VIDAFY
Life Converted

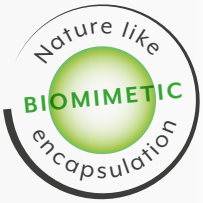
BIOMIMETIC PROPERTIES & SAFETY

NANOFY® encapsulation with Polysorbate 20/80

NATURAL ORIGIN & BROAD USE MAKE IT A RELIABLE CHOICE

— Source = palm oil & palm kernel oil
— Sole use certified sustainable palm oil products (RSPO Supply Chain Certification Standard)
— Broad use for decades worldwide
— Part of foodstuffs, beverages, cosmetics, pharma products

MODE OF ACTION ACCORDING TO NATURE *)



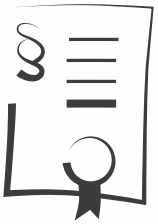
— BioMS® micellation = nature like ("biomimetic")
— Non-absorption of polysorbates confirmed

—"More is less": BioMS® encapsulation allows small ingredients dosages
— Competitor formulas of turmeric or curcumin:
  – Piperine or Cyclodextrin formulas use non natural bioavailability principle
  – Carrier free products
    = very high ingredient dosages
    = exceed tolerable upper limits

SAFETY EVIDENCE BY MANY STUDIES AND STANDARDS **)

— Food grade
— GRAS status
— Safety proven also in NovaSOL® trials
— Legal safety assessment / GRAS letter available
— Polysorbate content in NANOFY is less than 0.005% of the No Adverse Effect Level (NOAEL) established by the European Union
— Polysorbate micelle safety confirmed separately
— Tolerability proven even with ultimately sensitive organisms (Caco2 cells & C.elegans worms)
— Use in numerous human clinical trials approved by ethical commissions

CONSISTENT REGULATORY & LABEL ASSESSMENT ***)



— ADI of 25mg/kg bodyweight established with safety factor 100 on NOAEL
— Waiver of polysorbate labeling in foodstuffs approved by sworn experts
— Use officially confirmed with free sales certificates
— Multifold approval from regulatory bodies in EU, USA and Asia

07/2019

VIDAFY
Life Converted

BIOMIMETIC PROPERTIES & SAFETY

NANOFY® encapsulation with Polysorbate 20/80

*) — NANOFY: “Product micelle statement”
— Biesalski, Hans K.: “Statement non absorption product Micelle/Polysorbate”

**) — SISU: “Full Spectrum Curcumin NPN 80066894 – Regulatory label text”
— EFSA: “Scientific opinion re-evaluation Polysorbates”
— Frank, Jan: "Absorption of polysorbates from BioMS® micelles"
— Tawab, Mona: “Statement toxicity micellar solubilisation using Polysorbate”
— Abdel-Tawab, Mona: “Transepithelial Transport Curcumin in Caco2 cells”
— Kocher, Alexa: Highly bioavailable micellar Curcuminoides accumulate blood safety”
— Back, Evelyn I.: “Bioavailability fat soluble vitamins”
Back, Evelyn I.: “Bioavailability fat soluble vitamins - publication”
— Immel, Louise: “Fat malabsorption – solubilized vitamins”
Immel, Louise: “Hydrophilic formulation lipophilic vitamins Cystis Fibrosis”
Immel, Louise: “Effects solubilized vitamins fat malabsorption”
— Schiborr, Christina: “Bioavailability study Curcumin – part I”
— Kocher, Alexa: “Bioavailability study Curcumin – part II”

***) — EFSA: “Scientific opinion re-evaluation Polysorbates”
— NANOFY: “Statement Polysorbate ADI according WHO/JECFA”
— Gekeler, Walter: “Non declaration statement Polysorbate”
— Aurich, Sebastian: “Relevant EU regulation for use and labelling Polysorbate”

07/2019

UNIVERSITÄT HOHENHEIM

INSTITUT FÜR BIOLOGISCHE CHEMIE
UND ERNÄHRUNGSWISSENSCHAFT
Prof. Dr. med. Hans K. Biesalski

Universität Hohenheim (140), D-70593 Stuttgart

Hohenheim, 17.07.2007/db-a

An
AQUANOVA AG
Birkenweg 8-10

64295 Darmstadt

Gutachterliche Stellungnahme

AQUANOVA-Produktmicelle

Bei der oralen Aufnahme der AQUANOVA-Produktmicelle öffnet sich die Hülle der Micelle (Polysorbat-Partikel) an der Membrangrenzfläche des Dünndarms und ausschließlich der Inhalt (in der Struktur unveränderter Wirkstoff) wird aufgenommen.

Prof. Dr. Hans K. Biesalski

Expert Opinion

AQUANOVA-Product Micelle

With the oral admission of the AQUANOVA-Product Micelle the shell of the Micelle (Polysorbate molecule) opens in the membrane interface of the small intestine and exclusively the content (in the structure unchanged active substance) is taken up.

University of Hohenheim (140b) | 70593 Stuttgart

AQUANOVA AG
Birkenweg 8-10
64295 Darmstadt

Institute of Biological Chemistry and Nutrition
Biofunctionality and Safety of Food (140b)

Jan Frank, Ph.D.
Professor

Front desk: +49 711 459 24410
Phone: +49 711 459 24459
Fax: +49 711 459 23386
Email: jan.frank@nutres.de

Stuttgart, July 22, 2015

Your question regarding the potential absorption of polysorbate-80 micelles

Dear Mr. Behnam,

Thank you very much for enquiring about my opinion regarding the absorption of polysorbates from AQUANOVA micelles.

Absorption studies in humans and animals show that ingested or intravenously injected polysorbates are cleaved by endogenous enzymes into a free fatty acid and a polyoxyethylene sorbitan moiety. The released fatty acids are absorbed and metabolised identical to dietary fatty acids, whereas only a small fraction of the polyoxyethylene sorbitan moiety is taken up and the majority does not pass through the gastrointestinal barrier and is therefore excreted with the faeces. The small amount of polyoxyethylene sorbitan that is absorbed, however, is not retained in the body, but rapidly excreted with the urine.

Degradation of polysorbates by hydrolytic enzymes, however, requires access of the molecules to the catalytic centre of the enzyme. Free polysorbates are thus cleaved, while polysorbates that are part of a - relatively speaking – 'larger' micellar structure are spatially oriented in a way that probably prevents access of endogenous enzymes and thus cleavage into the polyoxyethylene sorbitan and fatty acid moieties.

Further support for the safety of AQUANOVA Curcumin comes from a consensus statement of an expert panel that evaluated the safety of polysorbate 80 micelles containing coenzyme Q_{10}. The expert panel came to the conclusion that no data exists to suggest any harmful effects of these coenzyme Q_{10}-polysorbate 80 micelles and therefore declared them to be generally recognised as safe (GRAS).

Please also see the recent Scientific Opinion of the European Food Safety Authority (EFSA) that confirms the safety of polysorbates for human consumption at doses of 25 mg/kg bodyweight, which is based on the 100-fold higher (2.5 g/kg bodyweight) no-

UNIVERSITY OF HOHENHEIM
Institute of Biological Chemistry and Nutrition (140b)
Garbenstr. 28
70599 Stuttgart

INTERNET
www.nutrition-research.de
www.facebook.com/Nutrition.Research
www.uni-hohenheim.de

DIRECTIONS
Tram
U3, Plieningen (Universität Hohenheim)
Bus
65, 70, 73, 74, 75, 76, 79

observed-adverse-effect-level (NOAEL) in rats (doi:10.2903/j.efsa.2015.4152).

The recently published Scientific Opinion of the EFSA can be accessed online at http://www.efsa.europa.eu/en/efsajournal/pub/4152.htm.

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food), 2015. Scientific Opinion on the re-evaluation of polyoxyethylene sorbitan monolaurate (E 432), polyoxyethylene sorbitan monooleate (E 433), polyoxyethylene sorbitan monopalmitate (E 434), polyoxyethylene sorbitan monostearate (E 435) and polyoxyethylene sorbitan tristearate (E436) as food additives. EFSA Journal 2015;13(7):4152, 74 pp. doi:10.2903/j.efsa.2015.4152

Based on the extensively documented safety of polysorbates and curcumin and our observations from human studies, I am convinced that AQUANOVA Curcumin is safe for human consumption.

Please do not hesitate to contact me, should you have further questions regarding the extremely low absorption of polysorbates or the safety of AQUANOVA Curcumin micelles.

Yours sincerely,

European Food Safety Authority

EFSA Journal 2015;13(7):4152

SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of polyoxyethylene sorbitan monolaurate (E 432), polyoxyethylene sorbitan monooleate (E 433), polyoxyethylene sorbitan monopalmitate (E 434), polyoxyethylene sorbitan monostearate (E 435) and polyoxyethylene sorbitan tristearate (E 436) as food additives[1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 10 December 2018, replaces the earlier version published on 10 June 2015.[4]

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food (ANS) re-evaluated the safety of polysorbate 20 (E 432), polysorbate 80 (E 433), polysorbate 40 (E 434), polysorbate 60 (E 435) and polysorbate 65 (E 436) as food additives. The Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) derived an Acceptable Daily Intake (ADI) of 25 mg/kg body weight (bw)/day (group ADI for polysorbates 20, 40, 60, 65 and 80) and the Scientific Committee on Food (SCF) derived a group ADI of 10 mg/kg bw/day. Small amounts of polyoxyethylene sorbitans are absorbed. Similar toxicokinetics would be expected for all polysorbates based on their similarities in structure and metabolic fate. The acute toxicity is very low. There is no concern regarding genotoxicity, carcinogenicity or developmental toxicity. From a limited number of studies, there is no indication of reproductive toxicity. The Panel considered the long-term carcinogenicity study in rats with a No Observed Adverse Effect Level (NOAEL) equivalent to 2 500 mg/kg bw/day – consistent with the NOAEL defined in subchronic studies – as the key study and allocated a group ADI of 25 mg/kg bw/day using an uncertainty factor of 100. The estimated exposure of toddlers at the highest level in non-brand loyal scenario remains very close to

[1] On request from the European Commission, Questions No EFSA-Q-2011-00523, EFSA-Q-2011-00524, EFSA-Q-2011-00525, EFSA-Q-2011-00526, EFSA-Q-2011-00527, and EFSA-Q-2012-00740, adopted on 10 June 2015.

