Romania (Dieta_Pilot_Adults)	128	76	171	65	156	61	146
Sweden (Riksmaten 2010)	367	114	235	93	192	57	102

–: 95th percentile of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a).

Appendix F – Refined estimated exposure assessment scenario considering food categories for which direct addition of glycerol is authorised (from Annex II to Regulation No 1333/2008), food categories which may contain glycerol due to carry-over and natural sources for which data were available (i.e. wines and other alcoholic beverages, and honey): data set 2

	Infants (12 weeks–11 month)	Toddlers (12–35 month)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Brand-loyal scenario						
Mean	9–126	138–453	199–369	102–248	59–151	66–124
95th percentile	223–435	400–796	420–786	217–500	141–325	156–274
Non-brand-loyal scenario						
Mean	6–100	89–308	112–240	74–163	51–104	57–83
95th percentile	153–267	261–418	222–460	149–298	116–201	102–154

Appendix G – Main food categories contributing to exposure to glycerol (E 422) using data set 2

Table G.1: Main food categories contributing to the exposure to glycerol (E 422) from its use as food additive according to Annex II to Regulation (EC) No 1333/2008 and from natural sources using the *brand-loyal refined exposure scenario* (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
03	Edible ices	–	14.0 (1)	8.8 (1)	–	–	–
05.2	Other confectionery including breath refreshening microsweets	–	–	41.2 (1)	45.8 (1)	8.0 (1)	–
07.1	Bread and rolls	30.4–93.0 (5)	11–84.7 (10)	22.6–66.7 (17)	29.1–55.5 (16)	32.4–72.4 (17)	34.8–84.1 (14)
07.2	Fine bakery wares	19.4–42.8 (3)	14.1–51.5 (9)	16.9–62.2 (16)	14.8–53.5 (15)	5.4–38.4 (16)	10.4–41 (12)
08.3	Meat products	7.7 (1)	5.6 (1)	–	–	–	–
12.6	Sauces	14.4–30.9 (3)	5.9–11.7 (3)	5–11.6 (7)	5.2–10.5 (9)	6.6–14 (9)	5.3–12.1 (7)
14.1.4	Flavoured drinks	8.4–29.4 (2)	6.5–40.3 (7)	7.8–40.8 (16)	8.6–43.1 (17)	5.1–31.7 (16)	6.3–9.8 (7)
14.2	Alcoholic beverages, including alcohol-free and low-alcohol counterparts	–	–	–	–	5.6–8.9 (5)	5.4–16 (8)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	36.2–76.1 (2)	27.6 (1)	9.2 (1)	–	5.1–9.7 (3)	5.9–9.4 (2)

–: Food categories not contributing or contributing less than 5% to the total mean exposure.
(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific population.

Table G.2: Main food categories contributing to exposure to glycerol (E 422) from its use as food additive according to Annex II to Regulation (EC) No 1333/2008 and from natural sources using the *non-brand-loyal refined exposure scenario* (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
05.2	Other confectionery including breath refreshening microsweets	–	–	36.8 (1)	41.0 (1)	5.7 (1)	–
07.1	Bread and rolls	47.9–94.7 (5)	23.3–86.3 (10)	6.3–74.8 (18)	45.5–74.5 (16)	47.8–80.7 (17)	54.1–86.9 (14)
07.2	Fine bakery wares	8.7–16.7 (3)	8.9–20.3 (9)	8.4–29.9 (16)	7.5–23.7 (15)	8.2–15.2 (14)	6.1–16.6 (11)
08.3	Meat products	16.9 (1)	12.0 (1)	5.0 (1)	–	–	–
12.6	Sauces	13.5–22.5 (3)	6.1–10.3 (5)	5.0–15.0 (10)	6.6–10.6 (10)	6.0–12.8 (10)	5.0–11.9 (9)
14.1.4	Flavoured drinks	6.6–17.6 (2)	5.4–26.6 (7)	5.7–28.8 (17)	7.8–33.3 (17)	6.2–20.6 (15)	5.3–7.6 (6)
14.2	Alcoholic beverages, including alcohol-free and low-alcohol counterparts	–	–	–	–	5.2–9.7 (9)	6.0–16 (10)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	26.0–65.2 (2)	27.8 (1)	10.3 (1)	–	5.7 (1)	5.1–6.3 (2)

–: Food categories not contributing or contributing less than 5% to the total mean exposure.
(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific population.

