Safety evaluation of certain food additives and contaminants

Food and Agriculture Organization of the United Nations

World Health Organization

Safety evaluation of certain food additives and contaminants

CONTENTS[1]

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[1]	Monographs on two contaminant groups (non-dioxin-like polychlorinated biphenyls and pyrrolizidine alkaloids) discussed at the eightieth meeting of the Joint FAO/WHO Expert Committee on Food Additives will be published as separate supplements in the WHO Food Additives series.

PREFACE

The monographs contained in this volume were prepared at the eightieth meeting of the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA), which met at FAO headquarters in Rome, Italy, on 16–25 June 2015. These monographs summarize the data on selected food additives reviewed by the Committee. Monographs on two contaminant groups discussed at the meeting will be published as separate supplements in the WHO Food Additives series.

The eightieth report of JECFA will be published by WHO in the WHO Technical Report series. Reports and other documents resulting from previous meetings of JECFA are listed in Annex 1. The participants in the meeting are listed in Annex 3 of the present publication. A summary of the conclusions of the Committee with respect to the food additives discussed at the meeting is given in Annex 4.

JECFA serves as a scientific advisory body to FAO, WHO, their Member States and the Codex Alimentarius Commission, primarily through the Codex Committee on Food Additives, the Codex Committee on Contaminants in Food and the Codex Committee on Residues of Veterinary Drugs in Foods, regarding the safety of food additives, residues of veterinary drugs, naturally occurring toxicants and contaminants in food. Committees accomplish this task by preparing reports of their meetings and publishing specifications or residue monographs and dietary exposure and toxicological monographs, such as those contained in this volume, on substances that they have considered.

The monographs contained in this volume are based on working papers that were prepared by WHO experts. A special acknowledgement is given at the beginning of each monograph to those who prepared these working papers. The monographs were edited by M. Sheffer, Ottawa, Canada.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the organizations participating in WHO concerning the legal status of any country, territory, city or area or its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the organizations in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biological or toxicological properties of or dietary exposure to the compounds evaluated in this publication should be addressed to: WHO Joint Secretary of the Joint FAO/WHO Expert Committee on Food Additives, Department of Food Safety and Zoonoses, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

SPECIFIC FOOD ADDITIVES

Benzoates: dietary exposure assessment

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1. Explanation

At the request of the Codex Committee on Food Additives (CCFA) at its Forty-sixth Session (FAO/WHO, 2014), the Committee evaluated dietary exposure to benzoic acid salts (benzoates). The Twenty-seventh Session of the Codex Alimentarius Commission (FAO/WHO, 2004) adopted the maximum level of benzoates (600 mg/kg) in Codex General Standard for Food Additives (GSFA) food category 14.1.4 on an interim basis with the understanding that a review would be conducted within 3 years. The safety of benzoates had been reviewed at the forty-sixth meeting of the Committee (Annex 1, reference *122*), and the group was assigned an acceptable daily intake (ADI) of 0–5 mg/kg body weight (bw), expressed as benzoic acid.

The fifty-first meeting of the Committee (Annex 1, reference *137*) assessed dietary exposure to benzoates from all categories of food based on maximum limits specified in national standards and in the GSFA. The estimates of national exposures for consumers at the mean, based on national maximum limits, were below the upper bound of the ADI, ranging from 0.18 mg/kg bw per day (in Japan) to 2.3 mg/kg bw per day (in the USA). The estimated exposures at higher percentiles, based on food additive levels in national standards, exceeded

the upper bound of the ADI in some cases (7.3 mg/kg bw per day, 150% of the upper bound of the ADI, in the USA; or 14 mg/kg bw per day, 280% of the upper bound of the ADI, in China). The Committee stated, "Because diets differ among countries, the foods that contribute most to benzoate intake would be expected to vary" (the present Committee noted that varying use levels in similar products across countries would also affect the order of importance of their contribution to dietary exposure to benzoates). For Australia, France, New Zealand, the United Kingdom and the USA, the GSFA food category that contributed the most to dietary exposure to benzoates was carbonated water-based flavoured drinks (GSFA food category 14.1.4.1). In Finland, 40% of the benzoates used in food was in soft drinks. Soya sauce was the main source in China and the second most important source in Japan.

2. Data submitted or available to the Committee

Maximum use levels of benzoates in 59 food categories from the GSFA have been adopted. Although all data necessary for an assessment of dietary exposure to benzoates were requested, only benzoate use levels reported from industries for non-alcoholic beverages (seven subcategories of GSFA food category 14.1) were submitted for this assessment. A total of 796 individual branded products from six countries – Australia ($n = 18$), Brazil ($n = 284$), China ($n = 33$), Mexico ($n = 152$), South Africa ($n = 42$) and the USA ($n = 267$) – were submitted by the International Council of Beverages Associations (ICBA). Table 1 summarizes the submitted data. Use levels of "0" were reported when there was no use of benzoates; the use level of benzoates in GSFA food category 14.1.5 (Coffee, coffee substitutes, tea, herbal infusions, and other hot cereal and grain beverages, excluding cocoa) was reported to be "0" by all six countries. The use level of benzoates in GSFA food category 14.1.3.4 (Concentrates for vegetable nectar) from Mexico was reported as "0"; the remaining countries did not submit any data for this food category. The "average typical" and "maximum" reported use levels of benzoates for GSFA food category 14.1.2.1 (fruit juice) were reported only from the USA – 126 mg/L and 229 mg/L, respectively. The average typical and maximum reported use levels of benzoates for GSFA food category 14.1.4 (water-based flavoured drinks) ranged from 83 to 209 mg/L and from 173 to 627 mg/L, respectively.

Norway submitted use level information for 86 products: soft drinks (weighted average 132.4 mg/L, maximum reported 148 mg/L), saft (a concentrate produced from fruit juice; weighted average 121.7 mg/L, maximum reported 173 mg/L) and flavoured water (weighted average 109 mg/L, maximum reported 131 mg/L). The data from Norway are also summarized in Table 1.

Table 1
Reported benzoate use levels submitted by ICBA and Norway

Country	GSFA food category	Number of entries	Average typical reported use level (mg/L)	Maximum reported use level (mg/L)
Australia	14.1.2.1 Fruit juice	0	NA	NA
	14.1.2.3 Concentrates for fruit juice	0	NA	NA
	14.1.3.1 Fruit nectar	0	NA	NA
	14.1.3.3 Concentrates for fruit nectar	0	NA	NA
	14.1.3.4 Concentrates for vegetable nectar	0	NA	NA
	14.1.4 Water-based flavoured drinks, including "sport", "energy", or "electrolyte" drinks and particulated drinks	18	103	173
	14.1.5 Coffee, coffee substitutes, tea, herbal infusions, and other hot cereal and grain beverages, excluding cocoa	0	NA	NA
Brazil	14.1.2.1 Fruit juice	0	NA	NA
	14.1.2.3 Concentrates for fruit juice	0	NA	NA
	14.1.3.1 Fruit nectar	101	17	339
	14.1.3.3 Concentrates for fruit nectar	0	NA	NA
	14.1.3.4 Concentrates for vegetable nectar	0	NA	NA
	14.1.4 Water-based flavoured drinks, including "sport", "energy", or "electrolyte" drinks and particulated drinks	179	209	371
	14.1.5 Coffee, coffee substitutes, tea, herbal infusions, and other hot cereal and grain beverages, excluding cocoa	4	0	0
China	14.1.2.1 Fruit juice	0	NA	NA
	14.1.2.3 Concentrates for fruit juice	0	NA	NA
	14.1.3.1 Fruit nectar	1	0	0
	14.1.3.3 Concentrates for fruit nectar	0	NA	NA
	14.1.3.4 Concentrates for vegetable nectar	0	NA	NA
	14.1.4 Water-based flavoured drinks, including "sport", "energy", or "electrolyte" drinks and particulated drinks	28	83	175
	14.1.5 Coffee, coffee substitutes, tea, herbal infusions, and other hot cereal and grain beverages, excluding cocoa	4	0	0
Mexico	14.1.2.1 Fruit juice	2	0	0
	14.1.2.3 Concentrates for fruit juice	0	NA	NA
	14.1.3.1 Fruit nectar	1	0	0
	14.1.3.3 Concentrates for fruit nectar	0	NA	NA
	14.1.3.4 Concentrates for vegetable nectar	4	0	0
	14.1.4 Water-based flavoured drinks, including "sport", "energy", or "electrolyte" drinks and particulated drinks	144	148	368
	14.1.5 Coffee, coffee substitutes, tea, herbal infusions, and other hot cereal and grain beverages, excluding cocoa	1	0	0

Table 1 (continued)

Country	GSFA food category	Number of entries	Average typical reported use level (mg/L)	Maximum reported use level (mg/L)
South Africa	14.1.2.1 Fruit juice	2	0	0
	14.1.2.3 Concentrates for fruit juice	0	NA	NA
	14.1.3.1 Fruit nectar	1	0	0
	14.1.3.3 Concentrates for fruit nectar	0	NA	NA
	14.1.3.4 Concentrates for vegetable nectar	0	NA	NA
	14.1.4 Water-based flavoured drinks, including "sport", "energy", or "electrolyte" drinks and particulated drinks	38	97	352
	14.1.5 Coffee, coffee substitutes, tea, herbal infusions, and other hot cereal and grain beverages, excluding cocoa	1	0	0
USA	14.1.2.1 Fruit juice	10	126	229
	14.1.2.3 Concentrates for fruit juice	2	64	168
	14.1.3.1 Fruit nectar	2	0	0
	14.1.3.3 Concentrates for fruit nectar	1	0	0
	14.1.3.4 Concentrates for vegetable nectar	0	NA	NA
	14.1.4 Water-based flavoured drinks, including "sport", "energy", or "electrolyte" drinks and particulated drinks	250	194	627
	14.1.5 Coffee, coffee substitutes, tea, herbal infusions, and other hot cereal and grain beverages, excluding cocoa	2	0	0
Norway	14.1.4 Soft drink, sugar	27	127[a]	147
	Saft, sugar	17	110[a]	173
	Soft drink, sweetener	15	142[a]	148
	Saft, sweetener	15	135[a]	173
	Nectar, sweetener	0	0[a]	0
	Flavoured water	12	109[a]	131

GSFA: Codex General Standard for Food Additives; ICBA: International Council of Beverages Associations; NA: not available
[a] Weighted average (mg/L).

The Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) database contains 68 measurements of benzoates in food category 14.1.2.1 (fruit juice, not detected [nd] – 580 mg/L), two measurements of benzoates in GSFA food category 14.1.3.1 (fruit nectar, nd) and 74 measurements of benzoates in GSFA food category 14.1.4 (water-based flavoured drinks, nd–150 mg/L), as provided by the Hong Kong Special Administrative Region (SAR) of China (see Appendix 1). Analytically determined average concentrations of benzoates in non-alcoholic beverages have been reported in the literature to range from 63.1 to 259 mg/L: Brazil (259 mg/L; Tfouni & Toledo, 2002), France (71.2 mg/L; Bemrah, Leblanc & Volatier, 2008), Lebanon (120 mg/L from 10 samples; Soubra et al., 2007) and New Zealand (63.1

mg/L in soft drinks; Cressey & Jones, 2009). Australia also measured benzoate concentrations in 18 soft drinks (nine cola and nine non-cola) in the 21st Australian Total Diet Study: 0–150 mg/L (average 17 mg/L) and 145–350 mg/L (average 220 mg/L), respectively (FSANZ, 2005).

Overall, from the review of submitted data and the literature, the Committee noted that out of the 59 food categories for which maximum benzoate use levels were adopted by CCFA in the GSFA, non-alcoholic beverages (GSFA food category 14.1) provide the primary source of dietary exposure to benzoates. Moreover, the Committee noted that the available data set (reported use levels from industries, analytical measurements from countries) reviewed at the meeting shows good consistency between the average concentrations of benzoates when they are used in the GSFA food category of water-based flavoured drinks (category 14.1.4) and in overall non-alcoholic beverages. The average typical reported concentrations from industries ranged from 83 to 209 mg/L (category 14.1.4); the published analytically quantified measurements for non-alcoholic beverages from various countries ranged from 63 to 259 mg/L. These concentrations were much lower than national maximum limits (150–400 mg/L) or limits for GSFA food category 14.1.4 (600 mg/L).

3. Assessment of dietary exposure

3.1 Dietary exposure to benzoates from non-alcoholic beverages

The Committee reviewed dietary exposure estimates submitted by the ICBA for four countries (Brazil, Mexico, South Africa and the USA), performed by combining individual consumption data with maximum reported use levels or national maximum permitted levels for non-alcoholic beverages. The Committee concluded that the information would not be appropriate for this assessment because maximum reported use levels or national maximum permitted levels were used in place of measured or average typical use levels. However, the Committee decided to make its own exposure estimates for these countries using consumption figures from the submitted data combined with average typical use levels.

Norway also submitted estimates of exposure to benzoates from soft drinks, saft and flavoured water. The consumption data on soft drinks, saft and flavoured water from two national food consumption surveys, Smabarnskost 2007 and Norkost 3, were combined with the weighted average benzoate use levels derived using Norwegian sales volumes to estimate benzoate exposure. The estimates of benzoate exposure for the general population were 0.73–1.1 mg/kg bw per day. The 95th percentile exposure estimates for children up to 2 years of

age were up to 3.4 mg/kg bw per day, whereas those for adult males reached 2.0 mg/kg bw per day.

The Committee also prepared international estimates of dietary exposure to benzoates using non-alcoholic beverage consumption levels from the FAO/WHO Chronic Individual Food Consumption Data – Summary statistics (CIFOCOss) database and information on average typical reported use levels of benzoates in water-based flavoured drinks (as non-alcoholic beverages in CIFOCOss consist primarily of water-based flavoured drinks) from various countries (83–209 mg/L). A total of 131 consumption data from 25 countries belonging to 10 GEMS/Food clusters were used, according to age/class in CIFOCOss. The weighted mean and the consumers-only weighted mean consumptions for beverages at FoodEx level 3 (European Food Safety Authority consolidated food consumption survey, classification for non-alcoholic beverages, not elsewhere specified) were estimated if several level 3 codes were presented from the same survey. The Committee was not able to take into account disparity in exposure estimates when different foods (beverages) were represented in the surveys that make up the FoodEx system. The Committee concluded that any differences would be small, as different beverages are generally consumed in similar volumes (e.g. lemonade versus carbonated beverages).

Table 2 summarizes the exposure estimates. None of the mean exposure estimates for consumers of non-alcoholic ("soft") beverages exceeded the upper bound of the ADI: 0.3–4.1 mg/kg bw per day for toddlers and young children, 0.2–2.7 mg/kg bw per day for other children, including adolescents, and 0.1–1.7 mg/kg bw per day for adults (see also Appendix 2). However, the Committee noted that the 95th percentile exposures for consumers-only reached or exceeded the upper bound of the ADI in some cases: up to 10.9 mg/kg bw per day for toddlers and young children and up to 7.0 mg/kg bw per day for other children, including adolescents.

3.2 **Total dietary exposure to benzoates**

The Committee conducted a review of the literature published since 2000 to obtain estimates of total dietary exposure to benzoates in 15 countries where benzoate use is legally permitted. The details are given in Appendix 3, and the estimates are summarized in Table 3. The Committee noted that the comparison of dietary exposures between studies is difficult because of differences in the methodologies used and assumptions made in the exposure assessments. The estimates were made by combining mean analytically measured levels (Australia, Austria, Belgium, Brazil, China, Denmark, Lebanon, New Zealand, the Republic of Korea, Saudi Arabia and Serbia) or means from use level surveys (France, Ireland, Italy and the United Kingdom) of benzoates in food with their consumption levels

Table 2

Benzoate exposure for consumers of non-alcoholic ("soft") beverages (CIFOCOss data plus Committee-prepared estimates)

Age group	Mean exposure (mg/kg bw per day)	95th percentile exposure (mg/kg bw per day)
Toddlers and young children (1–7 years)	0.3–4.1	1.7–10.9[a]
Other children, including adolescents (8–17 years)	0.2–2.7	0.5–7.0
Adults (18+ years)	0.1–1.7	0.2–4.2

bw: body weight; CIFOCOss: FAO/WHO Chronic Individual Food Consumption Data – Summary statistics
[a] 97.5th percentile exposure from South Africa.

Table 3

Literature-derived dietary exposure estimates for benzoates from all foods (including non-beverage uses)

Age group	General population exposure (mg/kg bw per day)		Consumers-only exposure (mg/kg bw per day)	
	Mean	95th percentile	Mean	95th percentile
Toddlers and young children (1–7 years)	1.5	3.9	0.9–6.8	2.0–9
Other children, including adolescents (8–17 years)	0.1–1.0	0.4–2.6	0.2–4	1.1–8
Adults (18+ years)	0.04–1.3	0.2–2.9	0.1–6.2	0.7–5.5

bw: body weight

from national consumption surveys (24-hour recall or intake record and/or food frequency questionnaire). Dietary exposure estimates from Austria were based on food consumption by consumers-only.

Most of the reported estimates for mean and high percentile exposures for the general population were below the upper bound of the ADI. Average analytically measured concentrations in samples with benzoate concentrations above the limit of quantification were used for exposure estimates, assuming that consumers only consume foods that could contain benzoates. Average concentrations of benzoates in all analysed samples were used for exposure estimates when assuming that consumers randomly consume foods that may or may not contain benzoates.

The estimated exposures to benzoates were below the upper bound of the ADI for preschool children (1.6 mg/kg bw per day, 31.8% of the upper bound of the ADI), males (1.6 mg/kg bw per day, 31.4% of the upper bound of the ADI) and females (1.8 mg/kg bw per day, 35.6% of the upper bound of the ADI) when assuming that consumers randomly consume foods that may contain benzoates. The estimated exposures to benzoates exceeded the upper bound of the ADI for

preschool children (6.8 mg/kg bw per day, 136% of the upper bound of the ADI), males (5.9 mg/kg bw per day, 118% of the upper bound of the ADI) and females (6.2 mg/kg bw per day, 124% of the upper bound of the ADI) when assuming that consumers only consume foods that could contain benzoates. Denmark reported similar results: exposures for 90th percentile consumers who always consume foods that contain benzoates exceeded the upper bound of the ADI, with the highest value of 9 mg/kg bw per day for boys aged 4–6 years. The Committee noted that the results are likely overestimates, because consumers do not select foods that contain benzoates all of the time.

Saudi Arabia had no national food consumption data, but estimated consumption was based on a quantitative frequency questionnaire completed by 100 students aged 18–25 years who recorded their food consumption during a week. The Committee noted that the exposures from Saudi Arabia exceeded the upper bound of the ADI because of a high-level consumption of non-alcoholic drinks (5.3 mg/kg bw per day) and yogurt rice dressing (5.2 mg/kg bw per day). More intake estimations, using a wide range of population groups and more food items, are needed in order to ascertain whether the exposure of the broader population is also above the upper bound of the ADI.

The largest contributor to estimated dietary exposure to benzoates was non-alcoholic beverages (up to 80% for the general population of Brazil) for most countries, but fish products (42.6–68.1%, Austria), meat products (68%, Italy, adults), soups and sauces (57%, United Kingdom), ketchup and tomato products (36.1%, Serbia) and seasoning (31.8%, China, 3- to 6-year-old female children) were also reported to be important contributors. China (Hong Kong SAR) also reported that soft drinks contributed to about 80% of dietary exposure to benzoates for children consuming the beverages and high beverage consumers (Ma et al., 2009). Therefore, the Committee noted that regional differences between diets should be taken into consideration, as well as different age groups with high consumption patterns.

Overall, from the literature review, the Committee noted that most of the reported estimates of dietary exposure to benzoates for the general population did not exceed the upper bound of the ADI. However, the dietary exposure for the consumers-only group reached or even exceeded the upper bound of the ADI, with dietary exposure of high percentile young consumers up to 9 mg/kg bw per day, owing primarily to their consumption of non-alcoholic beverages. For some adults, exceedance of the upper bound of the ADI in mean consumers (dietary exposure up to 6.2 mg/kg bw per day) was also noted, owing to the consumption of fish products, fruit and vegetable juices, and yogurt rice dressing.

4. Comments

4.1 Data submitted or available to the Committee

The Committee received data on "average typical" levels of benzoates in foods (796 individual branded products) for seven subcategories of GSFA food category 14.1 from six countries – Australia, Brazil, China, Mexico, South Africa and the USA – through the ICBA as well as use level data for 86 products from Norway. The average typical and maximum reported use levels of benzoates in GSFA food category 14.1.4 (water-based flavoured drinks) ranged from 83 to 209 mg/L and from 131 to 627 mg/L, respectively.

The Committee additionally evaluated published data on dietary exposures to benzoates from all foods at a national level. Information published since 2000 from 15 countries was considered. The estimates were made by combining mean analytically measured levels (Australia, Austria, Belgium, Brazil, China, Denmark, Lebanon, New Zealand, the Republic of Korea, Saudi Arabia and Serbia) or means from use level surveys (France, Ireland, Italy and the United Kingdom) of benzoates in food with their consumption levels from national consumption surveys (24-hour recall or intake record and/or food frequency questionnaire). Norway also submitted an estimate of benzoate exposure from soft drinks, saft (a concentrate produced from fruit juice) and flavoured water.

4.2 Assessment of dietary exposure

The Committee reviewed dietary exposure estimates submitted by the ICBA for four countries (Brazil, Mexico, South Africa and the USA), performed by combining individual consumption data with maximum reported use levels or national maximum permitted levels for non-alcoholic beverages. The Committee concluded that the information would not be appropriate for this assessment because maximum reported use levels or national maximum permitted levels were used in place of measured or average typical use levels. However, the Committee decided to make its own exposure estimates for these countries using consumption figures from the submitted data combined with average typical use levels. The Committee also prepared exposure estimates using food consumption data for non-alcoholic beverages from the CIFOCOss database. These estimates are summarized in Table 2 (see section 3.1). A total of 131 consumption data from 25 countries belonging to 10 GEMS/Food clusters were used. Additionally, as previously noted, the Committee also evaluated published estimates of total dietary exposure to benzoates (including non-beverage uses). These are summarized in Table 3 (see section 3.2).

Overall, the largest contributions to total estimated dietary exposure to benzoates were from non-alcoholic beverages (up to 80% for the general population of Brazil) for most countries.

5. Evaluation

Based on the available data set (reported use levels from industries and analytical measurements from the literature), the Committee noted that there is consistency in the average typical range of concentrations for benzoates used in the GSFA food subcategory for non-alcoholic ("soft") beverages (GSFA food category 14.1). For example, typical reported concentrations from industries ranged from 83 to 209 mg/L for water-based flavoured drinks (food category 14.1.4), and analytically quantified measurements ranged from 63 to 259 mg/L for non-alcoholic beverages (food category 14.1). These levels are lower than national maximum limits (150–400 mg/L) or limits for GSFA food category 14.1.4 (600 mg/L). The Committee also noted that most of the reported estimates for mean and high percentile per capita benzoate exposure were below the upper bound of the ADI, despite different methodologies and assumptions applied in the preparation of the exposure estimates.

None of the mean exposure estimates for consumers of non-alcoholic ("soft") beverages exceeded the upper bound of the ADI: 0.3–4.1 mg/kg bw per day for toddlers and young children, 0.2–2.7 mg/kg bw per day for other children, including adolescents, and 0.1–1.7 mg/kg bw per day for adults. However, the Committee noted that the 95th percentile exposures for the consumers-only group exceeded the upper bound of the ADI in some cases: up to 10.9 mg/kg bw per day for toddlers and young children and up to 7.0 mg/kg bw per day for other children, including adolescents. Additionally, the Committee noted that in some countries, the overall dietary exposure to benzoates for toddlers, young children and adolescents also exceeds the upper bound of the ADI at the high percentiles. Reduction of those exposures exceeding the upper bound of the ADI would require consideration of dietary patterns for both beverage and non-beverage foods containing benzoates and typical/allowed benzoate use levels in those countries.