[2] Panel members: Fernando Aguilar, Riccardo Crebelli, Alessandro Di Domenico, Birgit Dusemund, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy, Claude Lambré, Jean-Charles Leblanc, Oliver Lindtner, Peter Moldeus, Alicja Mortensen, Pasquale Mosesso, Agneta Oskarsson, Dominique Parent-Massin, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright and Maged Younes. Correspondence: fip@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group B on Food Additives and Nutrient Sources added to Food (2011-2014), Fernando Aguilar, Polly Boon Riccardo Crebelli, Birgit Dusemund, David Gott, Torben Hallas-Møller, Jürgen König, Oliver Lindtner, Daniel Marzin, Inge Meyland, Alicja Mortensen, Agneta Oskarsson, Iona Pratt (deceased), Paul Tobback, Ine Waalkens-Berendsen and Rudolf Antonius Woutersen, for the preparatory work on this scientific opinion and EFSA staff members: Anna Christodoulidou and Petra Gergelova for the support provided to this scientific opinion. EFSA wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

[4] In appendix C the values of the table were missing in the previous version and they have now been added.

Suggested citation: EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food), 2015. Scientific Opinion on the re-evaluation of polyoxyethylene sorbitan monolaurate (E 432), polyoxyethylene sorbitan monooleate (E 433), polyoxyethylene sorbitan monopalmitate (E 434), polyoxyethylene sorbitan monostearate (E 435) and polyoxyethylene sorbitan tristearate (E 436) as food additives. EFSA Journal 2015;13(7):4152, 74 pp. doi:10.2903/j.efsa.2015.4152

Available online: www.efsa.europa.eu/efsajournal

the ADI (24.5 mg/kg bw/day). The Panel is aware that for three food categories no reported uses have been obtained and that other dietary sources of exposure to polysorbates could not been considered in this opinion and therefore more data (usage and analytical data) are needed to decrease uncertainties in the refined exposure assessment scenario used.

KEY WORDS

food additive, polysorbate 20 (E 432), polysorbate 80 (E 433), polysorbate 40 (E 434), polysorbate 60 (E 435), polysorbate 65 (E 436)

SUMMARY

Following the request from the European Commission (EC), the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of polyoxyethylene sorbitan monolaurate (polysorbate 20, E 432), polyoxyethylene sorbitan monooleate (polysorbate 80, E 433), polyoxyethylene sorbitan monopalmitate (polysorbate 40, E 434), polyoxyethylene sorbitan monostearate (polysorbate 60, E 435) and polyoxyethylene sorbitan tristearate (polysorbate 65, E 436) used as food additives. Polysorbates (E 432–E 436) are authorised as food additives in the European Union (EU).

The Scientific Committee on Food (SCF) allocated a group Acceptable Daily Intake (ADI) of 10 mg/kg bw/day for polysorbates 20, 40, 60, 65 and 80 (SCF, 1985). The basis was a No Observed Effect Level (NOEL) equivalent to 1 460 mg/kg bw/day in the diet in the 90-day study in rats with polyoxyethylene sorbitan monostearate (polysorbate 60) (BIBRA, 1981; cited in SCF, 1985). A higher group ADI value of 0–25 mg/kg bw/day was allocated by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA, 1974a, b).

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

Specifications for the polysorbates have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (JECFA, 2006a).

The Panel considered the evaluation of polysorbates (E 432–E 436) as a group in one opinion because of their similarities in structure and metabolic fate. These additives are hydrolysed to oxyethylene sorbitans and the relevant fatty acids, the latter being normal constituents of the diet. From the toxicological data as described in this opinion, there is no indication of any relevant difference between the single polysorbates. Data on absorption and metabolic fate suggested hydrolysis of the ester bond between polyoxyethylene and the fatty acid of polysorbates in the gastro-intestinal tract after oral application. Intravenous data show that similar hydrolysis can occur in blood. Fatty acids are absorbed, metabolised and excreted in the same way as dietary fatty acids. Based on the similarity of the excretion in urine between compounds labelled in the polyoxyethylene and sorbitan moiety, cleavage of the polyoxyethylene and sorbitan bond does not occur. Only small amounts of polyoxyethylene sorbitans are absorbed. Similar toxicokinetics would be expected for all polysorbates.

The acute oral toxicity of all polysorbates was low. No mortality occurred in different species at high dose levels. Although the available data have limitations, the database was sufficient for the evaluation of this endpoint.

Subacute and subchronic oral studies were available for the polysorbates, but no studies performed in accordance or in line with current guidelines were published. Generally, the available studies were not sufficient for evaluating these endpoints. Subchronic studies with polysorbate 80 in rats (NTP, 1992a) suggested No Observed Adverse Effect Levels (NOAELs) equivalent to 4 500 mg/kg bw/day. In the most valid dietary subchronic study in rats (BIBRA, 1981), a NOAEL equivalent to 1 460 mg/kg bw/day was identified. This NOAEL was based on increased caecum weight and slightly increased haemoglobin levels, abnormalities which were not seen in rats exposed to doses up to the equivalent of 2 500 mg/kg bw/day for 24 months (NTP, 1992a). Increased caecum weight is a common observation in rodents consuming low-digestible carbohydrates. In addition, these NOAELs were compared with those of various other studies and a similar order of magnitude was obtained for all NOAELs.

The available data on genotoxicity *in vitro* did not show mutagenic potential as reported in a limited gene mutation assay in bacteria with polysorbate 80 (NTP, 1992a) but they were not sufficient for evaluation of the endpoints of gene and chromosome mutations in mammalian cells. However, the evaluation of structural alerts for genotoxicity in polysorbates with the Organisation for Economic Co-operation and Development (OECD) Quantitative Structure–Activity Relationship (QSAR) Toolbox 3.2, did not highlight alerts for DNA reactivity and carcinogenicity. Taking into account the overall information on structure–activity relationships, the Panel concluded that, despite the limited database, polysorbates do not give rise to concerns for genotoxicity.

The available long-term oral studies did not fulfil the requirements of current standards but these data were sufficient for evaluation. In male and female mice, forestomach squamous hyperplasia and inflammation and, in females, forestomach ulcers were induced by polysorbate 80 in the National Toxicology Program (NTP) study at a dose equivalent to 7 500 mg/kg bw/day; the NOAEL was calculated to be 3 750 mg/kg bw/day (NTP, 1992a). The carcinogenicity study in rats by NTP (1992a) indicated equivocal evidence for carcinogenic activity of polysorbate 80 based on increased incidences of benign phaeochromocytomas in the adrenal gland of males at a dose equivalent to 2 500 mg/kg bw/day. However, considering that (1) there was no evidence for *in vitro* genotoxicity (see section 3.2.3) or (2) for malignant tumour formation, and (3) that phaeochromocytomas were associated with exposure to poorly metabolised food additives at high doses and therefore are of no biological significance for humans (SCF 1995), a NOAEL equivalent to 2 500 mg/kg bw/day in the diet was considered by the Panel. The results of a limited long-term feeding study in rats (Oser and Oser, 1956a, 1957a, b) with polysorbate 80, polysorbate 60 or polysorbate 65 suggested treatment-related effects of all three tested polysorbates at a dose level of ≥ 10 % in the diet. Haematological parameters were not affected at concentrations up to 20 % in the diet. Overall, the Panel considered that the long-term studies in rats indicated a NOAEL of approximately 2 500 mg/kg bw/day.

Studies on reproductive toxicity are not sufficient for comprehensive evaluation of this endpoint. However, there is no indication of reproductive effects of polysorbates at dose levels inducing no laxative effects in the parental generation (< 10 % of the diet).

In contrast, the database on developmental toxicity was sufficient for evaluation. Oral studies in rats performed in accordance with current guidelines were available. No developmental effects were reported even at the highest dose levels tested. The NOAEL for polysorbate 20 and polysorbate 80 was 5 000 mg/kg bw/day. For polysorbate 60, the NOAEL for maternal and developmental toxicity was 7 700 mg/kg bw/day.

Data on toxic effects in humans were published; however, clinical studies performed in accordance with current standards are not available. The most valid study was performed by Waldstein et al. (1954). In this placebo-controlled study, the ingestion of 6 000 mg/day of polysorbate 60 for 28 days (equivalent to 100 mg/kg bw/day) produced no deleterious effects in humans.

In a recent study (Chassaing et al., 2015) the effects of emulsifiers, including polysorbate 80, have been discussed. The Panel considered that if such effects occurred with polysorbates, then an increase in body weights would have been expected in subchronic, chronic toxicity and carcinogenicity studies. No such increase has been observed, and therefore the relevance of the observed effects remains unclear. According to the authors, additional studies will be needed to show the relevance of the effects seen in mice for human health. The Panel agreed with this conclusion.

The Panel concluded that, based on the NOAEL of 2 500 mg/kg bw/day identified from an oral carcinogenicity study (NTP, 1992a) with polysorbate 80 in rats (consistent with the NOAEL defined in subchronic studies) and applying an uncertainty factor of 100, a group ADI of 25 mg/kg bw/day for polysorbates 20, 80, 40, 60 and 65 (E 432, E 433, E 434, E 435 and E 436, respectively) can be established.