Appendix H – Mean and 95th percentile of acute[a] exposure to glycerol (E 422) per food category and population group (mg/kg bw per meal)

Food category number	Food category name	High level[b] used in the calculation (in mg/kg)	Infants		Toddlers		Children		Adolescents		Adults		The elderly	
			Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95
02.1	Fats and oils essentially free from water (excluding anhydrous milkfat)	2.5[c]					< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
02.2	Fat and oil emulsions mainly of type water-in-oil	2.5[c]	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
03	Edible ices	20,000 (milk-based) 930 (water-based)[c]	25.4	129.0	55.1	127.3	49.1	124.2	25.9	64.2	17.7	44.9	15.9	37.1
04.2	Processed fruit and vegetables – dried fruit	250[d]	0.2	0.9	0.2	0.6	0.2	0.4	0.1	0.2	0.1	0.3	0.1	0.3
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	278[d]	0.2	0.4	0.2	0.6	0.2	0.5	0.1	0.4	0.1	0.3	0.1	0.2
05.2	Other confectionery including breath refreshening microsweets	30,000[c]	36.9	90.5	45.3	125.0	24.6	81.8	18.1	52.9	14.1	42.9	7.0	23.6
05.3	Chewing gum	100,000[c]			5.3	11.3	12.1	31.3	7.1	22.6	7.2	21.7	7.0	24.1
06.3	Breakfast cereals	0.0017[c]	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
07.1	Bread and rolls	31,000[c]	119.5	204.4	87.9	170.3	67.0	148.3	41.8	96.0	31.4	69.3	28.4	60.4
07.2	Fine bakery wares	58,000[c]	145.2	320.2	148.5	394.2	135.3	375.3	81.9	236.3	60.7	181.7	46.6	155.4
12.2	Herbs, spices, seasonings	48,800[c]	1.4	4.6	2.2	6.9	2.2	5.9	1.5	4.2	1.7	5.1	1.6	5.1
12.5	Soups and broths	10[c]	1.7	10.8	2.9	11.4	2.4	8.3	0.5	2.4	0.4	2.0	0.2	1.0
12.6	Sauces	50,000[c]	5.4	12.6	6.3	16.9	31.5	99.8	28.7	96.1	26.1	90.1	23.3	80.0
12.8	Yeast and yeast products	44[c]	0.3	1.7	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.1	< 0.01
14.1.2	Fruit and vegetable juices	880[d]	5.1	11.6	5.5	14.2	5.2	11.8	4.3	10.2	2.5	5.9	2.0	3.9
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	880[d]					7.3	13.5	3.9	7.6	5.6	21.6	2.2	5.2

Food category number	Food category name	High level[(b)] used in the calculation (in mg/kg)	Infants		Toddlers		Children		Adolescents		Adults		The elderly	
			Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95
14.1.4	Flavoured drinks	12,700[(d)]	178.0	331.3	135.2	254.0	91.0	224.1	66.8	155.5	51.7	120.0	34.6	80.4
16	Desserts excluding products covered in category 1, 3 and 4	6[(c)]			2.5	12.9	0.3	2.2	0.5	1.6	1.6	3.1	0.2	1.9

P95: 95th percentile.
(a): Acute exposure was calculated by combining a high concentration of glycerol (E 422) with the consumed amount of the food category per individual and meal.
(b): Max of the usage data or p95 of the analytical data.
(c): Usage levels.
(d): Analytical data.