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Appendix 1
GEMS/Food search results: individual records from China (Hong Kong Special Administrative Region)

Food category	Food name	Food code	Local food name	Result	Units	LOD	LOQ
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Malted soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Grape juice; prepackaged	580	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Apple juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Apple juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Apple juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Grape juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mixed fruit juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Pineapple juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Pear and lemon juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Grape juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Apple and mango juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Dark grape juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Orange juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Apple juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Orange juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mixed fruit juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Blackcurrant juice with aloe vera; prepackaged	140	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Orange juice with aloe vera; prepackaged	100	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10

Food category	Food name	Food code	Local food name	Result	Units	LOD	LOQ
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk with sesame; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Apple juice; prepackaged	170	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mixed vegetable juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Apple juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mixed fruit juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Orange juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mixed fruit and vegetable juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Apple juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Grape juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Grape juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Grape juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Grape juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	White grape juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mixed fruit juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit nectar	14.1.3.1	Guava nectar; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Grape juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Grape juice; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Red grape juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Carrot and lemon juice; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Carbonated red grape juice	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Apple drink; prepackaged	ND	mg/kg	5	10

Appendix 1 (continued)

Food category	Food name	Food code	Local food name	Result	Units	LOD	LOQ
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Orange and apple drink; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Orange drink; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Pear juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Tomato juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Pineapple juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mixed fruit juice; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Carbonated apple drink; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Carbonated grapefruit drink; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Carbonated orange drink; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Carbonated peach drink; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	140	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	140	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated light drink	56	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	120	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	130	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	130	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	130	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated light drink	130	mg/kg	5	10

Food category	Food name	Food code	Local food name	Result	Units	LOD	LOQ
Fruit and vegetable juices	Fruit juice	14.1.2.1	Apple juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Apple juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Dark grape juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Red grape juice; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink (caffeine free)	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Glucose drink; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated energy drink	150	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	150	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	150	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	150	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	150	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Lemon lime carbonated drink	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Tomato juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Tomato juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Orange and red grapefruit juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mixed fruit juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mixed fruit juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit nectar	14.1.3.1	Mango nectar; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Orange juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mixed fruit juice; prepackaged	ND	mg/kg	5	10

Appendix 1 (continued)

Food category	Food name	Food code	Local food name	Result	Units	LOD	LOQ
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Malted soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Chocolate flavoured soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Black soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Grape juice drink; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Melon flavoured soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Melon flavoured soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10

Food category	Food name	Food code	Local food name	Result	Units	LOD	LOQ
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Orange juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mixed fruit juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mulberry juice; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated light drink	150	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated light drink	150	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	150	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	150	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated light drink	140	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated energy drink	150	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated energy drink	150	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	140	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	140	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	130	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	130	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	ND	mg/kg	5	10

Appendix 1 (continued)

Food category	Food name	Food code	Local food name	Result	Units	LOD	LOQ
Fruit and vegetable juices	Fruit juice	14.1.2.1	Kiwifruit juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Strawberry juice; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Malted soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Fruit and vegetable juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Tomato juice; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Soya milk; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Cranberry juice; prepackaged	51	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Grape juice; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	140	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mixed fruit juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Fruit and vegetable juice drink; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Pineapple juice drink; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Lemon drink; prepackaged	ND	mg/kg	5	10
Non-alcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)	Soft drinks	14.1.4	Flavoured carbonated drink	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mixed vegetable juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Mixed fruit juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Pomelo juice; prepackaged	ND	mg/kg	5	10

Food category	Food name	Food code	Local food name	Result	Units	LOD	LOQ
Fruit and vegetable juices	Fruit juice	14.1.2.1	Chinese pear juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Orange juice; prepackaged	ND	mg/kg	5	10
Fruit and vegetable juices	Fruit juice	14.1.2.1	Red and white grape juice drink; prepackaged	ND	mg/kg	5	10
	Total	14.1.2.1	68	Mean-LB	15.31	208.2[a]	
				Mean-UB	24.57		
		14.1.3.1	2	Mean-LB	0.00	–	
				Mean-UB	10.00		
		14.1.4	74	Mean-LB	46.57	137.84[a]	
				Mean-UB	53.19		
	Total		144	Mean-LB	31.16	149.57[a]	
				Mean-UB	39.08		

LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; ND: not detected; UB: upper bound
[a] Average excluding non-detects.

Appendix 2
Benzoate exposure using CIFOCOss and reported average (typical) use level

Cluster	Age class	Country	Consumers only[a]			
			Mean-L	Mean-H	P95 max-L	P95 max-H
5	General population	Brazil	0.35	0.87	0.95	2.38
6	Other children	Greece	0.34	0.86	0.63	2.08
7	Adolescents	France	0.24	0.61	0.74	1.87
7	Adults	Finland	0.30	0.76	0.85	2.13
7	Adults	France	0.19	0.48	0.67	1.69
7	Adults	United Kingdom	0.24	0.60	0.73	1.84
7	Elderly	Finland	0.24	0.62	0.80	2.03
7	Elderly	France	0.09	0.23	0.45	0.66
7	Other children	Finland	0.51	1.28	0.51	6.51
7	Other children	France	0.40	1.00	1.31	3.29
7	Toddlers	Finland	0.82	2.07	2.89	7.28
7	Very elderly	France	0.09	0.24	0.36	0.91
8	Adolescents	Germany	0.56	1.42	1.66	4.17
8	Adolescents	Spain	0.44	1.10	1.18	2.98
8	Adults	Germany	0.47	1.18	1.46	3.68
8	Adults	Spain	0.34	0.87	1.01	2.55
8	Elderly	Germany	0.29	0.74	0.83	2.09
8	Other children	Germany	0.73	1.84	2.68	6.75
8	Other children	Spain	0.48	1.20	1.79	4.50
8	Toddlers	Germany	0.94	2.38	6.32	15.92
8	Very elderly	Germany	0.31	0.77	0.76	1.90
9	Children	China	0.35	0.89	0.85	2.13

Appendix 2 (continued)

			Consumers only[a]			
Cluster	Age class	Country	Mean-L	Mean-H	P95 max-L	P95 max-H
9	Children	Philippines	0.23	0.58	0.66	1.67
9	General population	China	0.15	0.39	0.53	1.34
9	General population	Thailand	0.03	0.18	–	–
10	Adolescents	Cyprus	0.24	0.61	0.69	1.73
10	Adolescents	Italy	0.27	0.67	0.63	1.59
10	Adolescents	Latvia	0.33	0.83	0.91	2.30
10	Adults	Italy	0.16	0.41	0.38	0.96
10	Adults	Latvia	0.21	0.54	0.67	1.69
10	Children	Japan	0.96	2.43	2.72	6.86
10	Children	Republic of Korea	0.51	1.28	1.04	2.62
10	Elderly	Italy	0.12	0.30	0.30	0.76
10	General population	Japan	0.29	0.74	0.98	2.48
10	General population	Republic of Korea	0.36	0.89	0.91	2.30
10	Infants	Bulgaria	1.33	3.36	–	–
10	Other children	Bulgaria	0.79	1.98	1.93	4.86
10	Other children	Italy	0.28	0.70	0.55	1.39
10	Other children	Latvia	0.48	1.22	1.22	3.07
10	Toddlers	Bulgaria	0.71	1.78	1.91	4.81
10	Toddlers	Italy	0.32	0.82	–	–
10	Very elderly	Italy	0.13	0.33	0.34	0.85
11	Adolescents	Belgium	0.70	1.77	1.72	4.34
11	Adults	Belgium	0.57	1.43	1.65	4.16
11	Adults	Netherlands	0.56	1.41	1.43	3.60
11	Elderly	Belgium	0.28	0.70	0.79	1.98
11	Other children	Belgium	0.92	2.32	2.77	6.97
11	Other children	Netherlands	0.49	1.24	2.69	6.77
11	Toddlers	Belgium	1.31	3.29	–	–
11	Toddlers	Netherlands	0.53	1.33	3.63	9.14
11	Very elderly	Belgium	0.27	0.67	0.87	2.20
13	Adult women	Burkina Faso	0.58	1.47	1.45	3.64
13	Children	Burkina Faso	1.09	2.74	1.66	4.18
15	Adolescents	Czech Republic	0.67	1.69	1.81	4.55
15	Adolescents	Denmark	0.49	1.23	1.24	3.12
15	Adolescents	Sweden	0.57	1.44	1.37	3.45
15	Adults	Czech Republic	0.40	1.00	1.10	2.78
15	Adults	Denmark	0.27	0.68	0.85	2.15
15	Adults	Hungary	0.27	0.69	0.72	1.81
15	Adults	Ireland	0.22	0.55	0.71	1.78
15	Adults	Sweden	0.27	0.68	0.81	2.03
15	Elderly	Denmark	0.14	0.35	0.58	1.45
15	Elderly	Hungary	0.17	0.42	0.41	1.03
15	Other children	Czech Republic	0.87	2.18	2.39	6.03

Cluster	Age class	Country	Consumers only[a]			
			Mean-L	Mean-H	P95 max-L	P95 max-H
15	Other children	Sweden	0.82	2.05	1.91	4.81
15	Very elderly	Denmark	0.08	0.20	0.19	0.48
15	Very elderly	Hungary	0.19	0.48	0.57	1.43
16	Adult women	Uganda	0.22	0.55	0.27	0.68
	Toddlers	USA[b]	1.47	3.70	3.31	8.33
	Children	USA[b]	0.90	2.27	1.97	4.97
	Adolescents – f	USA[b]	0.62	1.57	1.27	3.20
	Adolescents – m	USA[b]	0.70	1.77	1.34	3.37
	Adults – f	USA[b]	0.56	1.40	1.14	2.87
	Adults – m	USA[b]	0.66	1.67	1.32	3.33
	Toddlers	Mexico[b]	0.70	1.76	1.65	4.15
	Children	Mexico[b]	0.65	1.65	1.33	3.35
	Adolescents – f	Mexico[b]	0.47	1.19	1.01	2.56
	Adolescents – m	Mexico[b]	0.52	1.31	1.06	2.67
	Adults – f	Mexico[b]	0.32	0.80	0.70	1.76
	Adults – m	Mexico[b]	0.41	1.02	0.86	2.16
	Young children (1–5 years)	South Africa[c]	1.63	4.10	4.10	10.90
	Children	South Africa[c]	1.23	2.74	3.10	6.90
	10+ years	South Africa[c]	0.60	1.83	1.50	4.60

–: not applicable owing to the small number of consumers; f: female; H: high; L: low; m: male; max: maximum; P95: 95th percentile
[a] Mean-L, P95-L: based on low benzoate concentration (83 mg/L); mean-H, P95-H: based on high benzoate concentration (209 mg/L).
[b] Based on consumption figures from the submitted data.
[c] Based on the highest consumption figures of beverages from the submitted data.

Appendix 3
Literature-derived dietary exposure estimates for benzoates from all foods (including non-beverage uses)

Country	Food consumption data	Food sampling approach	Number of analyses	Exposure (mg/kg bw per day) – per capita	Exposure (mg/kg bw per day) – consumers only	Major food contribution to overall exposure	Reference
Australia[a]	Two non-consecutive 24 h recall (2007 ANCNPAS)	Targeted (stratified, TDS)	455	–	Mean 2–5 years 0.9 P90 2–5 years 2.3 Mean 6–12 years 0.9 P90 6–12 years 2.2 Mean 13–16 years 0.7 P90 13–16 years 1.8 Mean 17+ years 0.4 P90 17+ years 1.2	Non-cola soft drinks (32–55%), cordial (14–22%), juice and juice products (17–37%)	FSANZ (2005, unpublished data[b])
Austria[a]	24 h recall (2 581 adults), 3 d weighting method (151 preschool children)	Targeted	2 333	–	Mean females 6.2 Mean males 5.9 Mean preschool children 6.8	Fish and fish products (42.6–68.1%), non-alcoholic drinks (6.0–8.8%)	Mischek & Cermak (2012)
Belgium[a]	Two non-consecutive 24 h recalls in combination with a food frequency questionnaire	–	–	Mean adults 1.3 P95 adults 2.9	–	Non-alcoholic flavoured drinks (34.28%)	Vandevijvere et al. (2009)
Brazil[a]	–	Random	39	Mean 0.3–0.9	–	Soft drinks (>80%)	Tfouni & Toledo (2002)
China[a]	24 h recall in combination with a food frequency questionnaire	Targeted (stratified, TDS)	239	P50 0.2–2.4 P95 0.2–3.1	–	Seasoning, dumplings and pot stickers	Ling et al. (2015)
Denmark[a]	7 consecutive day food intake records	Random	1 526	–	Mean adults 1–2 P95 adults 2.5–5 Mean children 2–4 P95 children 5–8 Mean young children 4 P95 young children 8.5–9	Soft drinks (41.6–52.4%)	Leth, Christensen & Larsen (2010)

Country	Food consumption data	Food sampling approach	Number of analyses	Exposure (mg/kg bw per day) – per capita	Exposure (mg/kg bw per day) – consumers only	Major food contribution to overall exposure	Reference
France[c]	7 consecutive day food intake records	Random	121	Mean adults 0.04, P97.5 adults 0.2, Mean children 0.1, P97.5 children 0.4	Mean adults 0.1, P97.5 adults 0.7, Mean children 0.2, P97.5 children 1.1	Not reported	Bemrah, Leblanc & Volatier (2008)
France[c]	7 d food intake record	–	450	Mean adult 0.2, P97.5 adult 0.7, Mean children 0.3, P97.5 children 1.1	–	Adults: soup and sauces (35%), non-alcoholic beverages (50%); Children: non-alcoholic beverages	Vin et al. (2013)
Ireland[c]	7 d food intake record	–	450	Mean adults 0.5, P97.5 adults 1.4, Mean 5–12 years 0.9, P97.5 5–12 years 2.5, Mean 13–17 years 0.7, P97.5 13–17 years 1.9	–	Adults: non-alcoholic beverages (42%), soup and sauces (34%); Children: non-alcoholic beverages	Vin et al. (2013)
Italy[c]	3 d food intake record	–	450	Mean adults 0.2, P97.5 adults 0.9, Mean children 0.4, P97.5 children 1.5	–	Adults: meat products (68%); Children: non-alcoholic beverages	Vin et al. (2013)
Lebanon[a]	24 h recall in combination with a food frequency questionnaire (9–18 years, 230 teenagers)	Targeted (stratified, TDS)	113	Mean 1.9	Mean 1.9, P95 3.8	Canned juices (35%), sodas (20%)	Soubra et al. (2007)
New Zealand[a]	24 h recall (2002 CNS)	Stratified	300	Mean 0.04–0.4, P95 0.3–2.2	Mean 0.1–0.9, P95 0.7–3.0	Children: soft drinks (23%), soft drinks non-cola (72.3%); Adults: soft drinks (33.9%), soft drinks non-cola (61.5%)	Cressey & Jones (2009)
Republic of Korea[a]	24 h recall in combination with a food frequency questionnaire	Random	72	Mean 0.01–0.03	P90 0.2–1.9	Beverages, soya sauce	Yoon et al. (2003)

Appendix 3 (continued)

Country	Food consumption data	Food sampling approach	Number of analyses	Exposure (mg/kg bw per day) – per capita	Exposure (mg/kg bw per day) – consumers only	Major food contribution to overall exposure	Reference
Saudi Arabia[a]	Food frequency questionnaire from 100 students (18–25 years)	Random	50	–	Mean students 0.8 (fruit drink) 5.2 (yogurt rice dressing)	Non-alcoholic drinks, yogurt rice dressing	El-Ziney (2009)
Serbia[a]	Food frequency questionnaire	Random	748	Mean adults 0.32	Mean adults 0.4 P95 adults 1.1	Non-alcoholic beverages (43.1%), ketchup and tomato products (36.1%), domestic pickled vegetables (19.4%)	Lazarevic, Stojanovic & Rancic (2011)
United Kingdom[c]	4 d food intake record	–	450	Mean adults 0.4 P97.5 adults 1.4 Mean 1–4 years 1.5 P97.5 1–4 years 3.9 Mean 4–18 years 1.0 P97.5 4–18 years 2.6	–	Adults: soup and sauces (57%) Children: non-alcoholic beverages	Vin et al. (2013)

ANCNPAS: Australian National Children's Nutrition and Physical Activity Survey; CNS: Children's Nutrition Survey; P50: 50th percentile; P95: 95th percentile; P97.5: 97.5th percentile; TDS: Total Diet Study

[a] Based on the analytical data.

[b] Updated consumption data from the 2007 ANCNPAS.

[c] Based on the reported use level.

Lipase from *Fusarium heterosporum* expressed in *Ogataea polymorpha*

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1. Explanation

At the request of the Codex Committee on Food Additives at its Forty-sixth Session (FAO/WHO, 2014), the Committee evaluated the safety of lipase (triacylglycerol

lipase; Enzyme Commission No. 3.1.1.3) from *Fusarium heterosporum* expressed in *Ogataea polymorpha*, which it had not previously considered. *Ogataea polymorpha* was recently renamed from *Hansenula polymorpha* based on genetic analyses (Suh & Zhou, 2010). Lipase hydrolyses ester bonds in the 1- and 3-positions of fatty acids in triglycerides. The enzyme also has activity towards sn-1 ester bonds in other lipid components, including diacyl-phospholipids and diacyl-galactolipids. In this report, the expression "lipase" refers to the lipase enzyme and its amino acid sequence, the expression "lipase liquid enzyme concentrate" refers to the test material used in the toxicity studies evaluated, and the expression "lipase enzyme preparation" refers to the preparation formulated for commercial use. The lipase enzyme preparation is used as a processing aid in the manufacture of bakery products, pasta and noodles, in egg yolk and in the degumming of edible oil.

1.1 Genetic background

The host microorganism, *Ogataea polymorpha*, was recently renamed from *Hansenula polymorpha* based on genetic analyses (Suh & Zhou, 2010). Therefore, the name *H. polymorpha* still appears in many references. *Ogataea polymorpha* is a non-pathogenic and non-toxigenic yeast commonly used in commercial food enzyme production. In a previous evaluation by the Committee, it was determined to be the safe host microorganism for the production of hexose oxidase (Annex 1, reference *174*).

A uracil auxotroph of the wild-type *O. polymorpha* strain ATCC 34438, designated as RB11, was further genetically modified via plasmid transformation to produce a lipase originating from *F. heterosporum*. The transformation vector was created from a modified *Escherichia coli* pBR322 in which the genes encoding ampicillin resistance (*Apr*) and tetracycline resistance (*TCr*) had been removed. The synthetic lipase gene, containing a codon sequence optimized for maximum production in *O. polymorpha* of the native *F. heterosporum* lipase, combined with a promoter and a terminator from native *O. polymorpha*, was inserted into the vector. The *Saccharomyces cerevisiae* oritidine-5′-phosphate decarboxylase gene (*URA3)* was also inserted into the vector as a selectable marker. The resulting vector was used to transform the host strain RB11 to obtain the lipase production strain *O. polymorpha* GICC03251. The genetic construction was verified by Southern blot analysis to confirm that only the intended genetic modification to the *O. polymorpha* strain had been made. The production strain is stable with respect to the introduced DNA.

1.2 Chemical and technical considerations

Lipase is produced by submerged straight-batch or fed-batch pure culture fermentation of a genetically modified strain of *O. polymorpha* containing a

synthetic gene that encodes the same amino acid sequence as the native lipase gene from *F. heterosporum*. The fermentation broth carrying the enzyme is separated from the biomass by filtration and/or centrifugation. The liquid filtrate containing the enzyme is then concentrated by ultrafiltration, followed by polish filtration. Food-grade preservatives are added to the enzyme concentrate before spray-drying or agglomeration, and the product is formulated to the desired activity with food-grade ingredients. The lipase enzyme preparation conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm).

Lipase activity is measured in titratable phospholipase units (TIPU). One TIPU is defined as the amount of enzyme liberating 1 µmol free fatty acid from a lecithin substrate per minute under the assay conditions. The mean activity of lipase from three batches of the lipase enzyme concentrate was approximately 14 000 TIPU/g. The mean total organic solids (TOS) content of these three batches was 15%. TOS includes the enzyme of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism during the manufacturing process. The final commercial formulations can vary widely in activity and TOS content, depending on the use. The lipase enzyme preparation is used at concentrations up to 220 mg TOS/kg raw material, depending on the proposed food application. Lipase is expected to be inactivated in food or removed from the oil.

2. Biological data

2.1 Assessment of potential allergenicity

Lipase from *F. heterosporum* is a triacylglycerol lipase with a known amino acid sequence and a molecular weight of approximately 30 kDa. It was evaluated for potential allergenicity using the bioinformatics criteria recommended by FAO/WHO (2001, 2009), but modified at the present meeting. The amino acid sequence of lipase was compared with the amino acid sequences of known allergens in the AllergenOnline database (http://www.allergenonline.org/index.shtml) and in the Allermatch database (http://www.allermatch.org). A search for matches with greater than 35% identity over a window of 80 amino acids and a search for sequence identity of eight contiguous amino acids produced no match. Additionally, a full-length FASTA sequence search found two matches: Pha a 5 from *Phalaris aquatica* (identity 28.33% in 120 amino acid stretches) and Art v 2 from *Artemisia vulgaris* (identity 35.0% in 40 amino acid stretches). However, these two matches are identified as pollen allergens, not food allergens.

Based on these data, the Committee considered that dietary exposure to lipase from *F. heterosporum* is not anticipated to pose a risk of allergenicity.

2.2 Toxicological studies

Toxicological studies of lipase have been performed using either a lipase liquid enzyme concentrate (with TOS of 6.69% and protein content of 0.4%) or its freeze-dried powdered form (with TOS of 90.1% and protein content of 6.2%).

2.2.1 Acute toxicity

A good laboratory practice (GLP)–compliant acute oral toxicity study was performed in female Sprague-Dawley: Hsd:SD: Tu rats (Madetoja, 2005a). The test material was the freeze-dried powdered form of the lipase liquid enzyme concentrate suspended in sterile water at a concentration of 133 mg/mL. Ten millilitres of the test material was administered orally by gavage to rats that had been fasted overnight. In the sighting study, one female rat was administered a single dose of the test material at 1.33 g/kg body weight (bw) (equivalent to 1200 mg TOS/kg bw) and was observed for clinical signs for 14 days. With a lack of toxicity in the sighting study, the same single dose of the test material was administered to four additional female rats in the main study, followed by a 14-day observation period. At the end of the study, all the animals were weighed and terminated. A gross necropsy was performed, and all macroscopic signs were recorded.

No abnormal clinical signs were observed during the study, and all animals survived in good condition during the whole experiment. The body weight gains were normal in all animals during the observation period. No treatment-related macroscopic findings were noted at necropsy. Therefore, under these experimental conditions, oral administration of the freeze-dried powdered form of lipase liquid enzyme concentrate at 1.33 g/kg bw (equivalent to 1200 mg TOS/kg bw) to female rats did not result in acute toxicity (Madetoja, 2005a).

2.2.2 Short-term studies of toxicity

A GLP-compliant 13-week repeated-dose oral toxicity study in SPF Hsd: Sprague-Dawley rats was performed according to Organisation for Economic Co-operation and Development (OECD) test guideline 408 (Madetoja, 2005b). In this study, the lipase liquid enzyme concentrate was given to four groups of rats (10 of each sex per group) by gavage at a dose of 0 (sterile water), 67, 201 or 669 mg TOS/kg bw per day in a constant volume of 10 mL/kg bw. The animals were fed ad libitum and had free access to water. The general well-being of the animals and clinical signs were observed daily. A necropsy was performed at the end of the study.

All animals survived in good condition during the whole study. No abnormal treatment-related clinical signs, including changes in the eyes, were observed. Statistically significant differences in feed and water consumption were observed between the treated group and the control group. However, these changes are considered to be of no biological significance, as no changes in body weight or body weight gain were observed.

At necropsy, isolated incidences of macroscopic findings were noted (i.e. enlarged lymph nodes, hyperaemia in small intestine, erosion of stomach mucus). However, these occurrences were identified within the background values specific for rats of this strain and age. Additionally, none of these findings was dose related or could be attributed to exposure to the test material.

The total serum bilirubin level was statistically significantly increased in the two highest dose groups in both male and female animals at study termination. However, the values were still within the historical control range. No histopathological changes were identified in the liver.

No treatment-related effects on organ weights or urine analysis were noted. Macroscopic findings were minor and are considered to be of little biological significance. Microscopic examination of the liver and kidneys did not reveal any functional changes. The microscopic findings were considered to be incidental findings for animals of this strain and age. It was concluded that there were no observed treatment-related signs of clinical or systemic toxicity.

A no-observed-adverse-effect level (NOAEL) of 669 mg TOS/kg bw per day, the highest dose tested, was identified (Madetoja, 2005b).

2.2.3 Long-term studies of toxicity and carcinogenicity

No information was available.

2.2.4 Genotoxicity

The freeze-dried powdered form of the lipase enzyme concentrate, dissolved in water, was tested for genotoxicity using the bacterial reverse mutation test (Ames assay) and the in vitro chromosomal aberration assay. Both studies were GLP compliant and were conducted in accordance with the respective OECD test guideline (471 and 473, respectively). The results of these studies were negative (Table 1), indicating that the lipase enzyme preparation was unlikely to be genotoxic.

2.2.5 Reproductive and developmental toxicity

No information was available.

Table 1

Genotoxicity of lipase from _F. heterosporum_ expressed in _O. polymorpha_

End-point	Test system	Concentration	Result	Reference
In vitro				
Reverse mutation[a]	_Salmonella typhimurium_ TA97, TA98, TA100 and TA1535 and _Escherichia coli_ WP2uvrA	10–5 000 μg/plate ±S9[b]	Negative	Marhan (2005a)
Chromosomal aberrations[c]	Human lymphocytes	500–2 000 μg/mL ±S9[b]	Negative	Marhan (2005b)

S9: 9000 × _g_ supernatant fraction from rat liver homogenate

[a] The test was performed by the standard plate incorporation method. The positive controls used without S9 mix were _N_-ethyl-_N_-nitro-nitrosoguanidine (20 μg/plate) for TA97 and _E. coli_ WP2uvrA; 2-nitrofluorene (10 μg/plate) for TA98; and sodium azide (5 μg/plate) for TA100 and TA1535. The positive controls used with S9 mix were 2-aminoanthracene (10 μg/plate) for TA97, TA98 and TA100 and _E. coli_ WP2uvrA; and cyclophosphamide (100 μg/plate) for TA1535. A preliminary dose range–finding test was carried out using TA100, with and without S9, for doses up to 5000 μg/plate. In the main study, each of the five strains was tested using five doses of the test material, ranging from 10 to 5000 μg/plate, with and without S9. All tests were repeated twice in two independent assays; each was performed in triplicate. The positive control substances produced marked increases over the concurrent negative control values, and the test substance was not toxic to the test bacteria. There was no significant increase in the number of induced revertant colonies in any strain at any dose in the tests both with and without metabolic activation.

[b] The S9 mix, which acts as an exogenous metabolic activation system, was prepared from the liver of male Wistar rats induced with a single intraperitoneal injection of Aroclor 1254 (500 mg/kg bw) 5 days before S9 preparation.