Exposure assessment for food additives under re-evaluation was carried out by the ANS Panel based on (1) Maximum Permitted Levels (MPLs) set out in EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) the availability of adequate usage or analytical data (defined as the refined exposure assessment scenario).

Using the *regulatory maximum level exposure assessment scenario*, mean exposure to polysorbates from its use as a food additive ranged from 0.7 mg/kg bw/day in adults and the elderly to 25.0 mg/kg bw/day in toddlers. The high exposure to polysorbates using this scenario ranged from 2.1 mg/kg bw/day in the elderly to 63.7 mg/kg bw/day in children. The Panel noted that exposure estimates of polysorbates (E 432–E 436) did not exceed the ADI at the mean exposure level and did exceed the ADI for all age groups at the high level. The main contributing food categories to the total mean exposure estimates for all population age groups in this scenario were fine bakery wares and flavoured fermented milk products in toddlers, and fine bakery wares and food supplements in other all population groups.

Using the refined brand-loyal assessment exposure scenario, mean exposure to polysorbates from its use as a food additive ranged from 0.6 mg/kg bw/day in adults and the elderly to 18.1 mg/kg bw/day in children. The high exposure to polysorbates using this scenario ranged from 1.8 mg/kg bw/day in the elderly to 57.5 mg/kg bw/day in children. The Panel noted that exposure estimates of polysorbates (E 432–E 436) did not exceed the ADI for all age groups at the mean exposure level and did exceed the ADI for all age groups, except for adolescents at the high level. The main contributing food categories were fine bakery wares and food supplements, except for toddlers, for which, besides fine bakery wares, desserts were also very a important contributor to the total mean exposure to polysorbates.

Using the refined non-brand-loyal assessment exposure scenario, mean exposure to polysorbates from its use as a food additive ranged from 0.3 mg/kg bw/day in adults and the elderly to 9.6 mg/kg bw/day in toddlers. The high exposure to polysorbates using this scenario ranged from 1.1 mg/kg bw/day in the elderly to 24.5 mg/kg bw/day in toddlers. The Panel noted that exposure estimates of polysorbates (E 432–E 436) did not exceed the ADI for all age groups at both the mean exposure level and the high level. The main contributing foods were fine bakery wares, soups and desserts.

To date, the ANS Panel has used the maximum concentration value (maximum reported use level or maximum value from the analytical results) available for each authorised food category. However, given the extensive range of data that have been made available through the most recent calls, the ANS Panel considered that this should also be used in additional scenarios (brand-loyal and non-brand-loyal scenarios) of the exposure assessment approach intended to provide more realistic exposure estimates.

Overall, the Panel considered the regulatory maximum level exposure assessment scenario as conservative, as it assumes that in all processed foods and beverages polysorbates (E 432 – E 436) are used as the food additives at the level of MPLs. The Panel considered that the refined exposure assessment approach was a more realistic scenario, as it was based on the range of use level data and assumed that the processed foods and beverages contain the additive at the mean level for all products (non-brand-loyal consumer scenario) and considers one product containing polysorbates at the maximum level (brand-loyal consumer scenario). For this exposure assessment scenario, food categories for which no or inadequate reported use levels were available were not considered in the exposure assessment. Therefore, the Panel noted that if polysorbates are nevertheless used in those food categories that are not considered in the exposure estimate, the calculated refined exposure assessment might result in underestimation of exposure to polysorbates. The Panel also noted that the refined exposure estimates will not cover future changes in the level of use of polysorbates.

It should be mentioned that a high variability of use levels of food supplements, which may be dependent on the form (solid to be diluted, liquid, etc.) or by the specific brand of the product, could not be taken into consideration for the exposure assessment because of the lack of information and

FoodEx linkage. As a consequence, exposure to polysorbates of consumers with a long term use of food supplements with high polysorbate levels might be underestimated by the calculated exposure ranges.

Exposure due to permitted uses under Annex III to Regulation No 1333/2008 on additives to be used in other additives or flavourings and nutrients could not be considered. Exposure to polysorbates may result from other sources, such as via their use as cosmetic ingredients, in personal care products, textiles and pharmaceuticals.

The non-brand-loyal scenario shows that the highest exposure of toddlers to polysorbates as a food additive remains very close to the ADI. Overall, the Panel concluded that the uncertainties identified would tend to an overestimation of the real exposure to polysorbates (E 432–E 436) as food additives in European countries by the MPL scenario but might underestimate real exposure by the refined scenarios. The Panel is aware that for three food categories no reported uses have been obtained and that other dietary sources of exposure to polysorbates have not been considered in this opinion and therefore more data (usage and analytical data) are needed to decrease uncertainties and to exclude the risk of underestimation in the refined exposure assessment scenario.

Ethylene oxide is an impurity in polysorbates which is classified as 'carcinogenic to humans (Category 1)'. The highest exposure to polysorbates using the MPL scenario, which was found in children (64 mg/kg bw/day), will lead to an exposure to ethylene oxide of 12.7 ng/kg bw/day when the EU specification of 0.2 mg ethylene oxide/kg polysorbate is met.

For comparison, Benchmark Dose (Lower Confidence Limits; BMDLs) were calculated from the most sensitive animal studies using inhalation[5] and were converted to the oral equivalents of 18.7 mg/kg bw/day for mice and 14.4 mg/kg bw/day for rats (Appendix D). From the rat BMDL, a Margin of Exposure (MOE) for ethylene oxide of at least 1.1×10^6 could be calculated, which would be considered a low risk. As, at other ages, the amounts are lower, this is an underestimate of the true MOE. In reaching the conclusion that this route to extrapolation was valid, the Panel noted this was based on available data on the distribution of ethylene oxide and the patterns of tumours observed following oral and inhalation exposures. The Panel recognised that there was endogenous production of ethylene oxide, although data on tissue levels were limited. The Panel further agreed with the comment in the SCF opinion that it '...is likely to be significant loss of ethylene oxide from foods during cooking' (SCF, 2002b).

Regarding ethylene glycol impurities, the Tolerable Daily Intake (TDI) set by the SCF (2002b) is unlikely to be exceeded when the EU specification of 0.25 % ethylene glycols are met, taking into consideration the highest estimated exposures to polysorbates calculated in this opinion.

The Panel recommended that the maximum limits for the impurities of toxic elements (arsenic, lead, cadmium and mercury) in the EC specification for polysorbates (E 432–E 436) should be revised to ensure that polysorbates (E 432–E 436) as food additives will not be a significant source of exposure to these toxic elements in food.

As regards the request for extension of use of polyoxyethylene sorbitan monooleate (E 433) as a whipping agent added to emulsifiers intended for fine bakery wares to a level of 700 mg/kg in the final food, it was assumed that no additional exposure to E 433 will result from this use, further to the exposure from its currently authorised use in fine bakery wares.

[5] http://www.food.gov.uk/science/research/foodcomponentsresearch/t01programme/t01projlist/t01051

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

Regulation (EC) No 1333/2008[6] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[7]. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[8] of 2001. The report "Food additives in Europe 2000[9]" submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with the highest priority.

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

In 2012, the European Commission has received a request from an applicant for the extension of the use of polyoxyethylene sorbitan monooleate (polysorbate 80, E 433) as a whipping agent added to emulsifiers intended for fine bakery wares.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

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

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide technical assistance (intake assessment).

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

[7] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up the program for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19.

[8] Report from the Commission on Dietary Food Additive Intake in the European Union, Brussels, 01.10.2001, COM (2001) 542 final.

[9] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers. TemaNord 2002:560.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of polyoxyethylene sorbitan monolaurate (polysorbate 20, E 432), polyoxyethylene sorbitan monooleate (polysorbate 80, E 433), polyoxyethylene sorbitan monopalmitate (polysorbate 40, E 434), polyoxyethylene sorbitan monostearate (polysorbate 60, E 435) and polyoxyethylene sorbitan tristearate (polysorbate 65, E 436) when used as food additives.

Polysorbates (E 432–E 436) are authorised as food additives and used as emulsifiers and stabilisers in the European Union (EU) and were evaluated by the Joint FAO/WHO Expert Committee on Food Additives in 1973 (JECFA, 1974a,b) and the EU Scientific Committee on Food (SCF) in 1983 (SCF, 1985) and re-evaluated in 1993 (SCF, 1995). JECFA established a group Acceptable Daily Intake (ADI) of 0–25 mg/kg bw/day and the SCF established a group ADI of 10 mg/kg bw/day.

In 2012, the European Commission (EC) received a request from an applicant for the extension of the use of polyoxyethylene sorbitan monooleate (polysorbate 80, E 433) as a whipping agent added to emulsifiers intended for fine bakery wares. The EC considered that this proposed extension for use may result in a significant contribution to the total intake and requested that the European Food Safety Authority (EFSA) undertake an overall dietary exposure assessment by including the proposed extension of use. The current opinion on the re-evaluation of polysorbates (E 432–E 436) contains an up-to-date exposure assessment including the extension for use of polysorbate 80 as an emulsifier in certain emulsifiers for use in fine bakery wares.

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and reviews, additional literature that became available since then and the data available following a public call for data.[10] The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

2. Technical data

2.1. Identity of the substances

Polysorbates (E 432–E 436) constitute a class of surface active agents which are obtained by reaction of sorbitol, fatty acids and ethylene oxide. They are defined as mixtures of partial esters of sorbitol and its mono- and dianhydrides with edible commercial fatty acids which are condensed with ethylene oxide (Commission Regulation (EU) No 231/2012,[11] TemaNord, 2002; JECFA, 2006a; EFEMA, 2009). The identity of the different polysorbates is summarised in Table 1.

2.1.1. Nomenclature of polysorbates

The general names of the polysorbates are as follows:

- Polysorbate 20 (polyoxyethylene (20) sorbitan monolaurate)
- Polysorbate 40 (polyoxyethylene (20) sorbitan monopalmitate)
- Polysorbate 60 (polyoxyethylene (20) sorbitan monostearate)
- Polysorbate 80 (polyoxyethylene (20) sorbitan monooleate)
- Polysorbate 65 (polyoxyethylene (20) sorbitan tristearate)

[10] Call for scientific data on miscellaneous waxes permitted as food additives in the EU. Published 23 November 2009. Available online: http://www.efsa.europa.eu/en/dataclosed/call/ans091123.htm

[11] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.

The number 20 following the 'polyoxyethylene' part refers to the total number of oxyethylene –(CH_2CH_2O)– groups found in the molecule. The number following the 'polysorbate' part is related to the type of fatty acid associated with the polyoxyethylene sorbitan part of the molecule. Monolaurate is indicated by 20, monopalmitate is indicated by 40, monostearate is indicated by 60 and monooleate is indicated by 80 (Schiweck et al., 2012).

Table 1: Identity of polysorbates (E 432–E 436)

	Polyoxyethylene sorbitan monolaurate (polysorbate 20) (E 432)	**Polyoxyethylene sorbitan monooleate (polysorbate 80) (E 433)**	**Polyoxyethylene sorbitan monopalmitate (polysorbate 40) (E 434)**	**Polyoxyethylene sorbitan monostearate (polysorbate 60) (E 435)**	**Polyoxyethylene sorbitan tristearate (polysorbate 65) (E 436)**
Synonym (a)	Tween 20 (b)	Tween 80 (b)	Tween 40 (b)	Tween 60 (b)	Tween 65
CAS Registry Number	9005-64-5	9005-65-6	9005-66-7	9005-67-8	9005-71-4
EINECS (c)	500-018-3	500-019-9	–	500-020-4	–
EC number (d)	500-018-3 618-897-8	500-019-9 618-569-4	618-421-9	500-020-4 618-984-0	618-424-5
Approximate molecular formula (e)	$C_{58}H_{114}O_{26}$	$C_{64}H_{124}O_{26}$	$C_{62}H_{122}O_{26}$	$C_{64}H_{126}O_{26}$	$C_{100}H_{194}O_{28}$
Approximate molecular weight (g/mol) (e)	1 228	1 310	1 284	1312	1845

CAS, Chemical Abstracts Service; EINECS, European Inventory of Existing Commercial chemical Substances.
(a): Most common synonyms/brand name.
(b): European Pharmacopoeia, 7th edition.
(c): From the European chemical Substances Information System (ESIS).
(d): From SciFinder.
(e): Calculated based on the formulae presented in Figure 1.

The polysorbates are also known by many other synonyms (e.g. ethoxylated sorbitan esters, polyoxyethylene (POE) span 20, polyethylene glycol (PEG) sorbitan esters, Tween) or brand names (e.g. Armotan, Emasol, Emsorb, Glycosperse, Rheodol).

Polysorbates (E 432–E 436) are coloured (see Table 2) oily liquids or waxy solids with a faint characteristic odour (Commission Regulation (EU) No 231/2012; JECFA, 2006a). They have a warm, somewhat bitter, taste (FCC, 1996). An overview of the physico-chemical properties of polysorbate 20 is given in Table 2. Due to their long polyoxyethylene chains, polysorbates are very soluble in water. The pH of a 5 % w/v aqueous solution is reported to be 6.0–8.0 (Rowe, 2009). The good solubility of polysorbates 80 and 20 in most solvents is based on their ability to act as both hydrogen bond donors and hydrogen bond acceptors (Pollard et al., 2006). Measured values of the partitioning between octanol and water (log Po/w) are not available based on the literature search in Toxline, Medline and SciFinder. One of the starting materials of the polysorbates is sorbitol, which can be present in a linear or cyclic form (anhydride) (for molecular structures of sorbitol and its anhydrides, refer to section 2.3, Figure 2). Consequently, the polysorbates (E 432–E 436) can occur in the linear form, as cyclic furanose (five-ring), as pyranose (six-ring) or as isosorbide. The molecular structures of the polysorbates are given in Figure 1, and the identity parameters are summarised in Table 1. Note that, although four structural formulae are known for sorbitol, the polysorbates are represented exemplarily as the 1,4-sorbitan ester (furanose form) as usually found in the literature. Therefore, the structural formulae given in Figure 1 should be considered representatives of only the typical chemical (sub-) structures.

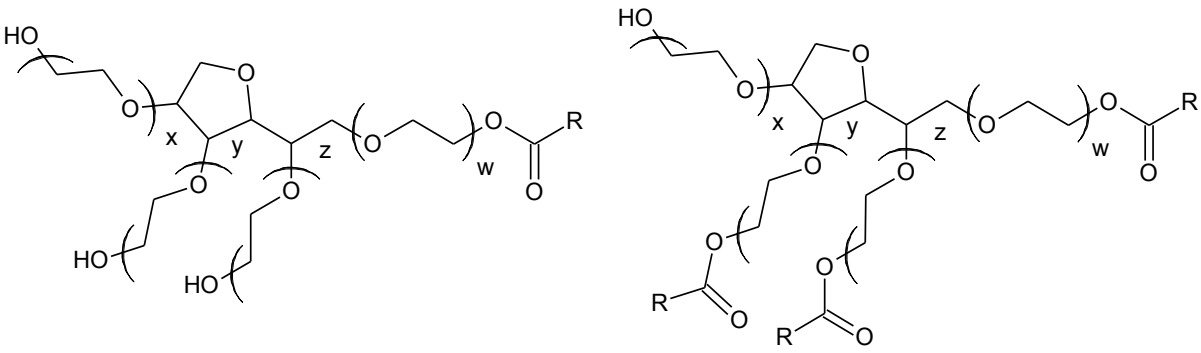


Figure 1: Structural formulae (only furanose form) of polysorbate 20 (E 432), polysorbate 80 (E 433), polysorbate 40 (E 434), polysorbate 60 (E 435) and polysorbate 65 (E 436)

The unique chemical characteristic of each polysorbate is attributed to the different fatty acid ester group present in each molecule. Polysorbates 20, 40, 60 and 80 contain only one fatty acid group per molecule, while, in polysorbate 65, three stearate groups are present. The content of oxyethylene groups is more uniform, with approximately 20 moles per molecule overall (corresponding to w, x, y and z in Figure 1) (Commission Regulation (EU) No 231/2012; JECFA, 2006a).

The commercial polysorbate products are not chemically pure compounds, but are random polydispersed compounds (Cottrell and van Peij, 2004). The variety of components can be explained by the composition of the starting materials (e.g. edible commercial fatty acids usually contain more than just the fatty acid principle named) and by the manufacturing process (see section 2.3, Figure 2).

2.2. Specifications

Specifications have been defined in Commission Regulation (EU) No 231/2012[12] and by JECFA (JECFA, 2006a) (Tables 2–6).

[12] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.

Table 2: Commission Regulation (EU) No 231/2012 and JECFA (2006a) specifications of polyoxyethylene sorbitan monolaurate (polysorbate 20) (E 432)

	Commission Regulation (EU) No 231/2012	**JECFA (2006a)**
Definition	A mixture of the partial esters of sorbitol and its mono- and dianhydrides with edible commercial lauric acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides	Consists of a mixture of the partial esters of sorbitol and its mono- and dianhydrides (which have an acid value below 7 and a water content below 0.2 %) with edible commercial lauric acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides
Assay	Content not less than 70 % of oxyethylene groups, equivalent to not less than 97.3 % of polyoxyethylene (20) sorbitan monolaurate on the anhydrous basis	Not less than 70.0 and not more than 74.0 % of oxyethylene groups, equivalent to not less than 97.3 and not more than 103.0 % of polyoxyethylene (20) sorbitan monolaurate calculated on the anhydrous basis
Description	A lemon- to amber-coloured oily liquid at 25 °C with a faint characteristic odour	Lemon- to amber-coloured oily liquid at 25 °C, with a faint characteristic odour
Identification parameter		
A. Solubility	Soluble in water, ethanol, methanol, ethyl acetate and dioxane. Insoluble in mineral oil and petroleum ether	Soluble in water, ethanol, methanol, ethyl acetate and dioxane. Insoluble in mineral oil and petroleum ether
B. Infrared absorption spectrum	Characteristic of a partial fatty acid ester of a polyoxyethylated polyol	The infrared spectrum of the sample is characteristic of a partial fatty acid ester of a polyoxyethylated polyol
C. Colour reaction	–	[test]
D. Test for fatty acids	–	[test]
E. Saponification	–	100 g of the sample yields approximately 16 g of fatty acids and 81 g of polyol
Purity		
Water	Not more than 3 % (Karl Fischer method)	Not more than 3 % (Karl Fischer method)
Acid value	Not more than 2	Not more than 2
Saponification value	Not less than 40 and not more than 50	Not less than 40 and not more than 50
Hydroxyl value	Not less than 96 and not more than 108	Not less than 96 and not more than 108
1,4-Dioxane	Not more than 5 mg/kg	–
Ethylene oxide	Not more than 0.2 mg/kg	–
Ethylene glycols (mono- and di-)	Not more than 0.25 %	–
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–
Sulphated ash	–	Not more than 0.25 %

Table 3: Commission Regulation (EU) No 231/2012 and JECFA (2006a) specifications of polyoxyethylene sorbitan monooleate (polysorbate 80) (E 433)