[c] Positive controls used in the chromosomal aberration assay were thiopeta (1 μg/mL) and cyclophosphamide (100 μg/mL), with and without S9 mix. The result was considered positive if the test substance increased the average percentage of aberrant cells to more than twice that of the negative control value (water) and a dose–response relationship was observed. In a dose range–finding test (1000–5000 μg/mL), cell toxicity was observed at doses of 3000 μg/mL and above. Therefore, the two main tests were carried out with the test material at 500, 1000 and 2000 μg/mL. Cells were treated for 4 hours (with a 24-hour incubation time) in the first main test and 48 hours in the repeated main test. Two slides from each culture were prepared, and 50 well spread metaphases on each slide were examined. The negative and positive controls worked properly in all tests. The test material did not produce an increase in the frequency of aberrant cells more than twice the control values at any dose.

2.3 Observations in humans

No information was available.

3. Dietary exposure

3.1 Introduction

The Committee evaluated one submission received from DuPont Industrial Biosciences, a manufacturer of the lipase enzyme preparation.

The lipase enzyme preparation is used internationally, being approved for use in Australia, Brazil, Denmark, France, Mexico and New Zealand, and is generally recognized as safe for use in food processing in the USA. The lipase enzyme preparation is used across a number of food products, and therefore an upper-bounding dietary exposure assessment approach was taken. A theoretical "worst-case" dietary exposure estimate was made, assuming that 100% of food products in which lipase could be used would be manufactured using the product at its maximum proposed use levels and that 100% would remain in the final food products.

The Committee concluded that a tiered approach to assessing potential dietary exposure to lipase was not necessary, as the upper-bounding exposure estimate was evaluated.

3.2 Dietary exposure assessment

Three broad uses were considered individually to arrive at a total dietary exposure estimate.

3.2.1 Bakery products, pasta and noodles

The lipase enzyme preparation is used to modify lipids used in flour/cereals for the production of bakery products, pasta and noodles. The sponsor used information on the consumption of flour from wheat, rye, corn, oat and barley in the USA to prepare its estimate of the dietary exposure from this use. Per capita consumption of these cereals was 79.2 kg/person per year (217 g/person per day, 3.6 g/kg bw per day for a 60 kg individual) in 2012 (USDA, 2012). If it is assumed that the lipase enzyme preparation is applied at the highest suggested rate of 44 mg TOS/kg flour, the estimated dietary exposure is 0.16 mg TOS/kg bw per day.

Reference to the 17 consumption cluster diets of the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) (WHO, 2012) reveals that cluster G06 (including a number of Middle Eastern countries) has the highest apparent consumption of total cereal grains and flour, at 479.2 g/day (cluster G10, which includes the USA, has an apparent consumption of 260 g/day). If the lipase enzyme preparation is applied at the highest suggested rate of 44 mg TOS/kg flour, the dietary exposure for consumption of 479.2 g/day would be 21.1 mg TOS/day, or 0.35 mg TOS/kg bw per day for a 60 kg individual.

3.2.2 Egg yolks

The lipase enzyme preparation is used in the modification of egg yolk lipids at a maximum rate of 220 mg TOS/kg egg. To estimate the contribution from egg yolks, the consumption of eggs by the population of the USA was used (USDA, 2012). If an egg consumption of 14.6 kg/person per year (40 g/person per day, or 0.67 g/kg bw per day for a 60 kg individual) is assumed and the maximum rate of 220 mg TOS/kg egg is used, dietary exposure to lipase enzyme preparation would be 0.15 mg TOS/kg bw per day.

Reference to the 17 GEMS/Food cluster diets (WHO, 2012) reveals that cluster G11 (Belgium and the Netherlands) has the highest apparent consumption of eggs, at 42.1 g/day (cluster G10, which includes the USA, has an apparent consumption of 17.6 g/day). The Committee concluded that the use of the cluster

diet information for cluster G11 would not give a dietary exposure estimate significantly different from that provided by the sponsor.

3.2.3 Degumming food oils

The lipase enzyme preparation is used as a degumming agent in the manufacture of food oils. The maximum intended use level of the lipase enzyme preparation is 22 mg TOS/kg crude oil. The sponsor used food oil consumption, as dairy fat, from the USA (USDA, 2012). If it is assumed that oil consumption is 13.4 g/day (0.22 g/kg bw per day for a 60 kg individual) and that the lipase enzyme preparation is used at the maximum intended use level, dietary exposure would be estimated to be 0.005 mg TOS/kg bw per day.

Reference to the 17 GEMS/Food cluster diets (WHO, 2012) reveals that cluster G08 (Austria, Germany, Poland and Spain) has the highest apparent consumption of total milk, mammalian and plant fats, at 89.8 g/day (cluster G10, which includes the USA, has the highest plant fat consumption, at 59.9 g/day, and cluster G15, primarily European countries, has the highest consumption of mammalian fat, at 14.1 g/day). If it is conservatively assumed that all fats would be degummed using the lipase enzyme preparation at its highest intended use level, dietary exposure as high as 0.03 mg TOS/kg bw per day could be estimated.

3.2.4 Assessment of total dietary exposure

The combination of the maximum dietary exposure from each of the three uses of lipase (0.35 + 0.15 + 0.03 mg TOS/kg bw per day) results in a potential total dietary exposure of 0.53 mg TOS/kg bw per day. This conservative estimate assumes maximum use concentrations, 100% market penetration for the production of products using the lipase enzyme preparation and the presence of the enzyme following the production processes. The Committee noted that under these conditions, the major contribution to total dietary exposure to lipase enzyme preparation would be from the consumption of bakery products, pasta and noodles.

4. Comments

4.1 Assessment of potential allergenicity

Lipase from *F. heterosporum* was evaluated for potential allergenicity using the bioinformatics criteria recommended by FAO/WHO (2001, 2009), but modified at the present meeting. The amino acid sequence of lipase from *F. heterosporum* was compared with the amino acid sequences of known allergens in publicly

available databases. A search for matches with greater than 35% identity over a window of 80 amino acids and a search for sequence identity of eight contiguous amino acids produced no match. Therefore, the Committee considered that dietary exposure to lipase from *F. heterosporum* is not anticipated to pose a risk of allergenicity.

4.2 Toxicological studies

An acute oral toxicity study using a freeze-dried powdered form of a lipase liquid enzyme concentrate demonstrated no sign of toxicity at 1.33 g/kg bw in rats (Madetoja, 2005a). In a 13-week oral toxicity study in rats, no treatment-related adverse effects were observed when the lipase liquid enzyme concentrate was administered by gavage at doses up to 669 mg TOS/kg bw per day (Madetoja, 2005b). The results from an in vitro bacterial reverse mutation assay (Marhan, 2005a) and an in vitro chromosomal aberration assay in human lymphocytes (Marhan, 2005b) using the powdered form of the lipase enzyme concentrate were both negative. The Committee concluded that the lipase enzyme preparation is unlikely to be genotoxic.

4.3 Assessment of dietary exposure

An estimate of the theoretical dietary exposure to this lipase enzyme preparation was made by the Committee based on the level of TOS in the lipase enzyme preparation and its maximum use levels in bakery products, pasta and noodles (44 mg TOS/kg flour) and egg yolk (220 mg TOS/kg egg) and in the degumming of edible oil (22 mg TOS/kg crude oil). The combination of these maximum levels with per capita food consumption data from the USA (supplied by the sponsor) or from the GEMS/Food cluster diets results in a potential total dietary exposure of 0.5 mg TOS/kg bw per day for a 60 kg individual. The Committee noted that the enzyme will be inactivated in baking and cooking steps and will be removed from the refined oil.

5. Evaluation

No treatment-related adverse effects were seen at the highest dose tested (669 mg TOS/kg bw per day) in the 13-week study of oral toxicity in rats (Madetoja, 2005b). A comparison of the dietary exposure estimate of 0.5 mg TOS/kg bw per day with the highest dose tested of 669 mg TOS/kg bw per day results in a margin of exposure of at least 1300. The Committee established an acceptable daily intake (ADI) "not specified" for lipase from *F. heterosporum* expressed in *O.*

polymorpha when used in the applications specified and in accordance with good manufacturing practice.

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Magnesium stearate

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1. Explanation

The commercial product called magnesium stearate is composed mainly of magnesium salts of stearic and palmitic acids, obtained from edible fats and oils.

In 2010, at the Forty-second Session of the Codex Committee on Food Additives (CCFA) (FAO/WHO, 2010), the deletion of magnesium salts of fatty acids from the International Numbering System (INS) had been proposed. The International Alliance of Dietary/Food Supplement Associations offered technological justification for the use of this additive. CCFA at its Forty-third Session in 2011 (FAO/WHO, 2011) assigned the new INS number 470(iii) to magnesium stearate and asked the Committee to conduct a safety assessment, assess dietary exposure and set specifications for magnesium stearate.

Magnesium salts of fatty acids, previously included in the INS as number 470 (salts of fatty acids), have been evaluated by the Committee at its seventeenth, twenty-ninth, forty-ninth and seventy-sixth meetings (Annex 1, references *32*, *70*, *131* and *211*). At the seventeenth meeting (1974), the Committee evaluated salts of palmitic and stearic acids and established acceptable daily intakes (ADIs) "not limited",[1] with notes that palmitic and stearic acids are normal products of the metabolism of fats and that their metabolic fate is well established. Provided that the contribution of cations such as magnesium does not add excessively to the normal body load, there would be no need to consider the use of these substances in any different light to that of dietary fatty acids.

At its twenty-ninth meeting (1986), the Committee was of the opinion that "ADIs for ionizable salts should be based on previously accepted recommendations for the constituent cations and anions". The Committee listed ADIs for a number of combinations of cations and anions, including those of magnesium stearate and magnesium palmitate (ADI "not specified"). The Committee was concerned that dietary exposure resulting from the use of magnesium salts as food additives may have a laxative effect. It was also noted that infants are particularly sensitive to the sedative effects of magnesium salts and that individuals with chronic renal impairment retained 15–30% of administered magnesium, which could cause toxicity. The Committee stated that fatty acids are normal constituents of coconut oil, butter and other edible oils and that they do not represent a toxicological problem. As the Committee had no information on the manufacture or use of the food-grade materials at that time, an ADI for magnesium stearate was not established.

At its forty-ninth meeting (1999), the Committee evaluated the safety of palmitic acid and stearic acid when used as flavouring agents and concluded that they would not present a safety concern under the proposed conditions of use.

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[1]   This term is no longer used by JECFA. It has the same meaning as ADI "not specified".

At its seventy-sixth meeting (2012), the Committee established an ADI "not specified" for a number of magnesium-containing food additives and recommended that total dietary exposure to magnesium from food additives and other sources in the diet should be assessed. This was in the context of the evaluation of magnesium dihydrogen diphosphate, in which the estimated chronic dietary exposure to magnesium from the proposed uses was up to twice the background exposures from food previously noted by the Committee and may be in the region of the minimum laxative effective dose.

For the present evaluation, a range of published studies together with three reports on genotoxicity testing of magnesium stearate were submitted to the Committee.

Magnesium stearate has been permitted for use in the European Union (EU, 2008) and is generally recognized as safe (GRAS) in the USA (CFR, 1985; FDA SCOGS, 2013). It is also permitted for use in China (NHFPC, 2014), Japan (FSC, 2003), and Australia and New Zealand (FSANZ, 2014).

1.1 Chemical and technical considerations

Magnesium stearate is an off-white to white, very fine powder that is greasy to the touch and practically insoluble in water. It is used as an anticaking agent, emulsifier and binder in food supplement tablets, capsules and powders, compressed and granulated mints and candy, chewing gum, herbs and spices, and bakery ingredients. According to the industry, the use levels in these categories range from 0.05% to 3% by weight.

The commercial product is manufactured by either a direct process, called fusion, in which fatty acids are directly reacted with a magnesium source, such as magnesium oxide, to form magnesium salts of the fatty acids; or an indirect process, called precipitation, in which a sodium soap is produced by the reaction of fatty acids with sodium hydroxide in water and the product is precipitated by adding magnesium salts to the soap.

The final product contains not less than 4.0% and not more than 5.0% magnesium, on a dried basis, and the fatty acid fraction contains not less than 40.0% stearic acid and not less than 90.0% of the sum of stearic acid and palmitic acid. Specifications for unsaponifiable matter are set to not more than 2%. In addition, the limits for cadmium, lead and nickel are specified.

According to the data provided by industry, magnesium stearate is a stable product for which no decomposition products are expected under normal storage conditions.

Magnesium distearate as such has the chemical formula $Mg(C_{18}H_{35}O_2)_2$ and a molecular weight of 591.27 g/mol. The molecular structure of magnesium distearate is shown in Fig. 1.

Fig. 1
Molecular structure of magnesium distearate
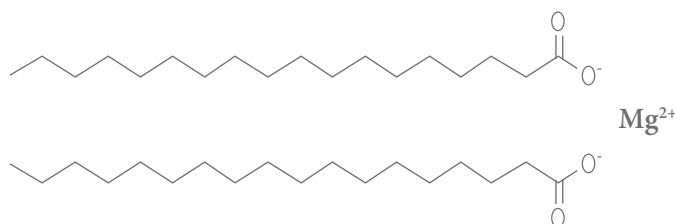
Besides magnesium salts of stearic acid and palmitic acid, other magnesium salts with fatty acids, such as lauric acid, myristic acid, pentadecanoic acid, margaric acid, oleic acid and arachic acid, may be present in the additive as minor components. The composition and distribution of the fatty acid fraction of commercial magnesium stearate depend on the vegetable or animal source of the fatty acid used as raw material for the production of the additive and the fractionation conditions to which the source material was subjected.

2. Biological data

2.1 Biochemical aspects

Magnesium stearate, under the acidic conditions of the stomach, is dissolved upon ingestion and separated into magnesium ion (cation) and stearic and palmitic acids (anions). The components are not necessarily absorbed in equal amounts.

Magnesium is an essential mineral, acting as a cofactor for many enzyme systems. It is involved in energy metabolism, the synthesis of proteins and nucleotides, and the metabolism and activation of vitamin D and parathyroid hormone. It is a normal constituent of the human body and is ubiquitous in foods, where it is commonly bound to phosphates. Recommended Dietary Allowances, which are considered to meet the nutrient needs of 97–98% of individuals in a population, have been set at 80–420 mg of magnesium per day for different age groups by the Institute of Medicine in the USA (Institute of Medicine, 1997; Annex 1, reference *211*).

Palmitic and stearic acids and their salts are constituents and products of the metabolism of edible oils and fats, for which the metabolic fate is well established. Stearic acid is the most poorly absorbed of the common fatty acids and is synthesized by the condensation of palmitoyl and acetyl coenzyme A

Table 1
Acute toxicity of magnesium stearate

Species	Sex	Route	LD$_{50}$ (g/kg bw) or LC$_{50}$ (mg/L)	Reference
Rat	Unknown	Oral	LD$_{50}$ >10	S.B. Penick & Co. (1977)
Rabbit	Unknown	Dermal	Non-corrosive	S.B. Penick & Co. (1977)
Rabbit	Unknown	Dermal	Non-irritating	S.B. Penick & Co. (1977)
Rabbit	Unknown	Ocular	Non-irritating	S.B. Penick & Co. (1977)
Rat	Unknown	Inhalation	LC$_{50}$ >2 mg/L	S.B. Penick & Co. (1977)

bw: body weight; LC$_{50}$: median lethal concentration; LD$_{50}$: median lethal dose

in the mitochondria. As palmitic and stearic acids undergo oxidation to give acetoacetic acid and ketone bodies, their β-oxidation yields two-carbon units, which enter the tricarboxylic acid cycle; the metabolic products are utilized and excreted (Cosmetic Ingredient Review, 1987; Annex 1, reference *70*). These fatty acids are of no toxicological concern and are therefore not further considered in this assessment.

2.2 Toxicological studies

2.2.1 Acute toxicity

(a) Magnesium stearate

The oral median lethal dose (LD$_{50}$) for magnesium stearate (composition unknown) in rats was greater than 10 g/kg body weight (bw), indicating that magnesium stearate is practically non-toxic. The results of acute toxicity tests for magnesium stearate administered to experimental species by different routes are summarized in Table 1.

2.2.2 Short-term studies of toxicity

(a) Magnesium stearate

(i) Rats

In a 90-day repeated-dose oral toxicity study, a commercial product of magnesium stearate was mixed with a semisynthetic diet and given to four groups of Wistar rats (20 animals of each sex per group) at a concentration of 0%, 5%, 10% or 20%. The test material was prepared according to Pharmacopoeia Nordica 1963, and the details of its composition were not available.

Decreased body weight gain was observed in males in the high-dose group (20%), and urolithiasis was found in eight males and seven females in the same group. Four males from the 20% dose group died within the first 2 months;

these deaths were related to stone formation in the lower urinary pathways. Reduced relative liver weight was seen in males in the 10% and 20% groups, and an increased amount of iron was found in the livers of rats of both sexes in the 20% group. Nephrocalcinosis was reduced in females in the 20% group. The authors concluded that the no-observed-effect level (NOEL), based on reduction in relative liver weight, was 5% magnesium stearate in the diet, equivalent to 2500 mg/kg bw per day, as determined by the authors (Sondergaard, Meyer & Würtzen, 1980).

The Committee concluded that this study was not relevant for the evaluation given the high concentration tested, which might lead to dietary imbalances, and the lack of information on the composition of the material tested.

(b) Magnesium

(i) Rats

At its seventy-sixth meeting, the Committee reviewed short-term studies of the toxicity of magnesium chloride hexahydrate in its evaluation of magnesium dihydrogen diphosphate. Groups of 10 male and 10 female F344 rats were fed magnesium chloride hexahydrate at a dietary concentration of 0%, 0.1%, 0.5% or 2.5% for 90 days. These dietary concentrations were equivalent to 0, 100, 500 and 2500 mg/kg bw per day, corresponding to, respectively, 0, 12, 60 and 300 mg/kg bw per day expressed as magnesium.

Transient soft faeces and a sustained increase in water consumption were observed in both high-dose males and females, and a slight reduction in body weight gain was also noted in the high-dose males. There were no changes in feed consumption, organ weights, haematology, biochemistry or histopathology. The no-observed-adverse-effect level (NOAEL) was reported to be 0.5% magnesium chloride hexahydrate in the diet, equivalent to 60 mg/kg bw per day expressed as magnesium (Takizawa et al., 2000).

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) Magnesium stearate

(i) Mice

Magnesium stearate pellets implanted in the urinary bladders of mice (bred at the test institute) for 30 weeks produced a tumour incidence of 5% (2/41 mice, one adenoma or papilloma and one carcinoma). This study is not considered relevant for the evaluation of the potential cancer risk from ingested stearates (Boyland et al., 1964).

(b) **Magnesium**

(i) Mice

At its seventy-sixth meeting, the Committee reviewed long-term studies of the toxicity and carcinogenicity of magnesium chloride hexahydrate in its evaluation of magnesium dihydrogen diphosphate. Groups of 50 male and 50 female B6C3F1 mice were given magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) at a dose level of 0% (control), 0.5% or 2% in the diet for 96 weeks, after which all animals received the control diet for 8 weeks and were then necropsied. The dietary concentrations were equivalent to 0, 750 and 3000 mg/kg bw per day, corresponding to, respectively, 0, 90 and 360 mg/kg bw per day expressed as magnesium.

A decrease in body weight was observed in females of the high-dose group. Survival rates did not differ between the treatment and control groups for males or females. Clinical signs and urinary, haematological and serum clinical chemistry parameters showed no treatment-related effects. With the exception of a significant decrease in the incidence of liver tumours among males of the high-dose group, no differences were noted in tumour incidence between the treated and control animals. Based on the decreased body weight gain in the high-dose females, the NOAEL was 0.5% magnesium chloride hexahydrate in the diet, equivalent to 90 mg/kg bw per day expressed as magnesium (Kurata et al., 1989).

2.2.4 **Genotoxicity**

(a) **Magnesium stearate**

In a series of in vitro and in vivo assays, no genotoxic potential of magnesium stearate was identified. The results of these studies of genotoxicity are summarized in Table 2.

2.2.5 **Reproductive toxicity**

(a) **Magnesium stearate**

(i) Rabbits

A tablet-coating vehicle containing 5.5% magnesium stearate was tested for teratogenicity in rabbits. Other components of this vehicle included polyethylene glycol 4000, starch, talcum and silica gel. Female rabbits were given this vehicle orally at a dose of 2.5 mg/kg bw 70 hours postcoitus ($n = 14$) and 192 hours postcoitus ($n = 13$). Fetal anomalies were observed in 9/86 and 11/90 offspring, respectively, compared with 12/112 anomalies in offspring from untreated control mothers ($n = 16$). The vehicle did not demonstrate teratogenicity at the dose tested in this study (Cosmetic Ingredient Review, 1982). The Committee noted that the vehicle tested was composed of many components with unknown

Table 2

Results of genotoxicity tests with magnesium stearate

Test system	Test object	Concentration/dose	Results	Reference
In vitro				
Reverse mutation[a]	*Saccharomyces cerevisiae* strain D4; *Salmonella typhimurium* strains TA1535, TA1537 and TA1538	1.25–5.0% (w/v) ±S9[b]	Negative	Brusick & Weir (1976)
Reverse mutation[c]	*S. typhimurium* strains TA98, TA100, TA1535 and TA1537; *Escherichia coli* WP2uvrA	Range finding: 5–5 000 µg/plate ±S9[d] Main: 156–5 000 µg/plate ±S9[d]	Negative	Saigoh (2001a)
Chromosomal aberration[e]	Chinese hamster lung (CHL/IU) cells	Range finding: 24 h treatment: 5–5 000 µg/mL Main: 6 h treatment: 1.56–50 µg/mL −S9; 31.3–1 000 µg/mL +S9[d] 24 h treatment: 0.313–10 µg/mL −S9 48 h treatment: 0.156–5 µg/mL −S9	Negative	Saigoh (2001b)
In vivo				
Micronucleus formation[f]	Male ICR mice (CD-1) femoral bone marrow	Preliminary: 31.3–2 000 mg/kg bw, oral (6 mice/group) Main: 500–2 000 mg/kg bw, oral (6 mice/group)	Negative	Saigoh (2001c)

bw: body weight; S9: 9000 × *g* supernatant fraction of liver homogenate from various species; w/v: weight per volume

[a] Negative control substance: dimethylformamide. Positive control substances without metabolic activation: ethylmethanesulfonate for TA1535 and D4, 2-nitrofluorene for TA1538, quinacrine mustard for TA1537. Positive control substances with metabolic activation: dimethylnitrosamine for TA1535 and D4, 8-aminoquinoline for TA1537, 2-aminoanthracene for TA1538. These positive controls produced the expected increase in the number of revertants. In this assay, the test substance is considered positive (mutagenic) if it produces at least a doubling of the mean number of revertants per plate in one or more strains compared with the solvent control, with or without metabolic activation.

[b] The S9 is the 9000 × *g* supernatant fraction of liver homogenate from male mice, male rats or male monkeys, without liver enzyme induction. The three S9 fractions, acting as an exogenous metabolic activation system, were used in separate experiments.

[c] Positive control substances without metabolic activation: 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide for TA100 and TA98, *N*-ethyl-*N*′-nitrosoguanidine for TA1535 and WP2uvrA, 9-aminoacridine hydrochloride monohydrate for TA1537. Positive control substance with metabolic activation for all test strains: 2-aminoanthracene. All positive control substances produced the expected increase in the number of revertants. In this assay, the test substance is considered positive (mutagenic) if it produces at least a doubling of the mean number of revertants per plate in one or more strains compared with the solvent control, with or without metabolic activation.

[d] The S9 is the 9000 × *g* supernatant fraction of liver homogenate from male rats treated with phenobarbital and 5,6-benzoflavone intraperitoneally. The S9 fraction acts as an exogenous metabolic activation system.

[e] Negative control substance: sodium carboxymethylcellulose. Positive control substance without metabolic activation: mitomycin. Positive control substance with metabolic activation: benzo[*a*]pyrene. All positive controls produced a statistically significant increase in the incidence of cells with chromosomal aberration. In this test, the test substance is considered positive (clastogenic) if the frequency of cells with chromosomal aberrations is significantly increased in the test substance group compared with the negative control group and if dose dependency or reproducibility was noted.

[f] Sampling times were 24, 48 and 72 hours. Negative control substance: sodium carboxymethylcellulose. Positive control substance: mitomycin. The test substance is considered positive (clastogenic) if the incidence of micronucleated polychromatic erythrocytes in the test substance group was significantly higher than that in the negative control group at a significance level of 5%.

proportions and that the percentage of magnesium stearate was only 5.5%. Owing to this limitation, the Committee concluded that this study was not relevant for the evaluation.

2.3 Observations in humans

2.3.1 Magnesium stearate

No information was available.

2.3.2 Magnesium

Adverse effects such as diarrhoea or other gastrointestinal effects have been seen with excessive magnesium intake as a consequence of the use of various magnesium salts for pharmacological or medicinal purposes. The United Kingdom Expert Group on Vitamins and Minerals reviewed a number of human studies on magnesium and concluded that there were insufficient data to establish a safe upper level for magnesium. For guidance purposes only, it suggested that supplemental magnesium at a dose of 400 mg/day would not be expected to result in any significant adverse effects (EVM, 2003).

Based on a series of human studies, the European Union's Scientific Committee on Food (SCF) concluded that no laxative effects have been observed in adult men and women, including during pregnancy and lactation, at doses up to 250 mg/day. This dose was considered to be the NOAEL for magnesium. Based on the NOAEL of 250 mg/day and an uncertainty factor of 1.0, the SCF established an upper level of 250 mg/day for readily dissociable magnesium salts (e.g. chloride, sulfate, aspartate, lactate) and compounds in nutritional supplements, in water or added to food and beverages (SCF, 2006).

The Institute of Medicine's Standing Committee on the Scientific Evaluation of Dietary Reference Intakes in the USA noted that the primary initial and the most sensitive toxic manifestation of excessive magnesium intake from non-food sources is diarrhoea. That committee reviewed several studies that reported mild diarrhoea and other gastrointestinal symptoms from uses of magnesium salts and identified a lowest-observed-adverse-effect level (LOAEL) of 360 mg/day for adults. Based on the LOAEL and an uncertainty factor of approximately 1.0, an upper level of 350 mg/day for magnesium from non-food sources was established (Institute of Medicine, 1997; Annex 1, reference *211*).