	Commission Regulation (EU) No 231/2012	**JECFA (2006a)**
Definition	A mixture of the partial esters of sorbitol and its mono- and dianhydrides with edible commercial oleic acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides	Consists of a mixture of the partial esters of sorbitol and its mono- and dianhydrides (which have an acid value below 7.5 and a water content below 0.2 %) with edible commercial oleic acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides
Assay	Content not less than 65 % of oxyethylene groups, equivalent to not less than 96.5 % of polyoxyethylene (20) sorbitan monooleate on the anhydrous basis	Not less than 65.0 and not more than 69.5 % of oxyethylene groups, equivalent to not less than 96.5 and not more than 103.5 % of polyoxyethylene (20) sorbitan monooleate, calculated on the anhydrous basis
Description	A lemon- to amber-coloured oily liquid at 25 °C with a faint characteristic odour	Lemon- to amber-coloured oily liquid at 25 °C, with a faint characteristic odour
Identification parameter		
A. Solubility	Soluble in water, ethanol, methanol, ethyl acetate and toluene. Insoluble in mineral oil and petroleum ether	Soluble in water, ethanol, methanol, ethyl acetate and toluene. Insoluble in mineral oil and petroleum ether
B. Infrared absorptions spectrum	Characteristic of a partial fatty acid ester of a polyoxyethylated polyol	The infrared spectrum of the sample is characteristic of a partial fatty acid ester of a polyoxyethylated polyol
C. Colour reaction	–	[test]
D. Test for fatty acids	–	[test]
E. Test for unsaturation	–	[test]
F. Gelatinisation	–	[test]
G. Saponification	–	100 g of the sample yields approximately 23 g of fatty acids and 75 g of polyols
Purity		
Water	Not more than 3 % (Karl Fischer method)	Not more than 3 % (Karl Fischer method)
Acid value	Not more than 2	Not more than 2
Saponification value	Not less than 45 and not more than 55	Not less than 45 and not more than 55
Hydroxyl value	Not less than 65 and not more than 80	Not less than 65 and not more than 80
1,4-Dioxane	Not more than 5 mg/kg	–
Ethylene oxide	Not more than 0.2 mg/kg	–
Ethylene glycols (mono- and di-)	Not more than 0.25 %	–
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg (E 432)
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–
Sulphated ash	–	Not more than 0.25 %

Table 4: Commission Regulation (EU) No 231/2012 and JECFA (2006a) specifications of polyoxyethylene sorbitan monopalmitate (polysorbate 40) (E 434)

	Commission Regulation (EU) No 231/2012	**JECFA (2006a)**
Definition	A mixture of the partial esters of sorbitol and its mono- and dianhydrides with edible commercial palmitic acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides	Consists of a mixture of the partial esters of sorbitol and its mono- and dianhydrides (which have an acid value below 7.5 and a water content below 0.2 %) with edible commercial palmitic acid condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides
Assay	Content not less than 66 % of oxyethylene groups, equivalent to not less than 97 % of polyoxyethylene (20) sorbitan monopalmitate on the anhydrous basis	Not less than 66.0 and not more than 70.5 % of oxyethylene groups, equivalent to not less than 97.0 and not more than 103.0 % of polyoxyethylene (20) sorbitan monopalmitate calculated on the anhydrous basis
Description	A lemon- to orange-coloured oily liquid or semi-gel at 25 °C with a faint characteristic odour	Lemon- to orange-coloured oily liquid or semi-gel at 25 °C, with a faint characteristic odour
Identification parameter		
A. Solubility	Soluble in water, ethanol, methanol, ethyl acetate and acetone. Insoluble in mineral oil	Soluble in water, ethanol, methanol, ethyl acetate and acetone. Insoluble in mineral oil
B. Infrared absorptions spectrum	Characteristic of a partial fatty acid ester of a polyoxyethylated polyol	The infrared spectrum of the sample is characteristic of a partial fatty acid ester of a polyoxyethylated polyol
C. Colour reaction	–	[test]
D. Test for fatty acids	–	[test]
E. Gelatinisation	–	[test]
F. Saponification	–	100 g of the sample yields approximately 20 g of fatty acids and 78 g of polyols
Purity		
Water	Not more than 3 % (Karl Fischer method)	Not more than 3 % (Karl Fischer method)
Acid value	Not more than 2	Not more than 2
Saponification value	Not less than 41 and not more than 52	Not less than 41 and not more than 52
Hydroxyl value	Not less than 90 and not more than 107	Not less than 90 and not more than 107
1,4-Dioxane	Not more than 5 mg/kg	–
Ethylene oxide	Not more than 0.2 mg/kg	–
Ethylene glycols (mono- and di-)	Not more than 0.25 %	–
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–
Sulphated ash	–	Not more than 0.25 %

Table 5: Commission Regulation No 231/2012/EC and JECFA (2014) specifications of polyoxyethylene sorbitan monostearate (polysorbate 60) (E 435)

	Commission Regulation (EU) No 231/2012	**JECFA (2014)**
Definition	A mixture of the partial esters of sorbitol and its mono- and dianhydrides with edible commercial stearic acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides	Consists of a mixture of the partial esters of sorbitol and its mono- and dianhydrides (which have an acid value below 10 and a water content below 0.2 %) with the food grade stearic acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides
Assay	Content not less than 65 % of oxyethylene groups, equivalent to not less than 97 % of polyoxyethylene (20) sorbitan monostearate on the anhydrous basis	Not less than 65.0 and not more than 69.5 % of oxyethylene groups, equivalent to not less than 97.0 and not more than 103.0 % of polyoxyethylene (20) sorbitan monostearate, on the anhydrous basis
Description	A lemon- to orange-coloured oily liquid or semi-gel at 25 °C with a faint characteristic odour	Yellow to orange-coloured oily liquid or semi-gel at 25 °C, with a faint characteristic odour
Identification parameter		
A. Solubility	Soluble in water, ethyl acetate and toluene. Insoluble in mineral oil and vegetable oils	Soluble in water, ethyl acetate, and toluene; insoluble in mineral oil and vegetable oils
B. Infrared absorptions spectrum	Characteristic of a partial fatty acid ester of a polyoxyethylated polyol (E 432)	The infrared spectrum of the sample is characteristic of a partial fatty acid ester of a polyoxyethylated polyol
C. Colour reaction	–	[test]
D. Test for fatty acids	–	[test]
E. Gelatinisation	–	[test]
F. Saponification	–	100 g of the sample yields approximately 25 g of fatty acids and 77 g of polyols
Purity		
Water	Not more than 3 % (Karl Fischer method)	Not more than 3 % (Karl Fischer method)
Acid value	Not more than 2	Not more than 2
Saponification value	Not less than 45 and not more than 55	Not less than 45 and not more than 55
Hydroxyl value	Not less than 81 and not more than 96	Not less than 81 and not more than 96
1,4-Dioxane	Not more than 5 mg/kg	Not more than 10 mg/kg
Ethylene oxide	Not more than 0.2 mg/kg	–
Ethylene glycols (mono- and di-)	Not more than 0.25 %	–
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–
Sulphated ash	–	Not more than 0.25 %

Table 6: Commission Regulation No 231/2012/EC and JECFA (2006a) specifications of polyoxyethylene sorbitan tristearate (polysorbate 65) (E 436)

	Commission Regulation (EU) No 231/2012	**JECFA (2006a)**
Definition	A mixture of the partial esters of sorbitol and its mono- and dianhydrides with edible commercial stearic acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides	Consists of a mixture of the partial esters of sorbitol and its mono- and dianhydrides (which have an acid value below 15 and a water content below 0.2 %) with edible commercial stearic acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides
Assay	Content not less than 46 % of oxyethylene groups, equivalent to not less than 96 % of polyoxyethylene (20) sorbitan tristearate on the anhydrous basis	Not less than 46.0 and not more than 50.0 % of oxyethylene groups, equivalent to not less than 96.0 and not more than 104.0 % of polyoxyethylene (20) sorbitan tristearate on the anhydrous basis
Description	A tan-coloured, waxy solid at 25 °C with a faint characteristic odour	Tan-coloured, waxy solid at 25 °C, with a faint characteristic odour
Identification parameter		
A. Solubility	Dispersible in water. Soluble in mineral oil, vegetable oils, petroleum ether, acetone, ether, dioxane, ethanol and methanol	Dispersible in water; soluble in mineral oil, vegetable oils, petroleum ether, acetone, ether, dioxane, ethanol and methanol
B. Congealing range	29–33 °C	29–33 °C
C. Infrared absorption spectrum	Characteristic of a partial fatty acid ester of a polyoxyethylated polyol (E 432)	The infrared spectrum of the sample is characteristic of a partial fatty acid ester of a polyoxyethylated polyol
D. Colour reaction	–	[test]
E. Test for fatty acids	–	[test]
F. Saponification	–	100 g of the sample yields approximately 43 g of fatty acids and 56 g of polyols
Purity		
Water	Not more than 3 % (Karl Fischer method)	Not more than 3 % (Karl Fischer method)
Acid value	Not more than 2	Not more than 2
Saponification value	Not less than 88 and not more than 98	Not less than 88 and not more than 98
Hydroxyl value	Not less than 40 and not more than 60	Not less than 40 and not more than 60
1,4-Dioxane	Not more than 5 mg/kg	Not more than 10 mg/kg
Ethylene oxide	Not more than 0.2 mg/kg	–
Ethylene glycols (mono- and di-)	Not more than 0.25 %	–
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–
Sulphated ash	–	Not more than 0.25 %

The JECFA specifications for polysorbates have an upper limit and a lower limit for the assay, whilst Commission specifications define only a lower limit. This is particularly relevant for ethylene oxide, ethylene glycol and heavy metals. The main organic impurities are discussed in section 2.9.5.

The Panel noted that according to the EC specifications for polysorbates (E 432–E 436), impurities of the toxic elements arsenic, lead, mercury and cadmium are accepted up concentrations of 3, 2, 1 and 1 mg/kg, respectively. Contamination at those levels would have a significant impact on the intake to these metals, for which the exposures are already close to the health-based guidance values established by EFSA (EFSA CONTAM Panel, 2009, 2009a, 2010, 2012a).