3. Assessment of dietary exposure

Magnesium stearate is used for diverse purposes, such as an anticaking agent, binder, drying agent, emulsifier, foaming agent, hydrophobation agent, lubricant

and/or thickener in confectionery (hard candy, pressed mint, mint pastilles), chewing gum, food supplements (tablets, capsules, powders), bakery wares (rusks, baking powder), herbs and spices. Magnesium stearate is also used in the pharmaceutical industry in the production of tablets, capsules and powders.

The Committee evaluated one submission by a sponsor (APAG, 2014). A survey on current food applications of magnesium stearate from 2013 was included in the submission and was used as a basis for an assessment of the expected dietary exposure to magnesium stearate (Table 3).

The submission estimated that magnesium stearate will be used in about 75% of all food supplement tablets and capsules, over 90% of confectionery candies and pastilles and up to 30% of chewing gum produced. The usage proportion in rusks, baking powder and herbs and spices is not known.

3.1 Assessments based on individual dietary records

An assessment of dietary exposure was performed using the above proposed maximum use levels in corresponding food categories and the data set of the European Food Safety Authority's (EFSA) Comprehensive European Food Consumption Database (EFSA, 2013) for consumption groups "Other confectionery", "Chewing gum", "Bakery wares", "Herbs, spices, seasonings" and "Food supplements". For the consumer groups "Children" (3–9 years) and "Adults" (18–64 years), consumption data from 13 and 14 European countries were available, respectively. The estimated dietary exposures to magnesium stearate (per country) for high-level consumption using the proposed maximum use levels were 21–44 mg/kg bw per day for children and 9–83 mg/kg bw per day for adults.

For children, estimates from four countries were higher than 40 mg/kg bw per day (Denmark: 44 mg/kg bw per day; United Kingdom: 43 mg/kg bw per day; Czech Republic: 42 mg/kg bw per day; Germany: 41 mg/kg bw per day); estimates from the majority of the remaining countries were between 25 and 35 mg/kg bw per day. The predominant source was "Bakery wares" (55–96%), followed by "Other confectionery" (2–40%), except for France, where "Other confectionery" was the main contributor (73%).

For adults, the majority of estimates for high-level consumers were in the range 10–20 mg/kg bw per day. Germany had the highest estimate, 83 mg/kg bw per day, followed by the Czech Republic, with 64 mg/kg bw per day, and one survey from Spain, with 37 mg/kg bw per day. The predominant source was "Bakery wares" (72–96%), followed by "Other confectionery" (1–25%), except for Finland, where "Other confectionery" and "Food supplements" were the main contributors (58% and 26%, respectively, of an estimated exposure of 18 mg/kg bw per day).

Table 3
Results of survey on use levels and food applications of magnesium stearate

GSFA category no.	GSFA food category	Technical functions	Proposed food uses	Average use level (mg/kg)	Maximum use level (mg/kg)
05.2	Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	Anticaking agent, lubricant, binder	Hard candy, pressed mint (05.2.1)	10 000	13 000
			Mint pastille (05.2.1)	5 000	9 500
05.3	Chewing gum	Emulsifier, anticaking agent, drying agent	Chewing gum	100–10 000	20 000
07.0	Bakery wares	Foaming agent, emulsifier	Rusks, baking powder	>500	2 500
12.2.1	Herbs and spices	Anticaking agent, hydrophobation agent	Spices, herbs	>500	10 000
13.6	Food supplements	Lubricant, anticaking agent, emulsifier, thickener	Food supplements: (chewable) tablets, capsules, powders	13 000	30 000

GSFA: Codex General Standard for Food Additives

In conclusion, the main contributing food category was bakery wares; however, the exposure estimate from this category is highly conservative when the use of the food additive is restricted to rusks and baking powder.

Based on the product composition and molecular weights, the dietary exposure to the ion components of magnesium stearate would be approximately 5% for magnesium and 95% for fatty acids (ratio 1:20). Thus, the estimated maximum dietary exposure to magnesium stearate of 44 mg/kg bw per day for children corresponds to 2 mg magnesium/kg bw per day and 42 mg fatty acids/kg bw per day. The estimated maximum dietary exposure to magnesium stearate of 83 mg/kg bw per day for adults corresponds to 4 mg magnesium/kg bw per day (240 mg/day for a 60 kg adult) and 79 mg fatty acids/kg bw per day.

Regarding magnesium, the Committee has stated previously that the background exposure to magnesium from food is 180–480 mg/day (Annex 1, reference *70*). The conservative estimates for the uses described in the present submission would contribute up to an additional 240 mg/day to this background exposure to magnesium from food. However, at the seventy-sixth meeting (Annex 1, reference *70*), the Committee also noted that an ADI "not specified" has been allocated individually to a number of magnesium-containing food additives and recommended that total dietary exposure to magnesium from food additives and other sources in the diet should be assessed.

4. Comments

4.1 Toxicological studies

The oral LD$_{50}$ for magnesium stearate of unknown composition administered to rats was greater than 10 g/kg bw (S.B. Penick & Co., 1977). The Committee reviewed a 90-day study in which rats were fed a diet containing 0%, 5%, 10% or 20% of a commercial product of magnesium stearate of unknown composition (Sondergaard, Meyer & Würtzen, 1980). The Committee concluded that this study was not relevant for the evaluation given the high concentrations tested, which might lead to dietary imbalances, and the lack of information on the composition of the material tested.

Magnesium stearate was not genotoxic in bacterial reverse mutation assays (Brusick & Weir, 1976; Saigoh, 2001a) and did not induce chromosomal aberrations in mammalian cells (Saigoh, 2001b). Magnesium stearate was also not genotoxic in an in vivo mouse micronucleus assay (Saigoh, 2001c).

4.2 Assessment of dietary exposure

An estimate of the theoretical dietary exposure to magnesium stearate was made by the Committee based on the proposed maximum use levels. The combination of these levels with consumption data using EFSA's Comprehensive European Food Consumption Database (EFSA, 2013) for consumption groups "Other confectionery", "Chewing gum", "Bakery wares", "Herbs, spices, seasonings" and "Food supplements" results in a potential total dietary exposure to magnesium stearate of 44 mg/kg bw per day for children and 83 mg/kg bw per day for adults, corresponding to 2 and 4 mg/kg bw per day expressed as magnesium, respectively. This would contribute up to an additional 240 mg/day to the background exposure to magnesium from food of 180–480 mg/day.

The Committee noted that the consumption of the food additive may lead to an additional dietary exposure to stearic and palmitic acids in the order of 5 g/day.

5. Evaluation

An ADI "not specified" has been established for a number of magnesium salts used as food additives. The Committee concluded that there are no differences in the evaluation of the toxicity of magnesium stearate compared with other

magnesium salts and confirmed the ADI "not specified" for magnesium salts of stearic and palmitic acids. However, the Committee was concerned that the use of magnesium salts in many food additives may result in combined exposure that may lead to a laxative effect.

5.1 Recommendation

Based on the present dietary exposure assessment, the Committee reiterates its earlier recommendation that total dietary exposure to magnesium from food additives and other sources in the diet should be assessed. This is important, as a large number of magnesium-containing food additives have been evaluated individually, but not collectively, in relation to their laxative effects.

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Maltotetraohydrolase from *Pseudomonas stutzeri* expressed in *Bacillus licheniformis*

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1. Explanation

At the request of the Codex Committee on Food Additives at its Forty-sixth Session (FAO/WHO, 2014), the Committee evaluated the safety of maltotetraohydrolase (glucan 1,4-α-maltotetraohydrolase; Enzyme Commission

No. 3.2.1.60) from *Pseudomonas stutzeri* expressed in *Bacillus licheniformis*, which it had not considered previously. The donor organism was recently reclassified from *Pseudomonas saccharophila*. Maltotetraohydrolase catalyses the hydrolysis of 1,4-α-D-glucosidic linkages in amylaceous polysaccharides. The reaction removes successive maltotetraose residues from the non-reducing chain ends. In this report, the expression "maltotetraohydrolase" refers to the modified maltotetraohydrolase enzyme and its amino acid sequence, the expression "maltotetraohydrolase liquid enzyme concentrate" refers to the test material used in the toxicity studies evaluated, and the expression "maltotetraohydrolase enzyme preparation" refers to the preparation formulated for commercial use. The maltotetraohydrolase enzyme preparation is commonly used as a processing aid in bakery products such as bread, bread buns, tortillas and crackers, as well as in the starch processing industry for the manufacture of corn sweeteners, such as high-fructose corn syrup (HFCS).

1.1 **Genetic background**

Maltotetraohydrolase is produced from a genetically modified strain of *B. licheniformis*, BML347, which is derived from *B. licheniformis* strain BRA-7. *Bacillus licheniformis* is a Gram-positive bacterium that is widely distributed in nature and is considered to be non-pathogenic and non-toxigenic. *Bacillus licheniformis* has a history of use in the production of enzymes used in food processing, including enzymes from genetically engineered strains of the organism. *Bacillus licheniformis* strain BRA-7 is also the production strain of pullulanase, which was previously evaluated by the Committee (Annex 1, reference *206*).

      The gene donor strain was originally identified as *Pseudomonas saccharophila* and was later reclassified as *Pseudomonas stutzeri*; thus, the name *P. saccharophila* still appears in most references (DuPont, 2014).

      Prior to the introduction of the maltotetraohydrolase gene from *P. stutzeri*, the host *B. licheniformis* strain was genetically modified through a series of deletions of genes encoding α-amylase, chloramphenicol acetyltransferase, subtilisin, glutamic acid–specific protease and the *spoIIAC* gene responsible for sporulation. The resulting strain was transformed using an expression cassette containing the maltotetraohydrolase *SAS3* gene, obtained from genetic cloning and a series of site-directed mutagenesis events. The maltotetraohydrolase *SAS3* gene encodes a variant of the wild-type *P. stutzeri* maltotetraohydrolase, with the C-terminal starch-binding domain removed, 16 amino acids changed and one methionine residue added at the N-terminus of the enzyme. These changes improved thermostability, baking performance and fermentation yield. Upon transformation, the maltotetraohydrolase expression cassette was integrated into the host *B. licheniformis* strain, and the rest of the plasmid was deleted by

recombinant excision. The final production strain was tested and found to be genetically stable.

1.2 Chemical and technical considerations

Maltotetraohydrolase is produced by submerged straight-batch or fed-batch pure culture fermentation of the genetically modified strain of *B. licheniformis.* The fermentation broth carrying the enzyme is separated from the biomass by filtration and/or centrifugation. The liquid filtrate containing the enzyme is then concentrated by ultrafiltration, followed by polish filtration. The resulting enzyme concentrate is either spray-dried and standardized to the desired activity with food-grade ingredients (powdered form) or treated with sodium benzoate and potassium sorbate to the desired activity (liquid form). The maltotetraohydrolase enzyme preparation conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm).

The activity of the maltotetraohydrolase enzyme is measured in betamyl units (BMU). One BMU is defined as the activity degrading 0.0351 mmol of blocked *p*-nitrophenyl-α-D-maltoheptaoside per minute in the presence of amyloglucosidase and α-glucosidase at 25 °C in a reaction mix for 5 minutes. The mean activity of maltotetraohydrolase from three batches of the enzyme concentrate prior to formulation was approximately 300 000 BMU/g.

A typical commercial formulation of the maltotetraohydrolase enzyme preparation will contain 32% total organic solids (TOS). TOS includes the enzyme of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism during the manufacturing process. The maltotetraohydrolase enzyme preparation is used at levels up to 23 mg TOS/kg raw material, depending on the proposed food application. The maltotetraohydrolase enzyme is expected to be inactivated during processing.

2. Biological data

2.1 Assessment of potential allergenicity

The maltotetraohydrolase under evaluation contains 429 amino acids with known sequence, and its molecular weight (both theoretical and as determined by liquid chromatography–mass spectrometry) is 47.6 kDa. Maltotetraohydrolase was evaluated for potential allergenicity using the bioinformatics criteria recommended by FAO/WHO (2001, 2009), but modified at the present meeting. The amino acid sequence of maltotetraohydrolase was compared with the amino

acid sequences of known allergens in the AllergenOnline database (http://www.allergenonline.org/index.shtml) and in the Allermatch database (http://www.allermatch.org). A search for matches with greater than 35% identity over a window of 80 amino acids and a search for sequence identity of eight contiguous amino acids produced no match.

Additionally, a full-length FASTA sequence search using *E*-value <0.1 as the cut-off revealed a match with Asp o 21, α-amylase from *Aspergillus oryzae* (TAKA-amylase A), with 23.7% sequence identity in a 325 amino acid stretch. TAKA-amylase A is not identified as a food allergen by the Allergen Nomenclature Sub-Committee of the World Health Organization/International Union of Immunological Societies. Sensitization to TAKA-amylase A is mainly from occupational exposure, such as working in a bakery (Green & Beezhold, 2011). There have been only four reported cases of allergy to TAKA-amylase A after ingestion (Losada et al., 1992; Baur & Czuppon, 1995; Kanny & Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004), among which three occurred in occupational workers. Furthermore, oral challenge studies conducted among patients with documented occupational or other allergy found no cases of allergy to TAKA-amylase A (Skamstrup Hansen et al., 1999; Bindslev-Jensen et al., 2006). As maltotetraohydrolase is also an amylase, amino acid sequence homology among these two enzymes is expected. However, based on the crystal structure of TAKA-amylase A and the sequence alignment result, there are large sequence differences in the loop regions, contributing to the low (<35%) homology. In contrast, the homologous regions are hydrophobic and do not locate on the enzyme surface area and thus are unlikely to be immunoglobulin E epitopes.

Based on the facts that there is no match found with 80 and eight amino acid searches and that maltotetraohydrolase shares only a low (23.7%) amino acid sequence homology with TAKA-amylase A, which is not a food allergen, maltotetraohydrolase does not seem to have the characteristics of a potential food allergen. Therefore, the Committee considered that dietary exposure to maltotetraohydrolase is not anticipated to pose a risk of allergenicity.

2.2 Toxicological studies

The acute and short-term toxicity and genotoxicity of maltotetraohydrolase have been tested using a liquid enzyme concentrate (protein content: 78.76 mg/mL; TOS: 9.09%; specific gravity: 1.028 mg/mL). This maltotetraohydrolase liquid enzyme concentrate was used as the test material in all the toxicological studies discussed in this section.

2.2.1 **Acute toxicity**

A good laboratory practice (GLP)–compliant acute oral toxicity study was performed in female Sprague-Dawley CD rats (Genencor, 2009) according to Organisation for Economic Co-operation and Development (OECD) test guideline 420. In the sighting study, one female rat was administered a single dose of maltotetraohydrolase liquid enzyme concentrate by oral gavage at 2 g/kg bw. In the absence of toxicity in this sighting study, four additional female rats were given the same dose orally in the main study. At the end of a 14-day observation period, all the animals were weighed and terminated. A gross necropsy was performed, and all macroscopic signs were recorded.

No mortality and no overt signs of systemic toxicity were observed during the study or at necropsy. It is thus concluded that under the study conditions, maltotetraohydrolase liquid enzyme concentrate orally administered to rats at 2 g/kg bw did not show evidence of acute toxicity (Genencor, 2009).

2.2.2 **Short-term studies of toxicity**

A GLP-compliant 13-week repeated-dose oral toxicity study in rats was performed according to OECD test guideline 408. In this study, four groups of Wistar Han[TM]:HsdRccHan[TM]:WIST rats (10 of each sex per group) were administered maltotetraohydrolase liquid enzyme concentrate by gavage at a dose of 0 (0.9% saline), 23.7, 47.4 or 79.0 mg total protein/kg bw in a constant volume of 5 mL/kg bw; these doses corresponded to 0, 28.0, 56.0 and 93.4 mg TOS/kg bw, respectively (Dhinsa & Brooks, 2008).

The animals were fed ad libitum and had free access to water. All animals were observed daily for mortality and signs of morbidity. Body weight and feed consumption were recorded weekly, and water consumption was recorded twice weekly for each cage. Ophthalmologic examination was performed on all animals prior to study initiation and in the control and high-dose groups prior to study termination. Haematology was conducted on day 90. A functional observational battery consisting of detailed clinical observation, reactivity to handling and stimuli, motor activity examination and forelimb/hindlimb grip strength as well as clinical chemistry were evaluated at study termination, prior to necropsy, on all groups. After a thorough macroscopic examination, selected organs were removed, weighed and processed for future histopathological examination. Microscopic examination was conducted on selected organs from control and high-dose animals. If a questionable finding was noted, the microscopic examination was extended to the low- and mid-dose groups. There were two interim deaths in this study, but both were considered to be unrelated to treatment. One low-dose female was killed in extremis on day 68 as a result of significant morbidity. The other death occurred on day 90 in a mid-dose female, which did not show any clinical signs prior to death.

Among the surviving animals, no abnormal clinical signs were observed in functional performance tests. There were no biologically or statistically significant differences between the control and treated groups with respect to body weight, feed consumption, water consumption, feed efficiency (ratio of body weight gain to dietary intake), haematology or ophthalmologic examinations.

Females from all treated groups showed higher serum potassium levels (i.e. 4.526, 4.469 and 4.433 nmol/L in the low-, mid- and high-dose groups, respectively) compared with concurrent controls (3.881 nmol/L). However, as these levels were still within the range of historical control values and the increase was not related to dose, it was considered to be unrelated to treatment.

Females from all treated groups showed lower absolute and relative ovary weights when compared with concurrent controls. On review of the historical control data, the control values for this parameter were higher than the expected ranges for female rats of the age and strain employed. In the absence of histopathological findings, these findings were not attributed to treatment.

Incidental macroscopic findings were noted (one high-dose male displayed small kidneys; one high-dose male exhibited hydronephrosis on the right kidney), but were not considered to be treatment related in the absence of relevant histopathological changes. Scattered histopathological findings were noted, but all morphological changes were those commonly observed in rats of the age and strain employed, and there were no differences in severity or incidence between the control and treated groups.

In conclusion, the 13-week rat study did not demonstrate adverse effects on clinical chemistry, haematology, functional observational tests or macroscopic and histopathological examinations. Under the conditions of this study, a no-observed-adverse-effect level (NOAEL) of 79.0 mg total protein/kg bw per day (corresponding to 93.4 mg TOS/kg bw per day), the highest dose tested, was identified (Dhinsa & Brooks, 2008).

2.2.3 Long-term studies of toxicity and carcinogenicity

No information was available.

2.2.4 Genotoxicity

The maltotetraohydrolase liquid enzyme concentrate was tested for genotoxicity using the bacterial reverse mutagenicity test (Ames assay) and the in vitro chromosomal aberration assay, conducted in accordance with OECD test guidelines 471 and 473, respectively. Both studies were certified for compliance with GLP and quality assurance. The results of both studies were negative (Table 1), indicating that the maltotetraohydrolase enzyme preparation is unlikely to be genotoxic.

Table 1

Genotoxicity of maltotetraohydrolase from *Pseudomonas stutzeri* expressed in *Bacillus licheniformis*

End-point	Test system	Concentration	Results	Reference
In vitro				
Reverse mutation[a]	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2uvrA	50–5 000 μg/plate ±S9[b]	Negative	Bowles (2008)
Chromosomal aberrations[c]	Human lymphocytes	1st experiment: 4 h exposure: 19.5–625 μg/mL ±S9[b] 2nd experiment: 4 h exposure: 19.5–625 μg/mL +S9[b] 24 h exposure: 19.5–312.5 μg/mL −S9[b]	Negative	Morris (2008)

S9: 9000 × *g* supernatant fraction from rat liver homogenate

[a] The test was performed by the standard plate incorporation method. The positive controls used for the assays without S9 mix were *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine (3 μg/plate for TA100, 5 μg/plate for TA1535, 2 μg/plate for WP2uvrA); 9-aminoacridine (80 μg/plate for TA1537); and 4-nitroquinoline-1-oxide (0.2 μg/plate for TA98). The positive controls used with S9 mix were 2-aminoanthracene (1 μg/plate for TA100, 2 μg/plate for TA1535 and TA1537, 10 μg/plate for WP2uvrA) and benzo[*a*]pyrene (5 μg/plate for TA98). A preliminary dose range–finding test was carried out using TA100 and WP2uvrA, with and without S9, with 11 concentrations of the test material (0–5000 μg total protein/plate). In the main study, each of the five strains was tested using five doses of the test material (50, 150, 500, 1500 and 5000 μg total protein/plate), with and without S9 mix. All tests were repeated in two independent assays; each was performed in triplicate. The positive control substances produced marked increases over the concurrent negative control values, and the test substance was not toxic to the test bacteria. There was no significant increase in the number of induced revertant colonies in any strain at any dose in the tests both with and without metabolic activation.

[b] The S9 mix was prepared from male Sprague-Dawley rats that received three consecutive daily doses of phenobarbitone/*p*-naphthoflavone (80/100 mg/kg bw per day). S9 acted as an exogenous metabolic activation system.

[c] A preliminary dose range–finding test was conducted with nine concentrations of the test material (19.53–5000 μg/mL). Two main experiments were subsequently conducted, using six concentrations (range shown in table), with only some concentrations selected for metaphase analysis (156.25–625 μg/mL in the 4-hour exposure and 156.25–312.5 μg/mL in the 24-hour exposure). Cells were either treated for 4 hours with or without the presence of S9 and followed by a 20-hour treatment-free incubation or continuously treated for 24 hours without S9. The average generation time of the cultured cells was approximately 17 hours, measured by bromodeoxyuridine incorporation. The negative control used was Eagle's minimal essential medium. Positive controls were mitomycin C (0.4 and 0.2 μg/mL for experiment 1 and experiment 2, respectively) in the absence of S9; and cyclophosphamide (5 μg/mL) in the presence of S9. Two slides from each culture were prepared, and 50 well spread metaphases on each slide were examined. The result was considered positive if the test substance markedly increased the average percentage of aberrant cells (excluding gaps) compared with the negative control value. A dose–response relationship was required only if the increase was moderate. The negative and positive controls worked properly in all tests. Maltotetraohydrolase liquid enzyme concentrate did not produce an increase in the frequency of aberrant cells more than twice the control values at any dose.

2.2.5 Reproductive and developmental toxicity

No information was available.

2.3 Observations in humans

No information was available.

3. Dietary exposure

3.1 Introduction

The Committee evaluated one submission received from DuPont Industrial Biosciences, a manufacturer of the maltotetraohydrolase enzyme preparation.

The maltotetraohydrolase enzyme preparation is used internationally, being approved for use in Australia, Canada, Denmark, France and New Zealand, and is generally recognized as safe for use in food processing in the USA. The enzyme is not expected to remain in downstream products, such as HFCS, following purification processes used in their manufacture, but would be present and inactivated in bakery products. The maltotetraohydrolase enzyme preparation is used across a number of food products, and therefore an upper-bounding dietary exposure assessment approach was taken. A theoretical "worst-case" dietary exposure estimate was made, assuming that 100% of food products in which maltotetraohydrolase enzyme preparation could be used would be manufactured using the product and that 100% would remain in the final food products.

The Committee concluded that a tiered approach to assessing potential dietary exposure to the maltotetraohydrolase enzyme preparation was not necessary, as the upper-bounding exposure estimate was evaluated.

3.2 Dietary exposure assessment

Two broad uses of maltotetraohydrolase enzyme preparation were nominated in the submission (manufacture of starch for sweeteners and baking). These were considered individually to arrive at a total dietary exposure estimate.

3.2.1 Starch production

The maltotetraohydrolase enzyme preparation is used to prepare starch for the production of HFCS. The Committee considered that the consumption of HFCS was likely to be higher for the population of the USA than elsewhere; therefore, it was assumed that HFCS consumption figures for the USA were sufficient to cover all possible use scenarios. For the USA, per capita consumption of HFCS was 20.9 kg/person per year (57.3 g/person per day, 0.96 g/kg bw per day for a 60 kg individual) in 2012 (USDA, 2012). Reference to the 17 consumption cluster diets of the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) (WHO, 2012) confirms that cluster G10, which includes the USA, has the highest apparent consumption of total sugars, at 130 g/day; however, as total sugars also includes other products, the USDA (2012) figure for HFCS consumption was used in the dietary exposure estimate.

If maltotetraohydrolase enzyme preparation is applied at the highest suggested rate of 20 mg TOS/kg starch, which is transformed quantitatively to HFCS, the dietary exposure would be 0.019 mg TOS/kg bw per day.

3.2.2 Bakery products

The maltotetraohydrolase enzyme preparation is used in the baking industry at the maximum rate of 23 mg TOS/kg flour. To estimate the contribution to dietary exposure from flour, the consumption of total flour for the population of the USA was used (USDA, 2012). If a consumption of flour of 79.2 kg/year (3.6 g/kg bw per day for a 60 kg individual) is assumed and the maximum rate of 23 mg TOS/kg flour is used, the dietary exposure to maltotetraohydrolase enzyme preparation would be 0.083 mg TOS/kg bw per day.

3.2.3 Assessment of total dietary exposure

The summation of the maximum dietary exposures from each of the two uses of maltotetraohydrolase enzyme preparation (0.019 + 0.083 mg TOS/kg bw per day) results in a potential total dietary exposure of 0.1 mg TOS/kg bw per day for a 60 kg individual. This estimate assumes maximum use levels, 100% market penetration for the production of HFCS and bakery products and the presence of the maltotetraohydrolase enzyme following the production process. The Committee noted that under these conditions, the major contributor to total dietary exposure to maltotetraohydrolase enzyme preparation would be the consumption of bakery products.