2.3. Manufacturing process

According to Cottrell and van Peij (2004), the most common method for producing sorbitan esters is the direct esterification of sorbitol with fatty acids using a mixture of an acidic catalyst (e.g. phosphoric acid) and a caustic soda-type catalyst to drive the reactions. In this process, blends of sorbitol and fatty acids are reacted and sorbitol is dehydrated resulting in a mixture of sorbitol, sorbitol monoanhydrides (sorbitans) and sorbitol dianhydrides (isosorbide) (Figure 2). The acidic catalyst drives the dehydration reaction while the caustic catalyst directs the esterification reaction. The authors indicate that in most commercial processes the dehydration of sorbitol and the esterification reactions occur concurrently. It is also indicated that the unique blends of catalyst systems and the reaction temperature contribute to the outcome of the reaction. Due to the variety of components in the reaction mixture and the process conditions used, the final sorbitan ester product is not a pure compound but a hetero-dispersed 'soup' of components all related to each other but dissimilar.

According to the same authors, sorbitan esters are reacted with ethylene oxide under pressure, using potassium hydroxide as a catalyst to yield polysorbates. During the reaction, the original esters are rearranged, resulting in a product with an assortment of positional isomers. The polysorbates contain an average of 20 moles of polymerised ethylene oxide per molecule, forming the hydrophilic portion of the emulsifier.

Commercial-grade fatty acids are not pure compounds but mixtures of several fatty acids. The purity of the acid can vary considerably depending on the original raw material source and the manufacturing process (Cottrell and van Peij, 2004).

Vu Dang et al. (2006) described a slightly different method in which the catalytic dehydration of sorbitol is performed at high temperature (i.e. 225–250 °C) to yield a mixture of isomers of sorbitol sorbitans and/or isosorbides. Following cyclisation, the mixture is condensed with ethylene oxide (polymerisation) and then esterified with fatty acids. Alternatively, the mixture can also be reacted with fatty acids and then condensed with ethylene oxide to produce commercial polysorbate formulations. The structural formulae of the different intermediates are shown in Figure 2.

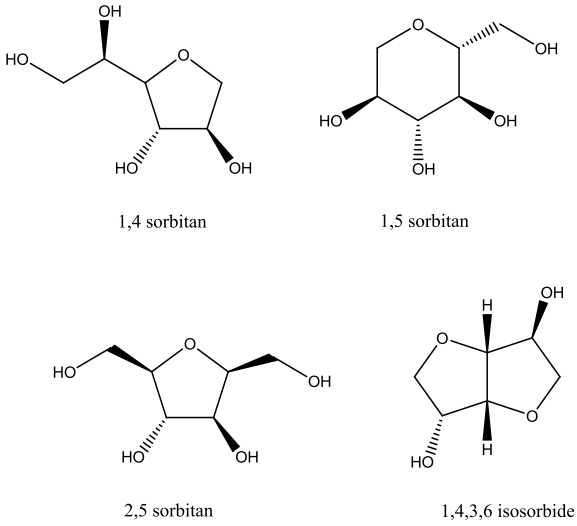


Figure 2: Structural formulae of intermediates in the synthesis of polysorbates

2.4. Methods of analysis in food

A large number of methods are available for the analysis of polysorbates in food products and other media. Analytical procedures are reviewed by Wood et al. (2004), while extraction methods are summarised in Burch et al. (2007).

In AOAC (2000) the American Organization of Analytical Chemists (AOAC) Official Method 974.11 (gravimetric method) is given for the analysis of polysorbate 60 in shortening, oils and dressings. The polysorbate is extracted from the food with chloroform, saponified with potassium hydroxide and acidified. The fatty acids are extracted with hexane. The aqueous polyol solution is desalted by ion exchange, and barium phosphomolybdate is used to precipitate the polyoxyethylated polyols which are determined gravimetrically. The method is applicable in the 0.1–1.0 % range of polysorbate 60. The method was described earlier by Smullin et al. (1971).

A gravimetric method similar to that of AOAC (2000) with slightly different extraction and purification procedures had been applied for the analysis of polysorbate 80 in bakery products and frozen desserts at levels of 0.05 % and 0.1 % (Hall, 1964). The glycol fraction is precipitated with phosphomolybdic acid in the presence of barium ions, followed by gravimetric measurements.

Alternatively, the glycol fraction can be determined gravimetrically as precipitate with silicotungstic acid (Barcklow, 1967). Recoveries from pickle relish and dill pickles containing from 0.01 to 0.02 % polysorbate 80 ranged from 80 to 98.5 %.

In a later publication, Smullin (1978) published the results of polysorbate 60 determination in non-standard salad dressings conducted in six laboratories. After extraction of the polysorbate from the food, saponification and removal of the acids, the polyoxyethylated polyols were precipitated as a highly insoluble heteropoly acid complex which was measured gravimetrically. Average recoveries from collaboration samples ranged from 105 to 130 %.

Lundquist and Meloan (1971) determined polysorbates in food products by reaction gas chromatography. The polysorbate extract from food was injected into a reactive column where the test substance is saponified and the acid salt is retained. The polyol is separated in a second column.

Kato et al. (1989) determined polysorbates in eight types of processed foods by colorimetric and thin-layer chromatography (TLC). Two different screening tests were developed: (1) after extraction of the polysorbates from the foods and purification, the extract is complexed with cobalt thiocyanate and measured photometrically at 620 nm; and (2) the extract is separated by TLC which is sprayed with

cobalt thiocyanate. The detection limit of the TLC method corresponds to 50 mg polysorbate 80/kg. The identity of the polysorbates was confirmed by infrared spectrophotometry, gas chromatography of the fatty acids and TLC of the residues after saponification.

Borrego et al. (1999) proposed a method for the determination of polysorbates in foods by formation of mixed micelles. Food samples can be analysed directly after dissolving the polysorbates from foodstuffs (baked bread, doughnuts, biscuits, butter, margarine, chocolate and noodle soup) with distilled water at room temperature. The test substance is titrated photometrically with Triton X-100, and Coomassie brilliant blue G is used for determination of critical micelle concentration values. The detection limits achieved for the polysorbates studied (polysorbates 20, 40, 60, 65 and 80) ranged from 0.05 to 0.08 µM.

Furthermore, polysorbate formulations were analysed using two-dimensional liquid chromatography (Abrar and Trathnigg, 2010), matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (Ayorinde et al., 2000) after hydrolysis and silylation by gas chromatography (Brueschweiler and Hautfenne, 1990), high-performance liquid chromatography (HPLC) of the free lauric acid after hydrolysis (Öszi and Pethö, 1998), HPLC with an ammonium cobalt thiocyanate complexation column (McKean et al., 1987), an automated fluorescence polarisation assay (Wenger et al., 2005) and shape selective mass spectrometry (Snelling et al., 2012).

2.5. Reaction and fate in food

No information on reaction and fate in food was available based on the literature searches in Toxline, Medline and SciFinder. However, information on the stability of polysorbates under different conditions is available.

Aqueous solutions of polysorbates undergo autoxidation, which occurs along the polyoxyethylene moieties and in unsaturated fatty acids (CIR, 1984; Donbrow et al., 1978; Kerwin, 2008; Yao et al., 2009). In an initiation step, an alkyl radical is formed, followed by reaction with oxygen to peroxyl radicals, which propagate the radical-chain reaction. Autoxidation is accelerated by light, elevated temperature and a copper sulphate catalyst. The degradation leads to changes in the peroxide number, pH, surface tension and cloud point.

Hydrolysis of the fatty acid ester bond results in formation of the long-chain fatty acids. The kinetics of the hydrolysis of polysorbate 80 in aqueous buffers was studied over the pH range 1.10 to 10.28. The hydrolysis was specific acid-catalysed at pH values below 3 and specific base-catalysed at pH values greater than 7.6 (Bates et al., 1973; CIR, 1984). Kishore et al. (2011) determined a half-life of 19 months at 25 °C and pH 5.5. Hewitt et al. (2011) observed that basic catalysed hydrolysis is dependent on the carbon chain length of the fatty acid, where shorter chain lengths have faster dissociation kinetics.

The relative importance of these degradation mechanisms revealed that storage of polysorbates at room temperature primarily results in hydrolysis of the fatty acid ester, while storage at higher temperatures also favours autoxidation of the polyoxyethylene chain. Storing the polysorbate solutions away from light, in the presence of nitrogen and at lower temperatures helps prevent degradation. Degradation can also be prevented by the addition of low concentrations of the antioxidant butylated hydroxytoluene (Donbrow et al., 1978; Kerwin, 2008).

2.6. Case of need and proposed uses

Maximum permitted levels (MPLs) of polysorbates (E 432- E 436) have been defined in Annex II to Regulation (EC) No 1333/2008 on food additives.

Currently, polysorbates (E 432- E 436) are food emulsifiers and stabilisers authorised in the EU with MPLs ranging from 500 to 10 000 mg/kg in foods. Further polysorbates (E 432-E 436) are authorised at *quantum satis* in food supplements.