4. Comments

4.1 Assessment of potential allergenicity

Maltotetraohydrolase was evaluated for potential allergenicity using the bioinformatics criteria recommended by FAO/WHO (2001, 2009), but modified at the present meeting. The amino acid sequence of the enzyme was compared with the amino acid sequences of known allergens in publicly available databases. A search for matches with greater than 35% identity over a window of 80 amino acids and a search for sequence identity of eight contiguous amino acids produced no match. Therefore, the Committee considered that dietary exposure to maltotetraohydrolase enzyme preparation is not anticipated to pose a risk of allergenicity.

4.2 **Toxicological studies**

Maltotetraohydrolase liquid enzyme concentrate administered to rats at 2 g/kg bw in an acute oral toxicity study demonstrated no sign of toxicity (Genencor, 2009). In a 13-week repeated-dose oral toxicity study in rats, no treatment-related adverse effects were observed when the maltotetraohydrolase liquid enzyme concentrate was administered by gavage at doses up to 93.4 mg TOS/kg bw per day (Dhinsa & Brooks, 2008). The results of an in vitro bacterial reverse mutation assay (Bowles, 2008) and an in vitro chromosomal aberration assay in human lymphocytes (Morris, 2008) using the maltotetraohydrolase liquid enzyme concentrate were both negative. The Committee concluded that maltotetraohydrolase enzyme preparation is unlikely to be genotoxic.

4.3 **Assessment of dietary exposure**

An estimate of the theoretical dietary exposure to maltotetraohydrolase enzyme preparation was made by the Committee based on the maximum level of TOS in the enzyme preparation and its maximum use levels in bakery products (23 mg TOS/kg flour) and HFCS production (20 mg TOS/kg starch). The combination of these levels with per capita food consumption data from the USA (supplied by the sponsor; corroborated with data from the GEMS/Food cluster diets) results in a potential dietary exposure of 0.1 mg TOS/kg bw per day for a 60 kg individual. The Committee noted that the enzyme will be inactivated in food processing and also removed from the HFCS final product during production.

5. Evaluation

No treatment-related adverse effects were seen at the highest dose tested (93.4 mg TOS/kg bw per day) in the 13-week study of oral toxicity in rats (Dhinsa & Brooks, 2008). A comparison of the dietary exposure estimate of 0.1 mg TOS/kg bw per day with the highest dose tested of 93.4 mg TOS/kg bw per day results in a margin of exposure of at least 900. The Committee established an acceptable daily intake (ADI) "not specified" for maltotetraohydrolase from *P. stutzeri* expressed in *B. licheniformis* when used in the applications specified and in accordance with good manufacturing practice.

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Mixed β-glucanase, cellulase and xylanase from *Rasamsonia emersonii*

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1. Explanation

At the request of the Codex Committee on Food Additives at its Forty-sixth Session (FAO/WHO, 2014), the Committee evaluated the safety of mixed β-glucanase (3-(1,3;1,4)-β-D-glucan 3(4) glucanohydrolase; Enzyme Commission No. 3.2.1.6), cellulase (4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase; Enzyme Commission No.

3.2.1.4) and xylanase (1,4-β-D-xylan xylanohydrolase; Enzyme Commission No. 3.2.1.8) from *Rasamsonia emersonii*. This enzyme preparation has not been evaluated previously by the Committee. *Rasamsonia emersonii* was recently renamed from *Talaromyces emersonii* based on genetic analyses (Houbraken, Spierenburg & Frisvad, 2012). The Committee evaluated several other enzyme preparations of β-glucanase, cellulase or xylanase at its thirty-first, thirty-fifth, thirty-ninth, sixty-first and sixty-third meetings and established an acceptable daily intake (ADI) "not specified" for their use in several applications, such as the preparation of beer and baking products (Annex 1, references *78*, *88*, *101*, *167* and *174*). An exception was cellulase from *Penicillium funiculosum*, for which no ADI was established, as no safety data were submitted (Annex 1, reference *77*). In this report, the expression "mixed β-glucanase, cellulase and xylanase liquid enzyme concentrate" is used when referring to the material tested in the toxicological studies evaluated; the expressions "β-glucanase", "cellulase" and "xylanase" are used when referring to the enzymes and their amino acid sequences; and the expression "mixed β-glucanase, cellulase and xylanase enzyme preparation" is used when referring to the commercial enzyme product.

β-Glucanase is an enzyme that catalyses the hydrolysis of 1,3- or 1,4-β-D-glucosidic linkages in β-D-glucans. Cellulase is an enzyme that catalyses the endo-hydrolysis of 1,4-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans and the hydrolysis of 1,4-linkages in β-D-glucans that also have 1,3-linkages. Xylanase is an enzyme that catalyses the hydrolysis of 1,4-β-xylosidic linkages in xylans.

The mixed β-glucanase, cellulase and xylanase enzyme preparation is intended to be used as a processing aid in brewing, potable alcohol (spirits) production and grain processing.

1.1 Genetic background

The β-glucanase, cellulase and xylanase enzymes are simultaneously produced at high levels from a strain of *R. emersonii*. *Rasamsonia emersonii* has been taxonomically identified to be from the genus *Rasamsonia* by the Dutch culture collection, the Centraalbureau voor Schimmelcultures. *Rasamsonia emersonii* is a filamentous eukaryotic thermostable fungus that is capable of growing at pH 3.5–5.5 and 45–50 °C. *Rasamsonia emersonii* is also referred to in the literature as *Penicillium emersonii*, *Geosmithia emersonii* and *Talaromyces emersonii*. *Rasamsonia emersonii* is a non-pathogenic microorganism with a history of use in commercial food enzyme production. The *R. emersonii* production strain has been demonstrated to be genetically stable under laboratory conditions, with no significant decrease in yield or change in appearance of morphological variants. It is derived from the original wild-type strain that has been used for large-scale

production of the mixed β-glucanase, cellulase and xylanase enzyme preparation since 1985. The production strain is a modification of the wild-type *R. emersonii* strain for increased enzyme production by classical mutagenesis and selection for higher enzyme productivity. Data indicate that the production strain does not produce mycotoxins under large-scale fermentation conditions, indicating that the production strain is non-toxigenic.

1.2 Chemical and technical considerations

The β-glucanase, cellulase and xylanase enzymes are produced by a controlled aerobic submerged fed-batch fermentation of a pure culture of *R. emersonii*. The enzymes are secreted into the fermentation broth and subsequently purified and concentrated. The enzyme concentrate is formulated with glycerol and sodium benzoate to achieve the desired activity and stability. The mixed β-glucanase, cellulase and xylanase enzyme preparation contains commonly used food-grade materials and conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm).

The β-glucanase and cellulase activity is expressed in beta-glucanase fungique (BGF) units, as defined in the specific assay that measures a change in viscosity of a glucan substrate solution in the presence of β-glucanase and cellulase. The xylanase activity is expressed in xylanase viscosity units (XVU), as defined in the specific assay that measures a change in viscosity of a xylan substrate solution in the presence of xylanase; however, the method described to determine this activity is proprietary and non-transferable. The mean activities of β-glucanase and cellulase and of xylanase from three batches of the mixed β-glucanase, cellulase and xylanase, prior to formulation, were reported to be approximately 500 000 BGF/g and 3800 XVU/g, respectively.

A typical commercial formulation of the mixed β-glucanase, cellulase and xylanase enzyme preparation will contain 5.4–17% total organic solids (TOS), depending on the use. TOS includes the enzymes of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism during the manufacturing process. The mixed β-glucanase, cellulase and xylanase enzyme preparation is used in brewing, potable alcohol (spirits) production and grain processing (production of non-alcoholic beverages [including soft drinks] and bakery ingredients) to reduce viscosity and improve filterability, yield and product consistency; it will be used at levels up to 25.5 mg TOS/kg raw material. The β-glucanase, cellulase and xylanase enzymes are expected to be inactivated during processing.

2. Biological data

2.1 Assessment of potential allergenicity

Rasamsonia emersonii is not listed in the World Health Organization/International Union of Immunological Societies allergen nomenclature (http://www.allergen.org/).

β-Glucanase, xylanase and cellulase from *R. emersonii* have commonly been found in food, and there are no indications for allergic reactions due to their ingestion. In addition, two β-glucanases, a xylanase (xylanase from *R. emersonii* expressed in *Aspergillus niger*, patent WO 20024926 A1) and a cellulase (cellulase from *R. emersonii* [formerly known as *T. emersonii*] from *R. emersonii*) have been evaluated for potential allergenicity using the bioinformatics criteria recommended by FAO/WHO (2001, 2009), but modified at the present meeting. A search for matches with greater than 35% identity over a window of 80 amino acids and a search for sequence identity of eight contiguous amino acids using the database AllergenOnline (available at http://www.allergenonline.org/, accessed 9 October 2013 and 27 November 2013) produced no match. Based on these data, the Committee concluded that dietary exposure to the β-glucanase, cellulase and xylanase enzyme preparation from *R. emersonii* is not anticipated to pose a risk of allergenicity.

2.2 Toxicological studies

The toxicological studies were performed with a mixed β-glucanase, cellulase and xylanase liquid enzyme concentrate (batch no. OP8017; dry matter content, 6.2% by weight; TOS content, 5.3% by weight), omitting stabilization and standardization. The liquid enzyme concentrate had activities of 92 000 BGF/g (β-glucanase and cellulase) and 520 XVU/g (xylanase).

2.2.1 Acute toxicity

No information was available.

2.2.2 Short-term studies of toxicity

In a 14-day range-finding study, groups of five male and five female CD rats were given the mixed β-glucanase, cellulase and xylanase liquid enzyme concentrate from *R. emersonii* at a dose equal to 0, 5.3, 26.5 or 133 mg TOS/kg body weight (bw) per day by oral gavage (Cooper, 2000a). Clinical signs, body weight, feed consumption, feed conversion efficiency, haematology, blood chemistry, weights of principal organs and macroscopic pathology were studied in all animals.

No treatment-related deaths or clinical signs were observed. Body weight, feed consumption and feed conversion efficiency were not adversely affected. Final body weights (up to +6%) and body weight gain (up to +11%) were slightly higher in treatment groups than in controls. Slightly lower haemoglobin concentrations (up to −7%, no dose–response relationship) were noted in all treated females, compared with controls. Also, haematocrit and erythrocyte counts were lower (up to, respectively, −7% and −8%) in treated females compared with controls, reaching significance in the low-dose group (haematocrit and erythrocyte counts) and/or the mid-dose group (haematocrit) only. The slightly higher alanine aminotransferase activities (+22–24%) seen in the mid-dose group (males only) and the high-dose group (both sexes) and the lower cholesterol levels (−14–15%) seen in females of the mid- and high-dose groups are considered to be of no toxicological concern. In addition, when compared with controls, plasma phosphorus concentrations were decreased (up to −16%) in all treatment groups, and calcium concentrations were lower (up to –5%) in treated males and females of the mid-dose group. Glucose concentrations were increased (up to +32%) in treated females and males of the mid-dose group. Potassium concentrations were statistically significantly increased in females of the high-dose group. All changes were small and not dose related and were therefore not considered to be of toxicological relevance. Organ weights were not affected by the treatment. Lower absolute and relative uterus and cervix weights were observed for females in the high-dose group, but statistical significance was not attained. No macroscopic findings related to treatment were observed. In the absence of adverse effects, the no-observed-adverse-effect level (NOAEL) for this study was 133 mg TOS/kg bw per day, the highest dose tested (Cooper, 2000).

In the subsequent 90-day study of toxicity, groups of 20 male and 20 female CD rats were given the mixed β-glucanase, cellulase and xylanase liquid enzyme concentrate from *R. emersonii* at a dose equal to 0, 5.3, 21.2 or 84.8 mg TOS/kg bw per day by oral gavage (in water obtained by reverse osmosis). The study was designed to meet the requirements of the United States Food and Drug Administration Redbook and was certified for compliance with good laboratory practice (GLP) and quality assurance (QA) (Cooper, 2000b). All animals were evaluated with respect to general clinical observations, body weight, feed consumption, feed conversion efficiency, ophthalmoscopic examination, haematology, clinical chemistry, weights of principal organs, macroscopic examination and histopathology of selected organs (control and high-dose groups) and histopathology of all lesions.

No treatment-related deaths or clinical signs were observed. Ophthalmoscopic, macroscopic and microscopic examinations did not reveal any abnormalities associated with the treatment.

Body weight gain of males of the high-dose group was slightly lower (−6%) than that of controls, and weekly feed consumption by males of the high-dose group was slightly lower than that of the controls (overall feed intake was 97% of feed intake of controls), but these effects were not statistically significant.

When compared with the controls, slightly longer prothrombin times (0.7 second longer) were recorded for both sexes of the high-dose group, with slightly longer activated partial thromboplastin times (3.6 seconds longer) also seen in the females. These slight changes in haematology are considered to be of no toxicological relevance.

Slightly lower aspartate aminotransferase activities were observed in females of the high-dose group (71 versus 85 units/L in controls). High levels of aspartate aminotransferase activity are markers for liver damage in rats, whereas low activity levels are considered to be of no toxicological significance. Glucose concentrations were increased in males of the high-dose group (7.54 versus 6.63 mmol/L in controls). Triglyceride concentrations were higher (up to +28%) in treated females, reaching statistical significance only in the low- and mid-dose groups. Sodium levels were slightly decreased in females of the high-dose group (140 versus 142 mmol/L in controls). As these effects were small and/or lacked a dose–response relationship, they were not considered to be toxicologically relevant.

Based on the absence of adverse effects in this study, the NOAEL was 84.8 mg TOS/kg bw per day, the highest dose tested (Cooper, 2000b).

2.2.3 Long-term studies of toxicity and carcinogenicity

No information was available.

2.2.4 Genotoxicity

The results of two studies of genotoxicity in vitro with the mixed β-glucanase, cellulase and xylanase liquid enzyme concentrate are summarized in Table 1. The first study followed Organisation for Economic Co-operation and Development (OECD) test guideline 471 (Bacterial Reverse Mutation Test, 1997), and the second study, OECD test guideline 473 (In Vitro Mammalian Chromosome Aberration Test, 1997). Both studies were certified for compliance with GLP and QA. Based on the results, the Committee concluded that the mixed β-glucanase, cellulase and xylanase enzyme preparation is unlikely to be genotoxic.

2.2.5 Reproductive and developmental toxicity

No information was available.

Table 1

Genotoxicity of the mixed β-glucanase, cellulase and xylanase liquid enzyme concentrate in vitro

End-point	Test system	Concentration	Results	Reference
In vitro				
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2uvrApKM101	100–10 000 µg/mL, ±S9	Negative[a]	May (1999)
Chromosomal aberrations	Human lymphocytes	1st experiment: 1 250, 2 500 or 5 000 µg/mL, ±S9	Negative[b]	Mason (1999)
		2nd experiment: 1 000, 3 000 or 4 000 µg/mL, −S9 1 250, 2 500 or 5 000 µg/mL, +S9		

S9: 9000 × *g* supernatant fraction from rat liver homogenate

[a] Two independent experiments were performed, the first using the plate incorporation method and the second using the preincubation assay. The same concentration range, with and without S9, was used in both experiments.

[b] In the first experiment, the cells were treated for 3 hours without and with S9 and were harvested 17 hours later. The highest tested concentration induced 16% mitotic inhibition in the presence of S9. In the absence of S9, no significant mitotic inhibition was observed. In the second experiment, the cells were exposed continuously for 20 hours without S9 and then harvested. With S9, the cells were treated for 3 hours and harvested 17 hours later. The highest tested concentration induced 60% mitotic inhibition in the absence of S9. In the presence of S9, no significant mitotic inhibition was observed. In the first experiment, no significant increase in chromosomal aberrations was observed. In the second experiment, in the absence of S9, a statistically significant increase in the proportion of cells with chromosomal aberrations was observed at 4000 µg/mL when gap-type aberrations were included in the analysis. The percentage of cells with aberrations including gaps was 10.5% versus 4.0% in controls. In the absence of a statistically significant increase in chromosomal aberrations when gaps were excluded, the results indicated that the material tested does not induce chromosomal aberrations.

2.3 Observations in humans

No information was available.

3. Dietary exposure

3.1 Introduction

The mixed β-glucanase, cellulase and xylanase enzyme preparation is intended to be used as a processing aid in brewing, potable alcohol (spirits) production and grain processing (production of non-alcoholic beverages [including soft drinks] and bakery ingredients [starch, fibres, flour]). The estimated maximum levels in final food products are 3.5 mg TOS/L in beer, 3 mg TOS/L in non-alcoholic beverages, 3 mg TOS/kg in bakery ingredients and 0 mg TOS/L in potable alcohol (spirits).

The enzymes are inactivated during the intended processes. In addition to the enzymes in question, the mixed β-glucanase, cellulase and xylanase enzyme preparation also contains some substances derived from the producing microorganism and the fermentation medium.

The Committee evaluated one submission by a sponsor (Reuvers, 2014) on dietary exposure to the additive using assessments based on individual dietary records.

3.2 Assessment of dietary exposure

The estimated daily intake (EDI) was calculated by the sponsor based on the estimated levels in the final food products and intake levels of beverages and solid food based on per capita food consumption data from the United States Department of Agriculture (USDA) (Wilkinson Enns, Goldman & Cook, 1997; Wilson et al., 1997). As a "worst-case situation", the 90th percentile of the intake level was taken except for alcoholic beverages, for which the 95th percentile was used. The EDI was estimated to be 0.001–0.02 mg TOS/kg bw per day from beer and ale using an estimated consumption per person of 360 mL beverage/day, 0.01–0.04 mg TOS/kg bw per day from non-alcoholic beverages (fruit drinks and ades; carbonated soft drinks; 838 mL/day) and 0.008–0.02 mg TOS/kg bw per day from grain products (cereal and pasta; quick breads, pancakes, French toast; cakes, cookies, pastries, pies; crackers, popcorn and chips; mixtures mainly grain, 502 g/day). The three estimates sum to 0.02–0.08 mg TOS/kg bw per day, or 1–5 mg/day for a 60 kg person. This estimate assumes that all cereal grain products, including beer and ale, are treated with the mixed β-glucanase, cellulase and xylanase enzyme preparation and would therefore be an overestimate.

Reference to the 17 consumption cluster diets of the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) (WHO, 2012) reveals that clusters G08, G11 and G15 (including a number of European countries) have the highest apparent consumption of beer (225–260 g/day). Cluster G10, which includes the USA, has an apparent consumption of 174 g/day. If the mixed β-glucanase, cellulase and xylanase enzyme preparation is present at the highest suggested level of 3.5 mg TOS/L beverage, the dietary exposure at the maximum consumption of 260 g/day would be 0.9 mg TOS/day (or 0.02 mg TOS/kg bw per day for a 60 kg person).

Cluster G02 (including a number of Eastern European/Central Asian countries) has the highest apparent consumption of non-alcoholic beverages (excluding milk-based beverages, stimulants and water) (75 g/day), followed by cluster G15 (including a number of European countries), at 56 g/day, and cluster G10, which includes the USA, at 47 g/day. If the mixed β-glucanase, cellulase and xylanase enzyme preparation is present at the highest suggested level of 3 mg TOS/L beverage, the dietary exposure at the maximum consumption of 75 g/day would be 0.2 mg TOS/day (or 0.004 mg TOS/kg bw per day for a 60 kg person). The estimate for consumption used here (75 g/day) is significantly lower than the estimate used by the sponsor (838 mL/day).

Clusters G01, G06 and G09 (including a number of countries from Asia, the Middle East and North Africa) have the highest apparent consumption of cereals and flour (370–480 g/day). If the mixed β-glucanase, cellulase and xylanase enzyme preparation is present at the highest suggested level of 3 mg TOS/kg product, the dietary exposure at the maximum consumption of 480 g/day would be 1.4 mg TOS/day (or 0.02 mg TOS/kg bw per day for a 60 kg person).

The three estimates (0.9, 0.2 and 1.4 mg TOS/day) sum to 2.5 mg TOS/day, or 0.04 mg TOS/kg bw per day for a 60 kg person. This estimate assumes that all cereal grain products, including beer and ale, are treated with the enzyme mixture and would therefore be an overestimate. The estimate is within the range of the EDI calculated by the sponsor using survey data from the USDA.

4. Comments

4.1 Assessment of potential allergenicity

β-Glucanase, xylanase and cellulase from *R. emersonii* have commonly been found in food, and there are no indications for allergic reactions due to their ingestion. In addition, two β-glucanases, a xylanase and a cellulase from *R. emersonii* have been evaluated for potential allergenicity using the bioinformatics criteria recommended by FAO/WHO (2001, 2009), but modified at the present meeting. A search for matches with greater than 35% identity over a window of 80 amino acids and a search for sequence identity of eight contiguous amino acids produced no match. Based on these data, the Committee concluded that dietary exposure to the β-glucanase, cellulase and xylanase enzymes from *R. emersonii* is not anticipated to pose a risk of allergenicity.

4.2 Toxicological studies

In a 13-week study of oral toxicity in rats, no treatment-related adverse effects were seen when the mixed β-glucanase, cellulase and xylanase liquid enzyme concentrate was administered by gavage at doses up to 84.8 mg TOS/kg bw per day (Cooper, 2000). The mixed β-glucanase, cellulase and xylanase liquid enzyme concentrate gave negative results in a bacterial reverse mutation assay (May, 1999) and an in vitro chromosomal aberration assay (Mason, 1999), and the Committee concluded that the mixed β-glucanase, cellulase and xylanase enzyme preparation is unlikely to be genotoxic.

4.3 Assessment of dietary exposure

The Committee estimated the theoretical dietary exposure to the mixed β-glucanase, cellulase and xylanase enzyme preparation based on the estimated maximum levels in final food products (3.5 mg TOS/L in beer, 3 mg TOS/L in non-alcoholic beverages [including soft drinks], 3 mg TOS/kg in bakery ingredients [starch, fibres, flour] and 0 mg TOS/L in potable alcohol [spirits]). The combination of these maximum levels with per capita food consumption data from the USA (supplied by the sponsor) and data from the GEMS/Food cluster diets results in a potential dietary exposure of 0.08 mg TOS/kg bw per day for a 60 kg person. The Committee noted that the enzymes will be inactivated in processed food and that the exposure estimate is conservative.

5. Evaluation

No treatment-related adverse effects were seen at the highest dose tested (84.8 mg TOS/kg bw per day) in the 13-week study of oral toxicity in rats (Cooper, 2000). A comparison of the dietary exposure estimate of 0.08 mg TOS/kg bw per day with the highest dose tested of 84.8 mg TOS/kg bw per day results in a margin of exposure of at least 1000. The Committee established an ADI "not specified" for the mixed β-glucanase, cellulase and xylanase enzyme preparation from *R. emersonii*, used in the applications specified and in accordance with good manufacturing practice.

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Mixed β-glucanase and xylanase from *Disporotrichum dimorphosporum*

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1. Explanation

At the request of the Codex Committee on Food Additives at its Forty-sixth Session (FAO/WHO, 2014), the Committee evaluated the safety of mixed β-glucanase (3-(1,3;1,4)-β-D-glucan 3(4) glucanohydrolase; Enzyme Commission No. 3.2.1.6) and xylanase (1,4-β-D-xylan xylanohydrolase; Enzyme Commission No. 3.2.1.8)

from *Disporotrichum dimorphosporum*. This enzyme preparation has not been evaluated previously by the Committee. The Committee evaluated several other enzyme preparations of β-glucanase or xylanase at its thirty-first, thirty-fifth, thirty-ninth, sixty-first and sixty-third meetings and established an acceptable daily intake (ADI) "not specified" for their use in several applications, such as the preparation of beer and baking products (Annex 1, references *78*, *88*, *101*, *167* and *174*). In this report, the expression "mixed β-glucanase and xylanase liquid enzyme concentrate" is used when referring to the material tested in the toxicological studies evaluated; the expressions "β-glucanase" and "xylanase" are used when referring to the enzymes and their amino acid sequences; and the expression "mixed β-glucanase and xylanase enzyme preparation" is used when referring to the commercial enzyme preparation.

β-Glucanase is an enzyme that catalyses the hydrolysis of 1,3- or 1,4-β-D-glucosidic linkages in β-D-glucans. Xylanase is an enzyme that catalyses the hydrolysis of 1,4-β-D-xylosidic linkages in xylans.

The mixed β-glucanase and xylanase enzyme preparation is intended to be used as a processing aid in brewing, potable alcohol (spirits) production and grain processing.

1.1 Genetic background

The β-glucanase and xylanase enzymes are simultaneously produced at high levels from a strain of *D. dimorphosporum*. *Disporotrichum dimorphosporum* has been taxonomically identified to be from the genus *Sporotrichum* by the Dutch culture collection, the Centraalbureau voor Schimmelcultures (Stalpers, 1984). *Disporotrichum dimorphosporum* is a saprophyte and a basidiomycete fungus; it is capable of growing at pH 3.5–5.5 and 28–32 °C. *Disporotrichum dimorphosporum* is a non-pathogenic microorganism with a history of use in commercial food enzyme production.

The *D. dimorphosporum* production strain is derived from the original wild-type strain that has been used for large-scale production of the mixed β-glucanase and xylanase enzyme preparation since 1999 after reisolation and subculturing. It has been demonstrated to be genetically stable under laboratory conditions, with no significant decrease in yield or change in appearance of morphological variants. Data indicate that the production strain does not produce mycotoxins under large-scale fermentation conditions, indicating that the production strain is non-toxigenic.

1.2 Chemical and technical considerations

The β-glucanase and xylanase enzymes are produced by a controlled aerobic submerged fed-batch fermentation of a pure culture of *D. dimorphosporum*. The

enzymes are secreted into the fermentation broth and subsequently purified and concentrated. Sodium benzoate and glycerol are added to the liquid enzyme concentrate to standardize and stabilize the enzyme preparation. The mixed β-glucanase and xylanase enzyme preparation contains commonly used food-grade materials and conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm).

The β-glucanase activity is expressed in beta-glucanase fungique (BGF) units, as defined in the specific assay that measures a change in viscosity of a glucan substrate solution in the presence of β-glucanase and cellulase. The xylanase activity is expressed in xylanase viscosity units (XVU), as defined in the specific assay that measures a change in viscosity of a xylan substrate solution in the presence of xylanase; however, the method described to determine this activity is proprietary and non-transferable. The mean activities of β-glucanase and xylanase from three batches of the mixed β-glucanase and xylanase, prior to formulation, were reported to be approximately 520 000 BGF/g and 3300 XVU/g, respectively.

A typical commercial formulation of the mixed β-glucanase and xylanase enzyme preparation will contain 11–17% total organic solids (TOS), depending on the use. TOS includes the enzymes of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism during the manufacturing process.

The mixed β-glucanase and xylanase enzyme preparation is used in brewing, potable alcohol (spirits) production and grain processing (production of non-alcoholic beverages [including soft drinks] and bakery ingredients) to reduce viscosity and improve filterability, yield and product consistency; it will be used at levels up to 36.5 mg TOS/kg raw material. The β-glucanase and xylanase enzymes are expected to be inactivated during processing.

2. Biological data

2.1 Assessment of potential allergenicity

Disporotrichum dimorphosporum is not listed in the World Health Organization/International Union of Immunological Societies allergen nomenclature (http://www.allergen.org/).

β-Glucanase and xylanase from *D. dimorphosporum* have commonly been found in food, and there are no indications for allergic reactions due to their ingestion. As these enzymes are not genetically modified, an assessment of their potential allergenicity is not required.

2.2 **Toxicological studies**

The toxicological studies were performed with a mixed β-glucanase and xylanase liquid enzyme concentrate (batch no. 8221; dry matter content, 21.8% by weight; TOS content, 19.9% by weight), omitting stabilization and standardization. The liquid enzyme concentrate had activities of 38 000 BGF/g (β-glucanase) and 3300 XVU/g (xylanase).

2.2.1 **Acute toxicity**

No information was available.

2.2.2 **Short-term studies of toxicity**

In a 14-day range-finding study, groups of five male and five female CD rats were given the mixed β-glucanase and xylanase liquid enzyme concentrate at a dose equal to 0, 20, 60 or 199 mg TOS/kg body weight (bw) per day by oral gavage (Cooper, 1999). Clinical signs, body weight, feed consumption, feed conversion efficiency, haematology, blood chemistry, weights of principal organs and macroscopic pathology were studied in all animals.

There were no deaths or clinical signs related to the treatment. Overall body weight gain (85–88% of controls), feed consumption (89–95% of controls) and feed conversion efficiency (93–97% of controls) were slightly, but not statistically significantly, lower in treated females compared with controls.

In males in the mid- and high-dose groups, haematological investigations revealed slightly increased mean cell haemoglobin concentrations (+3% in both groups, statistically significantly different at the middle dose only) and neutrophil counts (up to +88%) and slightly decreased mean cell volumes (up to −6%), compared with the controls. Other statistically significant changes were observed in males in the mid-dose group only. Females in the high-dose group had statistically significantly increased haematocrit (+2%) and haemoglobin concentrations (+5%), compared with the controls. As the changes observed were small and/or not consistent between the sexes, they were considered to be of no toxicological relevance. Examination of the blood plasma indicated a dose-dependent decrease in cholesterol levels in females (up to −27%), which is considered to reflect metabolic adaptation to exposure to the test compound. Other changes in biochemical parameters were minor and/or lacked a dose–response relationship and were therefore considered to be unrelated to treatment. Small, but statistically significant, decreases in absolute heart weight (−12%, females only) and absolute lung and bronchus weight (−11%, females; −12%, males) were observed in the high-dose group. Absolute and relative uterus and cervix weights were increased in all treated females, reaching statistical significance in the mid-dose group only. This increase was related to fluid distension observed in the

uterus of, respectively, one, three and two females of the low-, mid- and high-dose groups, a finding commonly encountered in CD rats. In the absence of adverse effects, the no-observed-adverse-effect level (NOAEL) in this study was 199 mg/kg bw per day, the highest dose tested (Cooper, 1999).

In the subsequent 13-week study of toxicity, groups of 20 male and 20 female CD rats were given the mixed β-glucanase and xylanase liquid enzyme concentrate at a dose equal to 0, 13, 50 or 199 mg TOS/kg bw per day by oral gavage. The study was designed to meet the requirements of the United States Food and Drug Administration Redbook and was certified for compliance with good laboratory practice (GLP) and quality assurance (QA) (Cooper, 2000).

All animals were evaluated with respect to general clinical observations, body weight, feed consumption, feed conversion efficiency, ophthalmoscopy (10 animals/group), haematology, clinical chemistry, weights of principal organs, macroscopic examination and histopathology of principal organs (control and high-dose groups) and histopathology of all lesions.

No treatment-related effects on mortality, body weight, feed consumption, feed conversion efficiency or ophthalmoscopy were observed. Salivation was seen shortly after dosing in several males and females of the high-dose group in the last 3 weeks of the treatment period. This finding is associated with the dosing procedure and is not considered to be of toxicological relevance. Lower incidences of hair loss on the head (in treated males) and on the forelimbs (in females of the mid- and high-dose groups) were observed. In females of the high-dose group, there was a higher incidence of brown staining of the head (eight animals compared with three animals in the control group). These signs are not considered to be of toxicological relevance.

Slightly shorter prothrombin times were recorded for males of the high-dose group (11.7 versus 12.8 seconds in controls), and slightly lower haematocrit values were noted for females (−5% and −3%, respectively, in the mid- and high-dose groups, compared with controls). Other statistically significant changes in haematological parameters were observed in the low- and/or mid-dose groups only. As these effects were seen in only one sex and were minor in nature, they are considered to be of no toxicological relevance. A dose-dependent decrease in alanine aminotransferase activities (up to −20%) was observed in treated females, and increased triglyceride concentrations (+53%) were observed in females of the high-dose group only. In the absence of other treatment-related findings in the liver, these effects were considered to be of no toxicological importance. A dose-dependent decrease in urea concentrations (up to −23%) was observed in treated females; however, in the absence of other effects on the kidney in these animals, this finding was not considered to be of toxicological relevance. In treated males, a small decrease in albumin/globulin ratio was observed (up to

−6%). Absolute organ weights were not affected by the treatment. Changes in relative organ weights comprised marginally higher kidney weights in males of the mid- and high-dose groups (+6%) and marginally higher ovary weights in females of the high-dose group (+16%), compared with controls. No treatment-related macroscopic changes were observed. Microscopic changes reported included a higher incidence of basophilic tubules in the kidney cortex (12 males in the high-dose group compared with five in the controls; with the exception of one animal, severity was recorded as minimal or slight). Interstitial inflammatory cell infiltration (six animals in the high-dose group compared with two in the controls; minimal severity) was seen in the kidneys of high-dose males. Also, chronic inflammation of the pancreas (minimal severity) was noted in three high-dose males, compared with zero control animals. These are common microscopic changes seen in rats of this age and strain and are therefore not considered to be treatment related.

The effects observed in this study were minor and not consistent between the sexes and were therefore not considered to be adverse effects. Therefore, the NOAEL was 199 mg TOS/kg bw per day, the highest dose tested (Cooper, 2000).

2.2.3 Long-term studies of toxicity and carcinogenicity

No information was available.

2.2.4 Genotoxicity

The results of three studies of genotoxicity in vitro and in vivo with the mixed β-glucanase and xylanase liquid enzyme concentrate are summarized in Table 1. The first study followed Organisation for Economic Co-operation and Development (OECD) test guideline 471 (Bacterial Reverse Mutation Test; 1997), the second, OECD test guideline 473 (In Vitro Mammalian Chromosome Aberration Test; 1997), and the third, OECD test guideline 474 (Mammalian Erythrocyte Micronucleus Test; 1997). All three studies were certified for compliance with GLP and QA.

In the in vitro chromosomal aberration assay (Mason, 1999a), the liquid enzyme concentrate did not induce a statistically significant increase in chromosomal aberrations in the presence of S9. In the absence of S9, the liquid enzyme concentrate induced a statistically significant increase in chromosomal aberrations (chromatid-type breaks) after exposure of the cells to the highest tested concentration for 20 hours. The author indicated that owing to the nature of the material tested, the liquid enzyme concentrate may have caused chromosome damage as a result of non-genotoxic mechanisms (Mason, 1999a). The Committee noted that the highest concentration caused a mitotic inhibition of 58%. Also, the percentage of cells with aberrations was only slightly higher than the upper

Table 1

Genotoxicity of mixed β-glucanase and xylanase liquid enzyme concentrate in vitro and in vivo

End-point	Test system	Route of administration	Concentration	Results	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2uvrApKM101	–	100–10 000 µg/plate, ±S9	Negative[a]	May (1999)
Chromosomal aberration	Human lymphocytes	–	1st experiment: 1 250, 2 500 or 5 000 µg/mL, ±S9	Equivocal[b]	Mason (1999a)
			2nd experiment: 750, 3 000 or 5 000 µg/mL, −S9		
			1 250, 2 500 or 5 000 µg/mL, +S9		
In vivo					
Micronucleus induction	Mouse; M	Gavage	500, 1 000 or 2 000 mg/kg bw	Negative[c]	Mason (1999b)

bw: body weight; M: male; S9: 9000 × *g* supernatant fraction from rat liver homogenate

[a] Two independent experiments were performed using the "treat-and-plate" method. The bacteria were first incubated in liquid culture – that is, with the test substance in solution, nutrient broth and buffer or S9 mix – for 1 hour. The bacteria were then washed before mixing with top agar and plating on minimal glucose agar plates.

[b] In the first experiment, the cell cultures were treated for 3 hours without and with S9 and were harvested 17 hours later. The highest tested concentration induced 15% mitotic inhibition in the absence of S9. In the second experiment, the cells were exposed continuously for 20 hours without S9 and then harvested. With S9, the cells were treated for 3 hours and harvested 17 hours later. The highest tested concentration induced 58% mitotic inhibition in the absence of S9. In the first experiment, no significant increase in chromosomal aberrations was observed. In the second experiment in the absence of S9, a statistically significant increase in chromosomal aberrations was observed at 5000 µg/mL. The percentage of cells with aberrations excluding gaps was 5% at the highest concentration versus 0% in the controls (upper 99% confidence limit of historical negative control data was 4.5%), whereas the percentage of cells with aberrations including gaps was 11% at the highest concentration versus 2.5% in the controls (upper 99% confidence limit of historical negative control data was 7.85%).

[c] Single dose administered by gavage. Animals were examined 24 hours after dosing, and additional examinations were performed 48 hours after dosing in the negative control group and the highest-dose group. The Committee noted that no evidence of bone marrow and/or systemic exposure was provided.

99% confidence limit of the historical negative control data (5.0% versus 4.5%), and the level of significance was enhanced by the fact that no aberrations were observed in the controls. Therefore, the Committee considered the results of the in vitro chromosomal aberration assay to be equivocal. In combination with the negative results of the in vitro reverse mutation assay, the Committee did not have concerns with respect to the genotoxicity of this enzyme preparation.

2.2.5 Reproductive and developmental toxicity

No information was available.

2.3 **Observations in humans**

No information was available.

3. Dietary exposure

3.1 **Introduction**

The mixed β-glucanase and xylanase enzyme preparation is intended to be used as a processing aid in brewing, potable alcohol (spirits) production and grain processing (production of non-alcoholic beverages [including soft drinks] and bakery ingredients). The estimated maximum levels in final food products are 6.2 mg TOS/L in beer, 28 mg TOS/L in non-alcoholic beverages, 28 mg TOS/kg in bakery ingredients and 0 mg TOS/L in potable alcohol (spirits).

The enzymes are inactivated during the intended processes. In addition to the enzyme protein in question, the mixed enzyme preparation also contains some substances derived from the producing microorganism and the fermentation medium.

The Committee evaluated one submission by a sponsor (Reuvers, 2014) on dietary exposure to the additive using assessments based on individual dietary records.

3.2 **Assessment of dietary exposure**

The estimated daily intake (EDI) was calculated by the sponsor based on the estimated levels in the final food products and the intake levels of beverages and solid food based on per capita food consumption data from the United States Department of Agriculture (USDA) (Wilkinson Enns, Goldman & Cook, 1997; Wilson et al., 1997). As a "worst-case situation", the 90th percentile of the intake level was taken except for alcoholic beverages, for which the 95th percentile was used. The EDI was estimated to be 0.004–0.04 mg TOS/kg bw per day from beer and ale using an estimated consumption per person of 360 mL beverage/day, 0.04–0.39 mg TOS/kg bw per day from non-alcoholic beverages (fruit drinks and ades; carbonated soft drinks; 838 mL/day) and 0.02–0.24 mg TOS/kg bw per day from grain products (cereal and pasta; quick breads, pancakes, French toast; cakes, cookies, pastries, pies; crackers, popcorn and chips; mixtures mainly grain; 502 g/day). The three estimates sum to 0.06–0.7 mg TOS/kg bw per day, or 4–40 mg/day for a 60 kg person. This estimate assumes that all cereal grain products, including beer and ale, are treated with the mixed β-glucanase and xylanase enzyme preparation and is therefore an overestimate.

Reference to the 17 consumption cluster diets of the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) (WHO, 2012) reveals that clusters G08, G11 and G15 (including a number of European countries) have the highest apparent consumption of beer (225–260 g/day). Cluster G10, which includes the USA, has an apparent consumption of 174 g/day. If the mixed β-glucanase and xylanase enzyme preparation is present at the highest suggested level of 6.2 mg TOS/L beverage, the dietary exposure at the maximum consumption of 260 g/day would be 1.6 mg TOS/day (or 0.03 mg TOS/kg bw per day for a 60 kg person).

Cluster G02 (including a number of Eastern European/Central Asian countries) has the highest apparent consumption of non-alcoholic beverages (excluding milk-based beverages, stimulants and water) (75 g/day), followed by cluster G15 (including a number of European countries), at 56 g/day, and cluster G10, which includes the USA, at 47 g/day. If the mixed β-glucanase and xylanase enzyme preparation is present at the highest suggested level of 28 mg TOS/L beverage, the dietary exposure at the maximum consumption of 75 g/day would be 2.1 mg TOS/day (or 0.04 mg TOS/kg bw per day for a 60 kg person). The estimate for consumption used here (75 g/day) is significantly lower than the estimate used by the sponsor (838 mL/day).

Clusters G01, G06 and G09 (including a number of countries from Asia, the Middle East and North Africa) have the highest apparent consumption of cereals and flour (370–480 g/day). If the mixed β-glucanase and xylanase enzyme preparation is present at the highest suggested level of 28 mg TOS/kg product, the dietary exposure at the maximum consumption of 480 g/day would be 13 mg TOS/day (or 0.2 mg TOS/kg bw per day for a 60 kg person).

The three estimates (1.6, 2.1 and 13 mg TOS/day) sum to 17 mg TOS/day, or 0.3 mg TOS/kg bw per day for a 60 kg person. This estimate assumes that all cereal grain products, including beer and ale, are treated with the mixed β-glucanase and xylanase enzyme preparation and would therefore be an overestimate. The estimate is within the range of the EDI calculated by the sponsor using survey data from the USDA.

4. Comments

4.1 Assessment of potential allergenicity

β-Glucanase and xylanase from *D. dimorphosporum* have commonly been found in food, and there are no indications for allergic reactions due to their ingestion. As these enzymes are not products of genetic modification, an assessment of their potential allergenicity was not required.

4.2 **Toxicological studies**

In a 13-week study of oral toxicity in rats, no treatment-related adverse effects were seen when the mixed β-glucanase and xylanase liquid enzyme concentrate was administered by gavage at doses up to 199 mg TOS/kg bw per day (Cooper, 1999). The mixed β-glucanase and xylanase liquid enzyme concentrate was not genotoxic in a bacterial reverse mutation assay (May, 1999). In an in vitro chromosomal aberration assay (Mason, 1999a), the liquid enzyme concentrate induced a small, but statistically significant, increase in chromosomal aberrations (chromatid-type breaks) after exposure of the cells to the highest concentration tested for 20 hours, in the absence of S9 only. However, as the effect was small, a mitotic inhibition of 58% was observed at the highest concentration tested and the level of statistical significance was related to the fact that no aberrations were observed in the controls, the Committee considered these results not to be of toxicological relevance. In combination with the negative results of the in vitro reverse mutation assay, the Committee did not have concerns with respect to the genotoxicity of the mixed β-glucanase and xylanase enzyme preparation.

4.3 **Assessment of dietary exposure**

The Committee estimated the theoretical dietary exposure to the mixed β-glucanase and xylanase enzyme preparation based on the estimated maximum levels in final food products (6.2 mg TOS/L in beer, 28 mg TOS/L in non-alcoholic beverages [including soft drinks], 28 mg TOS/kg in bakery ingredients [starch, fibres, flour] and 0 mg TOS/L in potable alcohol [spirits]). The combination of these maximum levels with per capita food consumption data from the USA (supplied by the sponsor) and data from the GEMS/Food consumption cluster diets results in a potential dietary exposure of 0.7 mg TOS/kg bw per day for a 60 kg person. The Committee noted that the enzymes will be inactivated in processed food and that the exposure estimate is conservative.

5. Evaluation

No treatment-related adverse effects were seen at the highest dose tested (199 mg TOS/kg bw per day) in the 13-week study of oral toxicity in rats (Cooper, 1999). A comparison of the dietary exposure estimate of 0.7 mg TOS/kg bw per day with the highest dose tested of 199 mg TOS/kg bw per day gives a margin of exposure of at least 280. The Committee established an ADI "not specified" for the mixed β-glucanase and xylanase enzyme preparation from *D. dimorphosporum*, used in the applications specified and in accordance with good manufacturing practice.

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Polyvinyl alcohol (PVA) – polyethylene glycol (PEG) graft copolymer

First draft prepared by

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1. Explanation

At the request of the Codex Committee on Food Additives at its Forty-sixth Session (FAO/WHO, 2014), the Committee evaluated the safety of polyvinyl alcohol (PVA) – polyethylene glycol (PEG) graft copolymer, which it had not evaluated previously. The individual components of the copolymer have been evaluated previously by the Committee. The use of PVA as a coating, binder, sealing and surface finishing agent in food products such as dairy-based desserts, confectionery, cereal products and food supplements was evaluated at the sixty-first meeting of the Committee, and the Committee established an acceptable daily intake (ADI) of 50 mg/kg body weight (bw) for PVA (Annex 1, reference *167*). At the twenty-third meeting, the Committee established an ADI of 10 mg/kg bw for polyethylene glycols (Annex 1, reference *51*).

PVA-PEG graft copolymer is a synthetic branched graft copolymer primarily intended for use in aqueous film coatings in the preparation and formulation of food supplements. It is currently approved for use for pharmaceutical applications in several regions, including the European Union, the USA and Japan. Recently, PVA-PEG graft copolymer was authorized as an additive for use in solid food supplements in the European Union, based on the evaluation by the European Food Safety Authority (EFSA, 2013).

1.1 Chemical and technical considerations

Graft copolymers are a form of copolymer where the side-chains are structurally different from the main chain. PVA-PEG graft copolymer is a synthetic, branched graft copolymer consisting of side-chains of PVA on a main chain of PEG. It consists of approximately 75% vinyl alcohol units ($-CH_2CH_2(OH)-$) and 25% ethylene glycol units ($-CH_2CH_2O-$).

PVA-PEG graft copolymer is a white to pale yellow free-flowing powder. It is manufactured by grafting polyvinyl acetate side-chains onto a PEG backbone that has an average molecular weight of 6000 Da. The polyvinyl acetate side-chains are then hydrolysed to form PVA side-chains. Based on the manufacturing conditions, PVA-PEG graft copolymer has an average of 2–3 PVA side-chains per PEG backbone. It has a weight-average molecular weight ranging from 40 000 to 50 000 Da. In the specifications, maximum limits have been set for a number of impurities, including vinyl acetate (20 mg/kg) and ethylene glycol and diethylene glycol (400 mg/kg, singly or in combination).

The predominant use in food supplements is that of glazing agent or, more specifically, as an aqueous film coating for food supplement tablets at a use level of up to 5% (weight per weight [w/w]). PVA-PEG graft copolymer also has

minor uses in food supplements as a stabilizer and binder for tablets at a use level of up to 10% (w/w).

2. Biological data

2.1 Biochemical aspects

2.1.1 Absorption, distribution, metabolism and elimination

The toxicokinetic properties of ^{14}C-labelled PVA-PEG graft copolymer (specific activity 65.8 MBq/g) were investigated in groups of four male and four female Wistar rats following administration of a single oral dose of 10 or 1000 mg/kg bw by gavage (Leibold & Hoffmann, 2001; published by Heuschmid et al., 2013a). The study design was in accordance with European Commission Directive 87/302/EEC, Organisation for Economic Co-operation and Development (OECD) test guideline 417 (Toxicokinetics; 1984) and United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) Health Effects Test Guideline OPPTS 870.7485 (Metabolism and Pharmacokinetics; 1998). The study was certified for compliance with good laboratory practice (GLP) and quality assurance (QA).

The excretion of radioactivity in faeces, urine and exhaled air (the latter in two males only) was monitored for up to 168 hours post-dosing, and the excretion of radioactivity in bile was monitored for up to 48 hours post-dosing. Cumulative values for the excretion of radioactivity for each route as percentages of the administered dose are presented in Table 1.

Table 1

Mean cumulative excretion of radioactive material following administration of single oral doses of ^{14}C-labelled PVA-PEG graft copolymer to male and female rats

Dose (mg/kg bw)	Mean cumulative excretion of radioactive material (% of administered dose)							
	Faeces		Urine		Bile		Exhaled air	
	Males	Females	Males	Females	Males	Females	Males	Females
48 h								
10	100.05	103.45	0.50	0.45	0.02	0.02	ND	ND
1 000	100.76	105.27	0.27	0.22	0.01	0.02	ND	ND
168 h								
10	100.14	103.63	0.53	0.47	ND	ND	0.15	ND
1 000	101.30	105.35	0.31	0.23	ND	ND	0.11	ND

bw: body weight; ND: not determined

Following administration of a single oral dose of 10 mg/kg bw of ^{14}C-labelled PVA-PEG graft copolymer, the cumulative amount of radioactivity recovered in the faeces of males and females at 48 and 168 hours post-dosing was approximately 100%. The total excretion of radioactivity via urine was negligible (range 0.45–0.53%). Excretion of ^{14}CO$_2$ via exhaled air (determined in two males only) was also negligible (0.15%). Limited amounts of remaining radioactivity were detected in the carcass (i.e. 0.02% in males and in females), and the concentration of radioactivity was below 0.1 µg equivalent (Eq)/g in all organs and tissues. Mean total recoveries of radioactivity were 101% in males and 104% in females.

At the high dose of 1000 mg/kg bw of ^{14}C-labelled PVA-PEG graft copolymer, the same pattern was observed. The cumulative amount of radioactivity recovered in the faeces of males and females at 48 and 168 hours post-dosing was approximately 100%. The total excretion of radioactivity via urine was negligible (range 0.22–0.31%). Excretion of ^{14}CO$_2$ via exhaled air (determined in two males only) was also negligible (0.11%). Limited amounts of remaining radioactivity were detected in the carcass (i.e. 0.13–0.16%), and the concentration of radioactivity was below 1 µg Eq/g in all organs and tissues (except the liver, with a concentration of 1.31 µg Eq/g). Mean total recoveries of radioactivity were 102% in males and 106% in females.

In bile duct–cannulated rats, cumulative biliary excretion of ^{14}C-labelled PVA-PEG graft copolymer 48 hours post-dosing was 0.01–0.02% of the administered radioactivity in males and females of the 10 and 1000 mg/kg bw dose groups.

Overall, based on the excretion data, the oral bioavailability of ^{14}C-labelled PVA-PEG graft copolymer was calculated to be less than 1% (Leibold & Hoffmann, 2001).

2.2 Toxicological studies

2.2.1 Acute toxicity

In a study of acute oral toxicity, three male and three female rats were given PVA-PEG graft copolymer (batch no. ZK 1440/236-1) at a single oral dose of 2000 mg/kg bw by gavage (Wiemann & Hellwig, 2000a). The study was certified for compliance with QA and GLP and was performed in accordance with OECD test guideline 423 (Acute Oral Toxicity – Acute Toxic Class Method; 1996) and USEPA Health Effects Test Guideline OPPTS 870.1100 (Acute Oral Toxicity; 1998). With the exception of two male rats with diffuse reddish discoloration and oedema of the lungs, no other abnormalities were noted at necropsy. The oral median lethal dose (LD$_{50}$ value) in this study was greater than 2000 mg/kg bw (Wiemann & Hellwig, 2000a).

PVA-PEG graft copolymer was not corrosive and did not lead to skin or eye irritation in New Zealand White rabbits (Wiemann & Hellwig, 2000b,c).

2.2.2 Short-term studies of toxicity

(a) Rats

In a 90-day oral toxicity study, groups of 10 male and 10 female Wistar rats were administered PVA-PEG graft copolymer (batch no. ZK 1440/236-1, purity >98.8%) at a concentration of 0 (vehicle control), 600, 3000 or 15 000 mg/L in the drinking-water, equal to, respectively, 0, 60, 300 and 1610 mg/kg bw per day for males and 0, 80, 370 and 2190 mg/kg bw per day for females. The study was designed according to OECD test guideline 408 (Repeated Dose 90-Day Oral Toxicity Study in Rodents; 1998) and was certified for compliance with GLP and QA. Observations included clinical signs, body weight, feed consumption, water consumption, a functional observational battery, motor activity, ophthalmoscopy, haematology, clinical chemistry, urine analysis, organ weights, and macroscopic and microscopic pathology (Mellert et al., 2001; published by Heuschmid et al., 2013b).