Table 7 summarises foods that are permitted to contain polysorbates (E 432 – E 436) and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

Table 7: MPLs of polysorbates (E 432–E 436) in foods according to Annex II of Regulation (EC)

FCS Category number	Foods	Restrictions/exceptions	Maximum level (mg/l or mg/kg as appropriate)
01.4	Flavoured fermented milk products including heat-treated products		1 000
01.8	Dairy analogues, including beverage whiteners	only milk and cream analogues	5 000 [(a)]
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	only fat emulsions for baking	10 000 [(a)]
03	Edible ices		1 000 [(a)]
04.2.4.1	Fruit and vegetable preparations excluding compote	only coconut milk	500 [(a)]
05.2	Other confectionery including breath freshening microsweets	only sugar confectionery	1 000 [(a)]
05.3	Chewing gum		5 000 [(a)]
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4		1 000 [(a)]
07.2	Fine bakery wares		3 000 [(a)]
12.5	Soups and broths	only soups	1 000 [(a)]
12.6	Sauces	only emulsified sauces	5 000 [(a)]
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		1 000 [(a)]
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)		1 000 [(a)]
16	Desserts excluding products covered in categories 01, 03 and 04		3 000 [(a)]
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms		*quantum satis*
17.2	Food supplements supplied in a liquid form		*quantum satis*
17.3	Food supplements supplied in a syrup-type or chewable form		*quantum satis*

FCS: Food Categorisation System (food nomenclature) presented in the Annex II to Regulation (EC) No 1333/2008.
(a): individually or in combination.

According to Annex III to Regulation (EC) No 1333/2008, polysorbates (E 432- E 436) are also authorised to be used at *quantum satis* (*QS*) as carriers in antifoaming agents, in colours and fat-soluble antioxidants, and in glazing agents for fruit, as well as additives other than carriers, in preparations of colours, contrast enhancers, fat soluble antioxidants and glazing agents for fruit. The substances are also authorised as carriers/additives for all flavourings up to 10 000 mg/kg in the flavourings, except when used for liquid smoke flavourings and flavourings based on spice oleoresins, where a maximum carry-over level of 1 000 mg/kg in the final food has been defined. For nutrients (except nutrients intended to be used in foodstuffs for infants and young children), the polysorbates are authorised as additives/carriers for beta carotene, lutein, lycopene and vitamin E preparations at *QS* and in vitamin A and D preparations at a maximum level of 2 mg/kg in final food.

According to the Codex Alimentarius (GSFA, 2011), polysorbates are used as emulsifiers. JECFA (2006a) states the functional class of the polysorbates to be emulsifier and dispersing agents, while FCC (1996) lists stabilisers as the functional class.

Polysorbates are also used in cosmetics, pharmaceuticals, textiles, personal care products, in animal feed as well as in industrial applications (CIR, 1984; Cottrell and van Peij, 2004; EFEMA, 2009).

As regards the request for extension of use of polyoxyethylene sorbitan monooleate (E 433) as a whipping agent added to emulsifiers intended for fine bakery wares at a level of 700 mg/kg in the final food, it was assumed that no additional exposure to E 433 will result from this use, further to the exposure from its currently authorised use in fine bakery wares. The use of polysorbates (E 432-436) in fine bakery wares is currently authorised at a level of 3 000 mg/kg, individually or in combination, thus it is likely that these levels already cover the use of polysorbates in this food category.Reported use levels of polysorbates (E 432–E 436) in foodstuffs

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. For those additives for which no MPL is set and which are authorised at *QS*, information on actual use levels is required for performing an exposure assessment. This is especially true for food additives for which no MPLs are set, but are authorised according to *quantum satis (QS)*.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Regulation (EU) No 257/2010[13] regarding the re-evaluation of approved food additives, EFSA issued a public call[14] for food additives usage data on polysorbates (present use and use patterns, i.e. which food categories and subcategories are used, the proportion of foods within categories/subcategories in which it is used and actual use levels (typical and maximum use levels), especially for those uses which are only limited by *QS*) in November 2009. In addition, more use levels were reported to EFSA in 2014 by industry.

Analytical data on the content of polysorbates (E 432-436) in food were not available.

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

Information on the actual use levels of polysorbates (E 432-436) in foods was made available to EFSA by industry, including the European Food Emulsifiers Manufacturers Association (EFEMA), the Confederation of Food and Drink Industries of the EEC (CIAA; now FoodDrinkEurope-FDE), Association of the European Self-Medication Industry (AESGP), Food Supplements Europe (FSE), the Specialised Nutrition Europe (SNE) and European Federation of Associations of Health Product (EHPM). The data provided cover the majority of the food categories in which this food additive is authorised; most data were provided for food supplements (FCS 17). No data resulting from non-authorised uses has been reported to EFSA.

In total, 248 use data on 14 out of the 17 food categories in which polysorbates (E 432-436) are authorised were submitted to EFSA by the data providers mentioned above.

Use data on food supplements (FCS 17) reported as niche products (n = 3) were not included in the exposure assessment. The Panel noted a high variability of use levels within the food supplements dataset, which may be given by the form (solid to be diluted, liquid etc.) or by the specific brand of the food supplement product. Therefore, Panel considered that the dietary exposure to polysorbates (E

[13] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up the program for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19.

[14] Call for scientific data on food additives permitted in the EU and belonging to the functional classes of emulsifiers, stabilisers and gelling agents. Published 23 November 2009. Available online: http://www.efsa.europa.eu/en/dataclosed/call/ans091123.pdf

432-436) estimated at brand level would give the most reliable results. Due to lack of information on brand name and lack of FoodEx linkage on form of food supplements in the consumption data this was not possible and may have led to underestimation of the exposure.

Appendix A provides data on the use levels of polysorbates (E 432- E 436) in foods as reported by industries.

2.7. Information on existing authorisations and evaluations

The SCF (1985) allocated an ADI of 10 mg/kg bw/day (group ADI for polysorbates 20, 40, 60, 65 and 80). The basis was a NOEL of 2 % in the diet in a 90-day study in rats with polyoxyethylene sorbitan monostearate (polysorbate 60) (BIBRA, 1981). The safety factor was not specified. No details from that study were presented in this evaluation. A re-evaluation of polysorbate 80 (SCF, 1995) was performed in view of the data published by NTP (1992a) without changes to the group ADI.

A higher group ADI value of 0–25 mg/kg bw/day was allocated by JECFA (1974a, b). The group ADI for polysorbates 20, 40, 60, 65 and 80 was based on a safety factor of 100 applied to a No Observed Adverse Effect Level (NOAEL) of 5 % in the diet (equivalent to 2 500 mg/kg bw/day) from long-term feeding studies in rats. In contrast to the SCF (1985), which presented no details of the studies, JECFA described the studies used for allocation of the ADI (see also section 3.2.4).

There has also been an evaluation by the Food Safety Commission of Japan in 2007.[15] The Food Safety Commission of Japan (2007) allocated an ADI for polysorbates 20, 60, 65 and 80 of 10 mg/kg bw/day as a group using a safety factor of 100. The basis was the occurrence of diarrhoea in a study in rats fed polysorbate 60 for 13 weeks (BIBRA, 1981). The NOAEL was calculated to be 2 % in the diet (equivalent to 1 000 mg/kg bw/day).

In the EU monitoring system, the polysorbates were examined at tier 2 and moved to tier 3 (EC, 2001). In the scientific cooperation (SCOOP) task report 4.2 (EC, 1997), an estimate of chronic intake was performed using the Theoretical Maximum Daily Intake (TMDI) value, based on the EFSA dietary database.

2.8. Exposure

2.8.1. Food consumption data used for exposure assessment

2.8.1.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database[16] (Comprehensive Database) has been populated with national information on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country.

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

For calculation of chronic exposure, intake statistics have been calculated based on individual average consumption over the total survey period excluding surveys with only one day per subject. High level consumption was only calculated for those population groups where the sample size was sufficiently

[15] http://www.fsc.go.jp/english/evaluationreports/foodadditive/polysorbate_report.pdf
[16] http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

large to allow calculation of the 95th percentile of total population. The Panel estimated chronic exposure for the following population groups: toddlers, children, adolescents, adults and the elderly. Calculations were performed using individual body weights.

Thus, for the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 different European countries, as mentioned in Table 8.

Table 8: Population groups considered for the exposure estimates of polysorbates (E 432–E 436)

Population	Age range	Countries with food consumption surveys covering more than one day
Toddlers	From 12 up to and including 35 months of age	Belgium, Bulgaria, Finland, Germany, Denmark, the Netherlands, Italy, Spain, the UK
Children[17]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, the Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, the Netherlands, Spain, Sweden, the UK
Adolescents	From 10 up to and including 17 years of age	Austria, Belgium, Cyprus, the Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, the Netherlands, Spain, Sweden, the UK
Adults	From 18 up to and including 64 years of age	Austria, Belgium, the Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, the Netherlands, Romania, Spain, Sweden, the UK
The elderly[16]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, the Netherlands, Romania, Sweden, the UK

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the Food Categorisation System (FCS) as presented in the Annex II to Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, FoodEx food codes were matched to the FCS food categories and the exposure was calculated by multiplying MPLs (Table 7) or usage levels reported (Appendix A) for each food category with their respective consumption amount per kilogram body weight separately for each individual in the database. The exposure per food category was subsequently summed to derive an individual total exposure per day. Finally, these exposure estimates were averaged over the number of surveys days, resulting in an individual average exposure per day for the survey period. This was carried out for all individuals in the survey and per age group, resulting in distributions of individual average exposure per survey and population group (Table 8). Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per population group.

2.8.1.2. Food items selected for the exposure assessment of polysorbates (E 432–E 436)

The food categories in which the use of polysorbates (E 432–E 436) is authorised were selected from the nomenclature of the Comprehensive Database (FoodEx classification system codes) (EFSA, 2011b), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

Some food categories or their restrictions/exceptions are not referenced in the EFSA Comprehensive Database and therefore could not be taken into account in the present estimate. This might result in an underestimation of the exposure. The food categories which were not taken into account are described below (in ascending order of FCS code):

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

- 02.2.2 Other fat and oil emulsions, including spreads, only fat emulsions for baking.
- 05.4 Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4.