There were no deaths or treatment-related clinical observations. No treatment-related effects were observed regarding feed consumption, feed efficiency, body weight (gain) and ophthalmoscopy or in the functional observational battery. No treatment-related changes in haematological and clinical chemistry parameters or urine analyses were noted. In males and females of the high-dose group, water consumption was higher (range 5–31% in males and 16–37% in females) than in controls throughout the study, reaching statistical significance on several days. Regarding the overall motor activity (summation of 12 time intervals), no significant deviations were seen. There were significantly decreased values in males of the mid- and high-dose groups at interval 10, but these isolated findings were not considered to be treatment related. A statistically significant increase (+10%) in absolute liver weight was observed in males of the low-dose group only. Mean relative brain weights of males of the low- and high-dose groups were 6% lower than in controls, but these can be attributed to the slightly, but not statistically significantly, higher mean body weights in these groups. These findings on organ weights were not considered to be treatment related. No treatment-related gross lesions or microscopic findings were noted.

The only treatment-related effect observed was an increase in water consumption in high-dose males and females. The reason for this increase is not known. In the absence of other treatment-related findings, this is not considered to be an adverse effect. The no-observed-adverse-effect level (NOAEL) in this study was therefore 1610 mg/kg bw per day, the highest dose tested (Mellert et al., 2001).

(b) **Dogs**

In an oral study of toxicity, groups of five male and five female Beagle dogs were given PVA-PEG graft copolymer (batch no. 99/401-2; purity >99%) for about 9 months. PVA-PEG graft copolymer was dissolved in drinking-water at a concentration of 0 (vehicle control), 3000, 10 000 or 30 000 mg/L. Control and test drinking-water solutions (400 mL) were added to 400 g feed and mixed into a paste. These concentrations were equal to mean intakes of, respectively, 0, 80, 260 and 780 mg/kg bw per day for males and 0, 80, 270 and 810 mg/kg bw per day for females. The study was designed following European Commission Directive 87/392/EEC and OECD test guideline 452 (Chronic Toxicity Studies; 1981). The study was certified for compliance with GLP and QA. Observations included clinical signs, body weight, feed consumption, ophthalmoscopy, haematology, clinical chemistry, urine analysis, organ weights, and macroscopic and microscopic pathology (Kaspers et al., 2002; published by Heuschmid et al., 2013b).

No animals died during the treatment period. Incidental clinical observations included a single case of diarrhoea in one female of the high-dose group on study day 56 and a palpable mass in the neck region of one female of the mid-dose group (from study day 279 onwards). Two females of the high-dose group had soft faeces between days 121 and 160. Owing to the incidental and/or transient nature of these findings, these were considered not to be substance related. Ophthalmological examinations did not reveal any deviations, except for slight cataract in both eyes in one control female. Feed consumption, body weight and feed efficiency were not affected by treatment. Several statistically significant differences were observed in haematology (white blood cells, activated partial thromboplastin time), clinical chemistry (aspartate aminotransferase, magnesium) and urine analysis (squamous cells in sediment). However, these were all small and/or occurred in the low- and/or mid-dose groups only and were therefore not considered to be of toxicological relevance.

In female dogs, the mean absolute weight of the ovaries was significantly increased in all dose groups (1450, 1508 and 1760 mg, respectively, in the low-, mid- and high-dose groups versus 906 mg in controls). The corresponding relative ovary weights were also increased, but these values did not reach statistical significance. Heuschmid et al. (2013b) reported that the absolute ovary weights of the three treatment groups were within the historical control range of the testing facility (range: 979–2258 mg; mean: 1378 mg), whereas the value of the control group was below the historical control range. Historical control data were not included in the report of Kaspers et al. (2002). The authors attributed the change in ovary weights to the different states of the sexual cycle in control and treated dogs. Based on histopathological investigations, all control dogs were in the

resting phase of the sexual cycle, whereas three low-dose, two mid-dose and four high-dose female dogs were in the active phase of the sexual cycle. The change in ovary weights was considered not to be treatment related. The Committee agreed with this view, given the lack of other histopathological findings in the ovaries in the dog study and the lack of adverse effects on reproductive organs in the 90-day toxicity study in rats and the reproductive toxicity study in rats.

In male dogs, absolute mean thyroid gland weight was significantly increased (+31%) in the low-dose group only. In the absence of similar changes at higher dose levels, this was considered to be an incidental finding. A few gross lesions (e.g. erosion/ulcer in glandular stomach, focus in lungs, urinary bladder, epididymides and prostate gland, cyst in pituitary and thyroid glands, and oedema in adipose tissue) were reported sporadically in all groups, including controls. With two exceptions (erosion/ulcer in the glandular stomach of two high-dose males and focus in the prostate of one high-dose male), these gross lesions occurred only, or at the same incidence, in control and/or mid-dose animals. The gross lesions were considered to be incidental and not substance related. Although no explanation for the occurrence of erosion/ulcer in the glandular stomach of the two high-dose males was provided, given that only two males were affected and in the absence of similar findings in females, this finding was not considered to be treatment related.

Histopathological examination showed chronic lymphocytic thyro-adenitis (thyroiditis) in two high-dose males. This type of chronic thyroiditis has been reported at an incidence of up to 20% in dogs (Fritz, Zeman & Zelle, 1970) and occurred in only one sex; therefore, it was considered not to be treatment related. Other microscopic findings noted were single observations, occurred in all or almost all groups at comparable incidences and graded severity, including control animals, and/or showed no dose–response relationship and therefore were considered to be unrelated to treatment.

In the absence of treatment-related adverse effects, the NOAEL was 780 mg/kg bw per day, the highest dose tested (Kaspers et al., 2002).

2.2.3 Long-term studies of toxicity and carcinogenicity

No long-term studies of toxicity and carcinogenicity were available for PVA-PEG graft copolymer.

2.2.4 Genotoxicity

The results of two studies of genotoxicity in vitro (a bacterial reverse mutation assay and a gene mutation assay) and one study of genotoxicity in vivo (a micronucleus assay) with PVA-PEG graft copolymer are summarized in Table 2. All three studies were certified for compliance with GLP and QA. The first study followed

Table 2

Genotoxicity of PVA-PEG graft copolymer in vitro and in vivo

End-point	Test system	Route of administration	Concentration	Results	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2uvrA	–	20–5 000 µg/plate, ±S9	Negative[a]	Engelhardt & Hoffmann (2000)
Gene mutation	Mouse lymphoma L5178Y TK$^{+/-}$ cells	–	First experiment: 312.5–5 000 µg/mL, ±S9	Negative[b]	Engelhardt & Hildebrand (2000a)
			Second experiment: 79–5 000 µg/mL, −S9 312.5–5 000 µg/mL, +S9		
In vivo					
Micronucleus induction	Mouse; M	Intraperitoneal	500, 1 000 and 2 000 mg/ kg bw	Negative[c]	Engelhardt & Hildebrand (2000b)

bw: body weight; M: male; S9: 9000 × *g* supernatant fraction from rat liver homogenate

[a] Two independent experiments were performed. The first experiment was performed using the plate incorporation method, and the second experiment was performed using the preincubation method. A slight decrease in the number of revertants and a slight reduction in bacterial growth were occasionally observed from 2500 µg/mL onwards.

[b] Two independent experiments were performed. Cells were exposed for 4 hours in the absence and presence of S9 (except in the second experiment, in which cells were exposed for 24 hours in the absence of S9), followed by an expression phase of 30–48 hours and a selection period of approximately 10 days. After an exposure period of 24 hours, relative total growth was decreased at 2500 µg/mL (−55%) and 5000 µg/mL (−37%).

[c] Two doses were administered intraperitoneally at 0 and 24 hours. Examinations were 24 hours after the last dosing (i.e. at 48 hours). No increases in group mean micronuclei were observed. The Committee noted that intraperitoneal administration is not a relevant route for human exposure to PVA-PEG graft copolymer.

OECD test guideline 471 (Bacterial Reverse Mutation Test; 1997) (Engelhardt & Hoffman, 2000), the gene mutation assay was conducted according to OECD test guideline 476 (In Vitro Mammalian Cell Gene Mutation Test; 1997) (Engelhardt & Hildebrand, 2000a) and the micronucleus test followed OECD test guideline 474 (Mammalian Erythrocyte Micronucleus Test; 1997). The genotoxicity studies were performed with batch no. ZK 1440/236-1 (purity about 98.8%). Based on the results of these tests, PVA-PEG graft copolymer is unlikely to be genotoxic.

2.2.5 Reproductive and developmental toxicity

(a) Reproductive toxicity

In a two-generation reproductive toxicity study performed in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Harmonized Tripartite Guideline S5A (Detection of Toxicity to Reproduction for Medicinal Products, dated 24 June 1993) and ICH Harmonized Tripartite Guideline S5B (Detection of Toxicity to Reproduction for Medicinal Products, Addendum to the Parent Guideline: Toxicity to Male Fertility, dated 29 November 1995), Wistar rats (25

of each sex per group for the F_0 and the F_1 generations) received PVA-PEG graft copolymer (batch no. Abl. Nr. 61-7156; purity >99%) by gavage at a dose of 0, 100, 300 or 1000 mg/kg bw per day. F_0 males were treated over a 4-week premating period, and F_0 females were treated over a 2-week period before mating and throughout the mating period. The females were also treated during gestation and lactation of the F_1 pups up to postnatal day (PND) 21. The F_1 litters were not culled. After weaning at PND 21, 25 weanling rats of each sex per dose were selected for producing the F_2 generation. The remaining weanlings were killed and checked for macroscopic anomalies. After weaning, the F_1 parental rats were raised without treatment. The F_0 and F_1 parental males were killed after mating. The F_0 females were killed after weaning of the F_1 pups. The F_1 parental females were killed on gestation days (GDs) 15–17. Mortality and clinical signs were recorded daily. Body weights of parental rats were recorded twice weekly during the premating and mating phases. In addition, females were weighed daily during gestation and lactation. Feed consumption was recorded weekly. Premating estrous cycle, fertility and mating indices of males and females, sperm parameters and gestation duration were determined. In F_1 females, the numbers of corpora lutea, live and dead fetuses, dead implantations, and early and late resorptions were recorded. All litters from the F_0 parents were examined for number of pups, sex of pups, number of stillbirths, number of live births and gross anomalies. All pups were checked daily for mortality and clinical signs and weighed on PNDs 0, 4, 7, 10, 14, 17 and 21. Pups that were not selected for breeding were killed and necropsied at PND 21. In all F_1 pups selected for breeding the F_2 generation, the days of vaginal opening and preputial separation were recorded. In addition, a learning and memory test (water maze) was performed on PNDs 34–36. The parental rats were necropsied, and testes, epididymides, prostate, seminal vesicles with coagulation glands, ovaries and uterus were weighed. Histopathology was not performed (Schneider et al., 2003; published by Heuschmid et al., 2013c).

No treatment-related mortality or clinical signs were observed. Body weight gain and feed consumption of the parental animals were not affected by treatment. The lower body weights early after weaning, but not later in the study, in mid- and high-dose F_1 pups selected for breeding were attributed to the larger F_1 litter sizes that were observed at these dose levels. Necropsy and organ weight measurements of parental rats revealed no effect of treatment. Fertility and reproduction parameters (estrous cycle data, mating behaviour, conception, gestation and parturition rates, number of corpora lutea, number of implantations and preimplantation losses) in F_0 and F_1 parental rats were not affected by treatment with PVA-PEG graft copolymer. A reduction in the total number of spermatids per gram testis in mid-dose males of the F_0 parental group was within the normal historical control range and, in the absence of a similar effect at the high dose or in the mid-dose F_1 males, was not considered treatment

related. In high-dose F_0 dams, postimplantation losses and total number of resorptions were reduced, and the percentage of live fetuses was increased. Litter size at the high dose (11.1) was increased compared with controls (9.5). The lower body weight at birth (3%) and at PND 21 (9%) of high-dose pups compared with control pups was attributed to the increased litter size. A slight delay in preputial separation in the high-dose F_1 pups (43.0 days versus 42.5 days in controls) was within the historical control range and may be related to the lower body weights of these pups. PVA-PEG graft copolymer had no influence on the sex ratio or on learning and memory of the F_1 generation. Furthermore, no effect of PVA-PEG graft copolymer on the intrauterine development and survival of the F_2 progeny was found. Necropsy of parental rats and F_1 pups revealed no effect of treatment.

The NOAEL for parental, offspring and reproductive toxicity was 1000 mg/kg bw per day, the highest dose tested (Schneider et al., 2003).

(b) Developmental toxicity

(i) Rats

In a developmental toxicity study performed in accordance with the ICH Guideline on Detection of Toxicity to Reproduction for Medicinal Products (United States Food and Drug Administration, dated 22 September 1994), groups of 25 female Wistar rats were treated orally, by gavage, with PVA-PEG graft copolymer (batch no. ZK 1440/236-1; purity 98.8%) at a dose of 0, 100, 300 or 1000 mg/kg bw per day from days 6 through 15 after mating (GD 0 = day on which sperm were detected in the vaginal smear). The vehicle was distilled water. The study was certified to comply with GLP and QA. Clinical signs and mortality were recorded daily. Body weights and feed consumption were measured on GDs 0 (body weights only), 1, 3, 6, 8, 10, 13, 15, 17 and 20. All females were killed on GD 20 and subjected to gross examination. The uterus was examined and weighed, and the numbers of live and dead fetuses, corpora lutea, implantations, and early and late resorptions were counted. Each fetus was weighed, sexed and examined macroscopically. About half of the fetuses from each litter were selected for skeletal examinations, and the other half for visceral examinations (Gamer, Schneider & Van Ravenzwaay, 2002; published by Heuschmid et al., 2013d).

At 0, 100, 300 and 1000 mg/kg bw per day, 23, 25, 21 and 24 females were pregnant, respectively. No treatment-related mortality, clinical signs or abortions were observed. Body weight gain and feed consumption were not affected by treatment. There were no treatment-related differences in the numbers of corpora lutea and implantation sites, preimplantation and postimplantation losses, and the numbers of resorptions and viable fetuses. External, visceral and skeletal examinations revealed no effect of treatment with PVA-PEG graft copolymer. No effects were observed on fetal sex ratio, placental weight or fetal weight.

Occasional findings of malformations or variations were generally within the historical control range or lacked dose dependency and were considered not treatment related.

The NOAEL for maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Gamer, Schneider & Van Ravenzwaay, 2002).

(ii) Rabbits

In a developmental toxicity study performed in accordance with the ICH Guideline on Detection of Toxicity to Reproduction for Medicinal Products (United States Food and Drug Administration, dated 22 September 1994), groups of 25 artificially inseminated Himalayan rabbits were treated orally, by gavage, with PVA-PEG graft copolymer (batch no. ZK 1440/236-1; purity 98.8%) in double distilled water at a dose of 0, 100, 300 or 1000 mg/kg bw per day from days 6 through 19 of gestation (the day after artificial insemination was designated as GD 0). The study was certified to comply with GLP and QA. The rabbits were checked daily for mortality and clinical signs. Body weight was measured on GDs 0, 2, 4, 6, 9, 11, 14, 16, 19, 21, 23, 25 and 29. Feed consumption was measured daily from GD 1 to GD 29. All females were killed on GD 29. All rabbits of all dose groups were examined macroscopically for abnormalities. The uterus was examined and weighed, and the numbers of live and dead fetuses, corpora lutea, implantations, and early and late resorptions were counted. Each fetus was weighed, sexed and examined macroscopically. All fetuses were subjected to skeletal and visceral examinations (Schneider, Hellwig & Van Ravenzwaay, 2002; published by Heuschmid et al., 2013d).

No treatment-related mortality was observed. One high-dose female aborted. No treatment-related effects on clinical signs, body weight gain or feed consumption were observed. Necropsy of the dams at day 29 revealed no treatment-related abnormalities. There were no treatment-related differences in the numbers of corpora lutea and implantation sites, preimplantation and postimplantation losses, or the numbers of resorptions and viable fetuses. External, visceral and skeletal examinations revealed no effect of treatment with PVA-PEG graft copolymer. No effects were observed on fetal sex ratio, placental weight or fetal weight. Occasional findings of malformations or variations were generally within the historical control range or lacked dose dependency and were considered not treatment related.

The NOAEL for maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Schneider, Hellwig & Van Ravenzwaay, 2002).

2.2.6 **Special studies**

(a) **Studies on vinyl acetate (impurity)**

The Committee noted that the use of PVA-PEG graft copolymer that complies with the proposed specifications could lead to exposure to vinyl acetate. Vinyl acetate is considered to be a possible human carcinogen (Group 2B) by the International Agency for Research on Cancer (IARC, 1995). Several oral long-term studies of toxicity and carcinogenicity with vinyl acetate are described in the literature (Lijinksy & Reuber, 1983; Lijinsky, 1988; Bogdanffy et al., 1994; Maltoni et al., 1997; Minardi et al., 2002; Umeda et al., 2004). The most recent study (Umeda et al., 2004) that complies with the relevant OECD test guideline is summarized in detail below, and the results are compared with the results of the studies by Bogdanffy et al. (1994), Maltoni et al. (1997) and Minardi et al. (2002). The studies of Lijinsky & Reuber (1983) and Lijinksy (1988) had several limitations (only 20 animals per group and decomposition of vinyl acetate in the drinking-water). Therefore, these are not considered further in this evaluation.

(i) Mice

In a carcinogenicity study, groups of 50 male and 50 female Crj:BDF1 mice were administered vinyl acetate in the drinking-water at a concentration of 0, 400, 2000 or 10 000 mg/L (equal to 0, 40, 200 and 990 mg/kg bw per day for males and 0, 60, 300 and 1420 mg/kg bw per day for females, respectively) for 104 weeks (Umeda et al., 2004). The study was reported to comply with GLP and to be performed according to OECD test guideline 453 (Combined Chronic Toxicity/ Carcinogenicity Studies, 1981). The animals were observed daily for clinical signs and mortality. Body weight, feed consumption and water consumption were measured weekly for the first 13 weeks and every 4 weeks thereafter. Urinary parameters were measured in urine samples collected in the last week of the study, and haematological and blood biochemical parameters were measured in blood samples collected at the end of the administration period. Necropsies and histopathological examinations were performed on all animals.

Survival rates were comparable among the groups, but several animals in the high-dose group died of tumours in the oral cavity, oesophagus, stomach or larynx. Terminal body weights were statistically significantly lower in high-dose males (−30%) and females (−18%). Average water consumption was lower (not statistically significantly) in the high-dose group. Mandibular nodules were observed in three males and five females of the high-dose group, whereas maxillary nodules were observed in three males and one female of the high-dose group. No treatment-related effects on the haematology or blood chemistry parameters were reported (results of the urine analysis were not reported). In high-dose males, statistically significant increases in squamous cell carcinomas and/or papillomas

were observed in the oral cavity (17/50 versus 0/50 in controls), oesophagus (7/50 versus 0/50 in controls) and forestomach (9/50 versus 1/50 in controls). In high-dose females, a statistically significant increase in squamous cell papillomas and/or carcinomas was observed in the oral cavity (18/50 versus 0/50 in controls) and the forestomach (4/50 versus 0/50). In addition, squamous cell carcinomas of the larynx were observed in males of the high-dose group (2/50) and females of the mid-dose (1/50) and high-dose (1/50) groups and were not observed in the control group. In these tissues, basal cell hyperplasia, squamous cell hyperplasia and/or epithelial dysplasia were also observed in several animals.

In addition, neoplastic lesions were observed in the tongue, liver, spleen, nasal cavity, lung and uterus, but these were not considered to be treatment related given the incidences in the control group and/or lack of a dose–response relationship.

(ii) Rats

In a carcinogenicity study, groups of 50 male and 50 female F344/DuCrj rats were administered vinyl acetate in the drinking-water at a concentration of 0, 400, 2000 or 10 000 mg/L (equal to 0, 20, 100 and 440 mg/kg bw per day for males and 0, 30, 150 and 580 mg/kg bw per day for females, respectively) for 104 weeks (Umeda et al., 2004). The study was reported to comply with GLP and to be performed according to OECD test guideline 453 (Combined Chronic Toxicity/Carcinogenicity Studies, 1981).

Survival rates were comparable among the groups, but two males in the high-dose group died of oral cavity tumours. An oral cavity nodule appeared in a low-dose female. Terminal body weights were statistically significantly lower in high-dose males (−7%). Average water consumption was lower (not statistically significantly) in the high-dose group. Mandibular nodules were observed in three males of the high-dose group and a female of the low-dose group, whereas a maxillary nodule was observed in one female of the high-dose group. No treatment-related effects on the haematology or blood chemistry parameters were reported (results of the urine analysis were not reported). Squamous cell carcinomas and/or papillomas were observed in the oral cavity (males: incidence was 7/50 in the high-dose group versus 0/50 in controls; females: incidences were 1/50, 1/50 and 3/50 in the low-, mid- and high-dose groups versus 0/50 in controls). In one high-dose female, a squamous cell carcinoma was observed in the oesophagus. In addition, several neoplastic lesions were observed in the pituitary, liver, uterus and thyroid, but these were not considered to be treatment related given the incidences in the control group and/or lack of a dose–response relationship.

(iii) Conclusion

In the study by Umeda et al. (2004), statistically significant increases in the incidences of squamous cell carcinomas in the oral cavity, oesophagus and/or forestomach were observed at the highest dose tested in male and/or female rats and mice (440–990 mg/kg bw per day).

In a study using lower dose levels, Bogdanffy et al. (1994) reported no neoplastic or non-neoplastic lesions in rats exposed to vinyl acetate via the drinking-water at doses up to 200 mg/kg bw per day for males and 300 mg/kg bw per day for females for 104 weeks. In contrast, Minardi et al. (2002) and Maltoni et al. (1997) found statistically significant increases in tumours after exposure of 17-week-old rats (Minardi et al., 2002) and mice (Maltoni et al., 1997) and 12-day-old embryos (offspring) of both species to vinyl acetate at 1000 or 5000 mg/L in the drinking-water for 104 and 74 weeks, respectively. The doses were equivalent to 50 and 250 mg/kg bw per day for rats and 90 and 450 mg/kg bw per day for mice, based on default values for converting drinking-water concentrations into doses, as reported by EFSA (2012).

In summary, in oral studies of long-term toxicity and carcinogenicity in rats and mice, statistically significant increases in incidences of tumours, mainly in the upper gastrointestinal tract, were observed at dose levels starting from 50–440 mg/kg bw per day (Maltoni et al., 1997; Minardi et al., 2002; Umeda et al., 2004).

2.3 Observations in humans

No information was available.

3. Dietary exposure

3.1 Introduction

PVA-PEG graft copolymer is primarily intended for use in aqueous film coatings for food supplement products. The copolymer has been globally approved for use in the preparation of pharmaceutical products in a number of regions. The copolymer can be used for the same applications as cellulosic polymer coating systems (e.g. hydroxypropyl methylcellulose–based coating systems). More broadly, however, it can be used for all applications for which a water-soluble flexible polymer is required. In addition to its use as an instant release coating, PVA-PEG graft copolymer can also be used in food supplements as a binder for tablets and a stabilizer.

The Committee evaluated one submission by a sponsor of the additive (Haber, 2014). In the dossier, the manufacturer assumes that PVA-PEG graft copolymer–based film coating formulations are applied to food supplement tablets or capsules at 2–4 mg/cm². The copolymer may comprise 60–100% of the dry coating and would typically constitute up to 5% of the weight of the tablet or capsule.

For technical uses other than film coating of tablets or capsules (i.e. binder or stabilizer), up to 10% of the weight of the food supplement is typically needed.

According to the chemical specifications from the sponsor, PVA-PEG graft copolymer can contain up to 20 mg vinyl acetate per kilogram and up to 400 mg ethylene glycols (ethylene glycol and diethylene glycol, singly or in combination) per kilogram as impurities.

3.2 Assessment of dietary exposure[1]

3.2.1 Assessments based on individual dietary records

Assuming that each tablet weighs 1 g and that all tablets are coated with PVA-PEG graft copolymer comprising 5% of the weight of the tablet (the maximum use level from its primary use as a coating for tablets), each tablet would contain 50 mg PVA-PEG graft copolymer. The exposure from the stated minor uses as stabilizer and binder for tablets at levels up to 10% is expected to be covered by the conservative estimates below.

Based on data from the dietary supplement questionnaire included in the National Health and Nutrition Examination Surveys (NHANES) of the National Center for Health Statistics of the USA (CDC, 2006, 2009; USDA, 2009), the manufacturer has estimated the consumption of dietary supplements in the form of tablets (including pills and capsules) as up to 15 tablets for adults (95th percentile for adult women) and up to nine tablets for children (95th percentile for children ages 3–11 years).

Based on data from the United Kingdom Food Standards Agency, EFSA (2013) found that the use of food supplements among high consumers (97.5th percentile) ranged from two tablets or capsules per day in children (4–18 years old) to seven tablets or capsules per day in adults.

The dietary exposure to PVA-PEG graft copolymer for high adult consumers would be 350–750 mg/day (7–15 tablets × 50 mg/day), i.e. 5.8–13 mg/kg bw per day for a 60 kg adult, and for children, 100–450 mg/day (2–9 tablets × 50 mg/day), i.e. 4.3–20 mg/kg bw per day for a 23 kg child.

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[1] In this section and in sections 4.2 and 5, "dietary exposure" refers to exposure from both food supplements and pharmaceutical products.

Assuming that there are similar levels of use and dietary exposure to PVA-PEG graft copolymer from both food supplements and pharmaceuticals, the Committee concluded that the combined intake from food supplements and pharmaceutical products for high consumers could be up to 25 mg/kg bw per day for adults and 40 mg/kg bw per day for children.

If it is assumed that the impurity vinyl acetate is present in PVA-PEG graft copolymer at a concentration up to 20 mg/kg, dietary exposure to vinyl acetate from both food supplements and pharmaceutical products for high consumers could be up to 0.0005 mg/kg bw per day for adults and 0.0008 mg/kg bw per day for children.

If it is assumed that the impurities ethylene glycol and diethylene glycol are present in PVA-PEG graft copolymer at a concentration up to 400 mg/kg, singly or in combination, dietary exposure to the glycols (singly or in combination) from food supplements and pharmaceutical products for high consumers could be up to 0.010 mg/kg bw per day for adults and 0.016 mg/kg bw per day for children.

The theoretical maximum daily dietary exposures estimated here are conservative, owing to the assumption that all food supplements and pharmaceutical products are coated with PVA-PEG graft copolymer and the fact that the exposure estimates are for high consumers of both food supplements and pharmaceutical products.

4. Comments

4.1 Toxicological studies

The toxicokinetic properties of ^{14}C-labelled PVA-PEG graft copolymer were investigated in rats following administration of a single oral dose of 10 or 1000 mg/kg bw by gavage (Leibold & Hoffmann, 2001; published by Heuschmid et al., 2013a). The cumulative percentage of radioactivity recovered in the faeces of males and females at 48 hours post-dosing was approximately 100%. Excretion via urine, exhaled air and bile was negligible at both dose levels. The bioavailability was calculated to be less than 1%. No study on toxicokinetics in humans was available, but the Committee concluded that in humans, PVA-PEG graft copolymer would also be expected to be mainly eliminated via the faeces and that the bioavailability of PVA-PEG graft copolymer would be negligible.

PVA-PEG graft copolymer had an LD$_{50}$ of greater than 2000 mg/kg bw in an acute oral toxicity study in rats (Wiemann & Hellwig, 2000a).

In a 90-day oral toxicity study in rats (Mellert et al., 2001; published by Heuschmid et al., 2013b), the only treatment-related effect observed was an increase in water consumption in high-dose males and females. In the absence of

other treatment-related findings, this was not considered an adverse effect. The NOAEL was 1610 mg/kg bw per day, the highest dose tested.

In a 9-month oral toxicity study in dogs (Kaspers et al., 2002; published by Heuschmid et al., 2013b), an increase in mean absolute ovary weights was reported in all female treatment groups. These values were within the historical control range of the testing facility (range: 979–2258 mg; mean: 1378 mg), whereas the value of the control group was below the historical control range. In the absence of changes in the reproductive organs in rats in the above-described study and because no effects were seen in the reproductive toxicity study (see below), it was concluded that these changes were not treatment related. Therefore, the NOAEL was 780 mg/kg bw per day, the highest dose tested.

PVA-PEG graft copolymer was tested for genotoxicity in a bacterial reverse mutation assay (Engelhardt & Hoffmann, 2000), a gene mutation assay in mouse lymphoma L5178Y TK$^{+/-}$ cells (Engelhardt & Hildebrand, 2000a) and an in vivo micronucleus assay in mice using intraperitoneal administration (Engelhardt & Hildebrand, 2000b). All three tests gave negative results. Therefore, the Committee concluded that PVA-PEG graft copolymer is unlikely to be genotoxic.

PVA-PEG graft copolymer has not been tested in a long-term toxicity and carcinogenicity study. Given the negative genotoxicity studies, the lack of adverse effects in the short-term studies and the negligible bioavailability of PVA-PEG graft copolymer, the Committee did not consider a study of long-term toxicity or carcinogenicity to be necessary for the safety evaluation of PVA-PEG graft copolymer.

In a two-generation reproductive toxicity study in rats (Schneider et al., 2003; published by Heuschmid et al., 2013c), no treatment-related effects were observed. The NOAEL for parental, offspring and reproductive toxicity was 1000 mg/kg bw per day, the highest dose tested.

In two developmental toxicity studies, no treatment-related maternal or developmental effects were observed when rats (Gamer, Schneider & Van Ravenzwaay, 2002) or rabbits (Schneider, Hellwig & Van Ravenzwaay, 2002) were given PVA-PEG graft copolymer at dose levels up to 1000 mg/kg bw per day during gestation. In both studies, the NOAELs for maternal toxicity and for embryo/fetal toxicity were 1000 mg/kg bw per day, the highest dose tested.

The Committee noted that the use of PVA-PEG graft copolymer that complies with the proposed specifications could lead to exposure to vinyl acetate, ethylene glycol and diethylene glycol. The Committee has not previously evaluated the safety of vinyl acetate. In oral long-term studies of the toxicity and carcinogenicity of vinyl acetate in rats and mice (Maltoni et al., 1997; Minardi et al., 2002; Umeda et al., 2004), statistically significant increases in incidences of

tumours, mainly in the upper gastrointestinal tract, were observed at dose levels starting from 50 mg/kg bw per day (Minardi et al., 2002).

Diethylene glycol was evaluated by the Committee at its twenty-third meeting (Annex 1, reference *50*). At that meeting, the Committee concluded that diethylene glycol was not suitable as a food additive because it produces renal damage, calcium oxalate stones and liver damage in a number of species, including humans, and is associated with bladder tumours in rats at higher levels. In view of the secondary nature of the bladder tumours produced and the relatively high levels of the substance required to produce kidney stones or liver damage, the Committee concluded that its presence as an impurity in food additives at low levels may be tolerated and that this should be evaluated on a case-by-case basis. Ethylene glycol has not been evaluated previously by the Committee. The SCF of the European Union derived a group tolerable daily intake (TDI) of 0.5 mg/kg bw for ethylene glycol and diethylene glycol in 1986 and confirmed it in 2002 (SCF, 1986, 2002).

4.2 **Assessment of dietary exposure**

An estimate of the theoretical dietary exposure to PVA-PEG graft copolymer was made by the Committee based on the estimated levels in food supplements (50 mg PVA-PEG graft copolymer for a 1 g tablet) from its primary use as a coating for tablets and the assumption that the exposures to PVA-PEG graft copolymer from pharmaceutical products and food supplements are the same. The exposure from the stated minor uses as a stabilizer and binder for tablets at levels up to 10% is expected to be covered by the conservative estimates below. If the levels of PVA-PEG graft copolymer in food supplements are combined with high consumption data for food supplements from the USA (supplied by the sponsor) and from the United Kingdom Food Standards Agency and if exposure to PVA-PEG graft copolymer from pharmaceutical products is included, potential total dietary exposures of 25 mg/kg bw per day for adults and 40 mg/kg bw per day for children can be calculated.

If it is assumed that the impurity vinyl acetate is present in PVA-PEG graft copolymer at a concentration up to 20 mg/kg, dietary exposure to vinyl acetate from both food supplements and pharmaceutical products for high consumers could be up to 0.0005 mg/kg bw per day for adults and 0.0008 mg/kg bw per day for children.

If it is assumed that the impurities ethylene glycol and diethylene glycol are present in PVA-PEG graft copolymer at a concentration up to 400 mg/kg, singly or in combination, dietary exposure to the glycols (singly or in combination) from both food supplements and pharmaceutical products for high consumers could be up to 0.010 mg/kg bw per day for adults and 0.016 mg/kg bw per day for children.

The theoretical maximum daily intakes estimated here are conservative, owing to the assumption that all food supplements and pharmaceutical products are coated with PVA-PEG graft copolymer and the fact that the exposure estimates are for high consumers of both food supplements and pharmaceutical products.

5. Evaluation

On the basis of the available studies, in which no treatment-related effects were seen at the highest doses tested, the Committee considered PVA-PEG graft copolymer to be a substance of low oral toxicity in rats, rabbits and dogs. The bioavailability of PVA-PEG graft copolymer in rats is negligible, and PVA-PEG graft copolymer is unlikely to be genotoxic and is not associated with reproductive or developmental toxicity. Therefore, the Committee concluded that calculation of a margin of exposure for PVA-PEG graft copolymer would not be meaningful.

Based on these data, the Committee would normally establish an ADI "not specified". However, the Committee decided not to establish an ADI "not specified" for PVA-PEG graft copolymer in view of the impurities present, some of which may also be impurities in other food additives. The Committee had concerns that establishing an ADI "not specified" could lead to additional uses beyond those considered in the present evaluation and consequently could increase exposure to the impurities.

The use of PVA-PEG graft copolymer that complies with the proposed specifications could lead to a dietary exposure to ethylene glycol and diethylene glycol from both food supplements and pharmaceutical products up to 0.016 mg/ kg bw per day for children (high consumers). This is 3% of the TDI of 0.5 mg/kg bw per day derived by the SCF, and therefore the exposure to ethylene glycol and diethylene glycol from the use of PVA-PEG graft copolymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used in the applications specified.

The use of PVA-PEG graft copolymer that complies with the proposed specifications could lead to dietary exposure to vinyl acetate from both food supplements and pharmaceutical products up to 0.0008 mg/kg bw per day for children. This dietary exposure estimate is at least 62 500 times lower than the dose levels at which increases in tumour incidences are observed in oral long-term studies of toxicity and carcinogenicity in rats and mice. Therefore, the exposure to vinyl acetate from the use of PVA-PEG graft copolymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used in the applications specified.

The Committee concluded that the use of PVA-PEG graft copolymer that complies with the specifications established at the current meeting is not of

safety concern when the food additive is used as a glazing agent (aqueous film coating), stabilizer and binder for tablets in the preparation and formulation of food supplements and in accordance with good manufacturing practice.

5.1 **Recommendation**

The Committee noted that ethylene glycol and diethylene glycol may also be present as impurities in other food additives, such as polyethylene glycols and polysorbates, and the total exposure to these compounds from food additives may be higher than from PVA-PEG graft copolymer alone. Currently, only the specifications monograph for polyethylene glycols contains maximum limits for ethylene glycol and diethylene glycol (2500 mg/kg, singly or in combination). The Committee recommends setting and/or revising maximum limits for ethylene glycol and diethylene glycol that may occur as impurities in food additives at a future meeting.

6. References

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ANNEX 1

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. General principles governing the use of food additives (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).

2. Procedures for the testing of intentional food additives to establish their safety for use (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).

3. Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants) (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. I. Antimicrobial preservatives and antioxidants, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).

4. Specifications for identity and purity of food additives (food colours) (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. II. Food colours, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).

5. Evaluation of the carcinogenic hazards of food additives (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).

6. Evaluation of the toxicity of a number of antimicrobials and antioxidants (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).

7. Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).

8. Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).

9. Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).

10. Specifications for identity and purity and toxicological evaluation of food colours. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.

11. Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).

12. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases. FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.

13. Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.

14. Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.

15. Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.

16. Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.

17. Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.

18. Specifications for the identity and purity of some antibiotics. FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.

19. Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.

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21. Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.

22. Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.

23. Toxicological evaluation of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.

24. Specifications for the identity and purity of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.

25. A review of the technological efficacy of some antimicrobial agents. FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.

26. Evaluation of food additives: some enzymes, modified starches, and certain other substances: Toxicological evaluations and specifications and a review of the technological efficacy of some antioxidants (Fifteenth report

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28. Specifications for the identity and purity of some enzymes and certain other substances. FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.

29. A review of the technological efficacy of some antioxidants and synergists. FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.

30. Evaluation of certain food additives and the contaminants mercury, lead, and cadmium (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.

31. Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbamate, and octyl gallate. FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.

32. Toxicological evaluation of certain food additives with a review of general principles and of specifications (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).

33. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.

34. Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers. FAO Food and Nutrition Paper, No. 4, 1978.

35. Evaluation of certain food additives (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.

36. Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.

37. Specifications for the identity and purity of some food colours, enhancers, thickening agents, and certain food additives. FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.

38. Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.

39. Toxicological evaluation of some food colours, thickening agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.

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41. Evaluation of certain food additives (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.

42. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 10, 1976.

43. Specifications for the identity and purity of some food additives. FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.

44. Evaluation of certain food additives (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.

45. Summary of toxicological data of certain food additives. WHO Food Additives Series, No. 12, 1977.

46. Specifications for identity and purity of some food additives, including antioxidants, food colours, thickeners, and others. FAO Nutrition Meetings Report Series, No. 57, 1977.

47. Evaluation of certain food additives and contaminants (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.

48. Summary of toxicological data of certain food additives and contaminants. WHO Food Additives Series, No. 13, 1978.

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50. Evaluation of certain food additives (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.

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55. Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives). FAO Food and Nutrition Paper, No. 17, 1980.

56. Evaluation of certain food additives (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.

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58. Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives). FAO Food and Nutrition Paper, No. 19, 1981.

59. Evaluation of certain food additives and contaminants (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.

60. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 17, 1982.

61. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 25, 1982.

62. Evaluation of certain food additives and contaminants (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.

63. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 18, 1983.

64. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 28, 1983.

65. Guide to specifications – General notices, general methods, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.

66. Evaluation of certain food additives and contaminants (Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710, 1984, and corrigendum.

67. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 19, 1984.

68. Specifications for the identity and purity of food colours. FAO Food and Nutrition Paper, No. 31/1, 1984.

69. Specifications for the identity and purity of food additives. FAO Food and Nutrition Paper, No. 31/2, 1984.

70. Evaluation of certain food additives and contaminants (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.

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74. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 21. Cambridge University Press, 1987.

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76. Principles for the safety assessment of food additives and contaminants in food. WHO Environmental Health Criteria, No. 70. Geneva, World Health Organization, 1987 (out of print). The full text is available electronically at www.who.int/pcs.

77. Evaluation of certain food additives and contaminants (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987, and corrigendum.

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87. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/2, 1990.

88. Evaluation of certain food additives and contaminants (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.

89. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 26, 1990.

90. Specifications for identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 49, 1990.

91. Evaluation of certain veterinary drug residues in food (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.

92. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 27, 1991.

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ANNEX 2

Abbreviations used in the monographs

ADI	acceptable daily intake
ANCNPAS	Australian National Children's Nutrition and Physical Activity Survey
BGF	beta-glucanase fungique
BMU	betamyl unit
bw	body weight
CCFA	Codex Committee on Food Additives
CIFOCOss	Chronic Individual Food Consumption Data – Summary statistics (FAO/WHO)
CNS	Children's Nutrition Survey
DNA	deoxyribonucleic acid
EDI	estimated daily intake
EFSA	European Food Safety Authority
Eq	equivalent
f	female
F_0	parental generation
F_1	first filial generation
F_2	second filial generation
FAO	Food and Agriculture Organization of the United Nations
GD	gestation day
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
GLP	good laboratory practice
GSFA	Codex General Standard for Food Additives
HFCS	high-fructose corn syrup
IARC	International Agency for Research on Cancer
ICBA	International Council of Beverages Associations
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
INS	International Numbering System
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LB	lower bound
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection

LOQ	limit of quantification
m	male
M	male
NA	not available
nd	not detected
ND	not detected
NHANES	National Health and Nutrition Examination Survey
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
OECD	Organisation for Economic Co-operation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances (USEPA)
P50	50th percentile
P95	95th percentile
P97.5	97.5th percentile
PEG	polyethylene glycol
PND	postnatal day
PVA	polyvinyl alcohol
QA	quality assurance
S9	$9000 \times g$ supernatant fraction of liver homogenate
SAR	Special Administrative Region
SCF	Scientific Committee on Food (European Union)
TDI	tolerable daily intake
TDS	Total Diet Study
TIPU	titratable phospholipase unit
TOS	total organic solids
UB	upper bound
USA	United States of America
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
w/v	weight per volume
w/w	weight per weight
XVU	xylanase viscosity unit

ANNEX 3

Joint FAO/WHO Expert Committee on Food Additives[1]

Rome, 16–25 June 2015

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ANNEX 4

Toxicological and dietary exposure information and information on specifications

Food additives considered for specifications only

Food additive	Specifications
Advantame	R[a]
Aluminium silicate	W[b]
Annatto extract (solvent-extracted bixin)	R[c]
Annatto extract (solvent-extracted norbixin)	R[c]
Calcium aluminium silicate	W[b]
Calcium silicate	R[d]
Glycerol ester of gum rosin	W[b]
Silicon dioxide, amorphous	R, T[e]
Sodium aluminium silicate	R, T[f]

R: existing specifications revised; T: tentative specifications; W: tentative specifications withdrawn

[a] The requested information was received, and the method of assay was revised. The tentative status of the specifications was removed.

[b] The requested information was not received.

[c] The specifications were revised to reflect the modification of the method for residual solvents by headspace gas chromatography and to include sample and standard preparation information.

[d] The requested information was received, and the specifications were revised to include information on functional uses, pH, loss on drying, loss on ignition, impurities soluble in 0.5 M hydrochloric acid and the assay. The tentative status of the specifications was removed

[e] Limited information was received. The specifications were revised to include information on pH, loss on drying, loss on ignition, impurities (lead and arsenic) soluble in 0.5 M hydrochloric acid and the assay for some forms of silicon dioxide. The tentative status of the specifications was maintained, and information was requested in order for the tentative specifications to be revised.

[f] Limited information was received. The specifications were revised to include information on Chemical Abstracts Service number, chemical formula, pH, loss on drying, loss on ignition and limits on impurities (lead and arsenic) soluble in 0.5 M hydrochloric acid. The tentative status of the specifications was maintained, and information was requested in order for the tentative specifications to be revised.

Food additives evaluated toxicologically and/or assessed for dietary exposure

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information
Benzoates: dietary exposure assessment	NA	Based on the available data set, the Committee noted that there is consistency in the average typical range of concentrations for benzoates reported to be used or analysed in non-alcoholic ("soft") beverages (Codex General Standard for Food Additives [GSFA] food category 14.1). For example, typical reported concentrations from industries ranged from 83 to 209 mg/L for water-based flavoured drinks (food category 14.1.4), and analytically quantified measurements ranged from 63 to 259 mg/L for non-alcoholic beverages (food category 14.1). These levels are lower than national maximum limits (150–400 mg/L) or limits for GSFA food category 14.1.4 (600 mg/L). The Committee also noted that most of the reported estimates for mean and high percentile per capita benzoate exposure were below the upper bound of the ADI of 0–5 mg/kg body weight (bw), expressed as benzoic acid, despite different methodologies and assumptions applied in the preparation of the exposure estimates.

Food additives evaluated toxicologically and/or assessed for dietary exposure (continued)

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information
		None of the mean exposure estimates for consumers of non-alcoholic ("soft") beverages exceeded the upper bound of the ADI: 0.3–4.1 mg/kg bw per day for toddlers and young children, 0.2–2.7 mg/kg bw per day for other children, including adolescents, and 0.1–1.7 mg/kg bw per day for adults. However, the Committee noted that the 95th percentile exposures for the consumers-only group exceeded the upper bound of the ADI in some cases: up to 10.9 mg/kg bw per day for toddlers and young children and up to 7.0 mg/kg bw per day for other children, including adolescents. Additionally, the Committee noted that in some countries, the overall dietary exposure to benzoates for toddlers, young children and adolescents also exceeds the upper bound of the ADI at the high percentiles. Reduction of those exposures exceeding the upper bound of the ADI would require consideration of dietary patterns for both beverage and non-beverage foods containing benzoates and typical/allowed benzoate use levels in those countries.
Lipase from *Fusarium heterosporum* expressed in *Ogataea polymorpha*	N	No treatment-related adverse effects were seen at the highest dose tested (669 mg total organic solids [TOS]/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the dietary exposure estimate of 0.5 mg TOS/kg bw per day (for a 60 kg individual) with the highest dose tested of 669 mg TOS/kg bw per day results in a margin of exposure (MOE) of at least 1300. **The Committee established an ADI "not specified"[a] for lipase from *F. heterosporum* expressed in *O. polymorpha* when used in the applications specified and in accordance with good manufacturing practice.**
Magnesium stearate	N	The Committee estimated the potential total dietary exposure to magnesium stearate based on the proposed maximum use levels: 44 mg/kg bw per day for children and 83 mg/kg bw per day for adults, corresponding to 2 and 4 mg/kg bw per day, expressed as magnesium, respectively. These dietary exposures would contribute up to an additional 250 mg/day to the background exposure to magnesium from food of 180–480 mg/day. The Committee noted that the consumption of the food additive may lead to an additional dietary exposure to stearic and palmitic acids in the order of 5 g/day. An ADI "not specified"[a] has previously been established for a number of magnesium salts used as food additives. The Committee concluded that there are no differences in the evaluation of the toxicity of magnesium stearate compared with other magnesium salts. **The Committee confirmed the ADI "not specified" for magnesium salts of stearic and palmitic acids.** However, the Committee was concerned that the use of magnesium salts in many food additives may result in combined exposure that could lead to a laxative effect. Therefore, the Committee reiterated its previous recommendation to undertake an exposure assessment for magnesium from use of food additives.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information
Maltotetraohydrolase from *Pseudomonas stutzeri* expressed in *Bacillus licheniformis*	N	No treatment-related adverse effects were seen at the highest dose tested (93 mg TOS/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the dietary exposure estimate of 0.1 mg TOS/kg bw per day (for a 60 kg individual) with the highest dose tested of 93 mg TOS/kg bw per day results in an MOE of at least 900. **The Committee established an ADI "not specified"[a] for malto-tetraohydrolase from *P. stutzeri* expressed in *B. licheniformis* when used in the applications specified and in accordance with good manufacturing practice.**
Mixed β-glucanase, cellulase and xylanase from *Rasamsonia emersonii*	N, T[b]	No treatment-related adverse effects were seen at the highest dose tested (84.8 mg TOS/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the dietary exposure estimate of 0.08 mg TOS/kg bw per day (for a 60 kg individual) with the highest dose tested of 84.8 mg TOS/kg bw per day results in an MOE of at least 1000. **The Committee established an ADI "not specified"[a] for the mixed β-glucanase, cellulase and xylanase enzyme preparation from *R. emersonii* when used in the applications specified and in accordance with good manufacturing practice.**
Mixed β-glucanase and xylanase from *Disporotrichum dimorphosporum*	N, T[b]	No treatment-related adverse effects were seen at the highest dose tested (199 mg TOS/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the dietary exposure estimate of 0.7 mg TOS/kg bw per day (for a 60 kg individual) with the highest dose tested of 199 mg TOS/kg bw per day gives an MOE of at least 280. **The Committee established an ADI "not specified"[a] for the mixed β-glucanase and xylanase enzyme preparation from *D. dimorphosporum* when used in the applications specified and in accordance with good manufacturing practice.**
Polyvinyl alcohol (PVA) – polyethylene glycol (PEG) graft copolymer	N	On the basis of the available studies, in which no treatment-related effects were seen at the highest doses tested, the Committee considered PVA-PEG graft copolymer to be a substance of low oral toxicity in rats, rabbits and dogs. The bioavailability of PVA-PEG graft copolymer in rats is negligible, and PVA-PEG graft copolymer is unlikely to be genotoxic and is not associated with reproductive or developmental toxicity. Therefore, the Committee concluded that calculation of an MOE for PVA-PEG graft copolymer would not be meaningful. Based on these data, the Committee would normally establish an ADI "not specified". However, the Committee decided not to establish an ADI "not specified" for PVA-PEG graft copolymer in view of the impurities present, some of which may also be impurities in other food additives. The Committee had concerns that establishing an ADI "not specified" could lead to additional uses beyond those considered at the current meeting and consequently could increase exposure to the impurities.

Food additives evaluated toxicologically and/or assessed for dietary exposure (continued)

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information
		The use of PVA-PEG graft copolymer that complies with the proposed specifications could lead to a dietary exposure to ethylene glycol and diethylene glycol from both food supplements and pharmaceutical products up to 0.016 mg/kg bw per day for children (high consumers). This is 3% of the tolerable daily intake (TDI) of 0.5 mg/kg bw per day derived by the Scientific Committee on Food of the European Union, and therefore the exposure to ethylene glycol and diethylene glycol from the use of PVA-PEG graft copolymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used in the applications specified.
		The use of PVA-PEG graft copolymer that complies with the proposed specifications could lead to a dietary exposure to vinyl acetate from both food supplements and pharmaceutical products up to 0.0008 mg/kg bw per day for children. This dietary exposure estimate is at least 62 500 times lower than the dose levels at which increases in tumour incidence are observed in oral studies of long-term toxicity and carcinogenicity in rats and mice. Therefore, the dietary exposure to vinyl acetate from the use of PVA-PEG graft copolymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used in the applications specified.
		The Committee concluded that the use of PVA-PEG graft copolymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a glazing agent (aqueous film coating), stabilizer and binder for tablets in the preparation and formulation of food supplements and in accordance with good manufacturing practice.

N: new specifications; NA: not applicable (dietary exposure assessment only); T: tentative specifications

[a] ADI "not specified" is used to refer to a food substance of very low toxicity that, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary exposure to the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice – i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

[b] Information is required in order for the tentative specifications to be revised.

This volume contains monographs prepared at the eightieth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met in Rome, Italy, from 16 to 25 June 2015.

The toxicological and dietary exposure monographs in this volume summarize the safety and/or dietary exposure data on seven food additives (benzoates: dietary exposure assessment; lipase from *Fusarium heterosporum* expressed in *Ogataea polymorpha*; magnesium stearate; maltotetraohydrolase from *Pseudomonas stutzeri* expressed in *Bacillus licheniformis*; mixed β-glucanase, cellulase and xylanase from *Rasamsonia emersonii*; mixed β-glucanase and xylanase from *Disporotrichum dimorphosporum*; and polyvinyl alcohol (PVA) – polyethylene glycol (PEG) graft copolymer). Monographs on two contaminant groups (non-dioxin-like polychlorinated biphenyls and pyrrolizidine alkaloids) discussed at the eightieth meeting will be published as separate supplements in the WHO Food Additives series.

This volume and others in the WHO Food Additives series contain information that is useful to those who produce and use food additives and veterinary drugs and those involved with controlling contaminants in food, government and food regulatory officers, industrial testing laboratories, toxicological laboratories and universities.