For the following food category, the restrictions which apply to the use of polysorbates (E 432–E 436) could not be taken into account, and therefore the whole food category was considered for the exposure estimations. This results in a slight overestimation of the exposure:

- 17.1/17.2/17.3 Food supplements, in solid, liquid, syrup-type or chewable form.

Overall, two food categories were not taken into account in the exposure assessment because they are not referenced in the EFSA Comprehensive Database. One food category was included in the exposure assessment without considering the restrictions/exceptions as set in Annex II to Regulation No 1333/2008. For the remaining food categories, the refinements considering the restrictions/exceptions as set in Annex II to Regulation No 1333/2008 were applied. Finally, 15 food categories were considered in the present exposure assessment to polysorbates (E 432–E 436).

Owing to the assumption that exposure from the food category of '02.2.2 Fat emulsions' could be neglected, since it corresponds to a very specific use (fat emulsions only for baking) which is already covered to a great extent in category '07.2 Fine bakery wares', there might be a slight underestimation of the exposure via this food category.

2.8.2. Exposure to polysorbates (E 432–E 436) from their use as food additives

Dietary exposure to polysorbates (E 432–E 436) from their use as food additive was estimated using the approach adopted by the Panel at its 52nd plenary meeting.[18] This approach is to be followed to assess the exposure as part of the safety assessment of food additives under re-evaluation with the use of the food consumption data available within the EFSA Comprehensive Database, as presented in Table 8, and with the limitations described above.

Exposure assessment for food additives under re-evaluation is carried out by the ANS Panel based on (1) MPLs set down in the EU legislation (defined as the *regulatory maximum level exposure assessment* scenario); and (2) the availability of adequate use levels or analytical data (defined as the *refined exposure assessment* scenario).

2.8.2.1. Regulatory maximum level exposure assessment scenario

The *regulatory maximum level exposure assessment scenario* is based on the MPLs as set in Annex II to Regulation No 1333/2008 and listed in Table 7. As no MPLs are set for food category 17 (food supplements), a maximum level exposure assessment scenario has been performed based on the maximum use levels of data provided to EFSA.

The exposure estimates derived following this scenario should be considered as the most conservative since it assumes that the consumer will be continuously (over a lifetime) exposed to polysorbates (E 432–E 436) present in the food at the MPLs.

2.8.2.2. Refined exposure assessment scenario

The refined exposure assessment scenario is based on information on reported use levels by industry and analytical results submitted to EFSA by Member States. This exposure scenario can only consider food categories where the above data were available to the Panel.

For polysorbates (E 432–E 436) no analytical levels were available; thus, the refined scenario was based only on reported use levels. Appendix B summarises the concentration levels of polysorbates

[18] http://www.efsa.europa.eu/en/events/event/140701a-m.pdf

(E 432–E 436) used in the refined exposure assessment scenario. The Panel calculated two estimates based on different model populations:

- The brand-loyal consumer scenario: This assumes that a consumer is exposed long term to polysorbates (E 432–E 436) present at the maximum reported use levels for one food category. This exposure estimate is calculated as follows:
  - Combining food consumption with the maximum of the maximum reported use levels for the main contributing food category at the individual level.
  - Using the mean of the typical reported use levels for the remaining food categories.
- The non-brand-loyal consumer scenario: This assumes that the population is exposed long term to polysorbates (E 432–E 436) present at the mean reported use levels in food. This exposure estimate is calculated using the mean of the typical reported use levels for all food categories.

In the refined exposure assessment scenarios, the concentration levels considered by the Panel were extracted from the whole dataset received. The mean of typical reported use levels for each food category was calculated. If the typical use level was reported as a range, a normal distribution of values within the food category was assumed and the mean of two values, representing lower and upper range, was calculated without considering values reported as zero at the lower range.

Food categories for which none or inadequate reported use levels were available were not considered in the exposure assessment. This concerns the following food categories:

- Flavoured fermented milk products (FCS 01.4),
- Fruit and vegetable preparations, excluding compote (FCS 04.2.4.1),
- Decorations, coatings and fillings (FCS 054.4).

The Panel noted that if polysorbates (E 432–E 436) are nevertheless used in those food categories for which reported use/analytical levels were not available, the calculated refined exposure assessment might result in underestimation of exposure to polysorbates (E 432–E 436).

2.8.2.3. Anticipated exposure to polysorbates (E 432–E 436)

Table 9 summarises the estimated exposure to polysorbates (E 432–E 436) from their use as food additives of all five population groups. The exposure estimates by age group and survey are presented in detail in Appendix C.

Table 9: Summary of anticipated exposure to polysorbates (E 432–E 436) from their use as a food additive using the regulatory maximum level exposure assessment scenario and refined exposure scenarios, in five population groups (minimum–maximum across the dietary surveys in mg/kg bw/day)

	Toddlers (12–35 months)	**Children (3–9 years)**	**Adolescents (10–17 years)**	**Adults (18–64 years)**	**The elderly (> 65 years)**
Regulatory maximum level exposure assessment scenario					
Mean	5.0–25.0	3.8–23.6	1.8–11.9	0.7–17.7	0.7–18.5
High level	19.4–58.0	11.1–63.7	5.1–31.0	2.6–41.6	2.1–47.7
Refined estimated exposure scenario					
Brand-loyal scenario					
Mean	1.4–15.0	2.2–18.1	1.1–8.5	0.6–16.9	0.6–17.3
High level	5.1–37.4	5.8–57.5	3.2–22.8	2.2–38.5	1.8–42.9

	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (> 65 years)
Non-brand-loyal scenario					
Mean	0.5–9.6	1.3–8.0	0.6–4.1	0.3–2.7	0.3–2.9
High level	2.3-24.5	3.3-20.7	1.8-10.1	1.2-7.0	1.1-7.0

2.8.3. Main food categories contributing to exposure to polysorbates (E 432–E 436) using the regulatory maximum level exposure assessment scenario

Table 10 lists the main food categories contributing the most to the total mean exposure of polysorbates (E 432–E 436) using MPLs. Only food groups with a contribution of at least 5 % are reported. Based on this scenario, the food categories 'Fine bakery wares' and 'Flavoured fermented milk products' contributed most to the exposure of polysorbates (E 432–E 436) in toddlers, and 'Fine bakery wares' and food supplements in other population groups.

Table 10: Main food categories contributing to exposure to polysorbates (E 432–E 436) using MPLs (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

FCS category no	Foods	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]				
01.4	Flavoured fermented milk products, including heat-treated products	14.2–70.4 (9)	5.4–30.3 (15)	5.6–18.2 (11)	5.6–20.1 (9)	5.2–20.6 (6)
1.8	Dairy analogues, including beverage whiteners	11.1 (1)	–	–	–	–
03	Edible ices	6.5–8.7 (2)	5.3–13.8 (8)	6.1–12.9 (7)	5.2–9.4	9.7 (1)
05.2	Other confectionery with added sugar (only sugar confectionery considered)	–	6.2–12.5 (2)	7.0–14.5 (3)	10.9 (1)	5.2 (1)
05.3	Chewing gum	–	–	7.4 (1)	6.4 (1)	–
07.2	Fine bakery wares	6.1–91.6 (10)	22.2–91.0 (17)	23.3–85.5 (16)	9.7–67.4 (17)	11.6–87.1 (14)
12.5	Soups and broths (only soups considered)	6.5–20.7 (5)	9.7–34.6 (5)	5.9–34.4 (6)	7.8–43.2 (6)	9.2–38.8 (7)
12.6	Sauces (only emulsified sauces considered)	–	5.9–14.1 (2)	5.3–17.7 (6)	5.2–21.0 (7)	6.3–18.0 (6)
16	Desserts, excluding products covered in categories 01, 03 and 04	13.0–34.7 (7)	6.6–26.5 (12)	5.2–19.4 (11)	5.4–16.7 (4)	5.8–18.0 (7)
17	Food supplements	–	12.2–71.0 (6)	10.1–60.4 (6)	13.9–80.0 (11)	5.2–82.3 (10)

(a): The total number of surveys may be greater than total number of countries as listed in Table 8, as some countries submitted more than one survey for a specific age range.

2.8.4. Main food categories contributing to exposure to polysorbates (E 432–E 436) using the refined exposure assessment scenarios

Regarding the refined scenarios, Tables 11 and 12 list the main food categories contributing the most to the total mean exposure of polysorbates (E 432–E 436) when mean typical and maximum of maximum use levels are considered. 'Fine bakery wares' was the main contributor in all age groups, followed by food supplements (brand-loyal scenario; Table 11) and soups and desserts (non-brand-loyal scenario; Table 12).

Table 11: Main food categories contributing to exposure to polysorbates (E 432–E 436) using the brand-loyal refined exposure scenario (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

FCS category no	Foods	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]				
1.8	Dairy analogues, including beverage whiteners	38.5 (1)	–	–	–	–
03	Edible ices	7.3–14.3 (3)	5.0–17.4 (6)	5.4–13.7 (5)	8.6 (1)	9.6 (1)
05.2	Other confectionery with added sugar (only sugar confectionery considered)	5.2 (1)	11.0 (1)	12.5–13.1 (2)	8.5 (1)	–
05.3	Chewing gum	–	5.1 (1)	11.4 (1)	9.1 (1)	–
07.2	Fine bakery wares	19.7–97.6 (10)	25.7–95.3 (17)	32.0–90.0 (16)	9.4–85.8 (17)	9.9–94.6 (14)
12.5	Soups and broths (only soups considered)	5.3–29.6 (6)	8.5–33.9 (5)	5.3–35.2 (7)	7.7–44.8 (6)	8.6–39.4 (7)
12.6	Sauces (only emulsified sauces considered)	7.8 (1)	19.0 (1)	5.3–22.7 (6)	6.9–24.6 (5)	5.9–19.2 (6)
16	Desserts, excluding products covered in categories 01, 03 and 04	19.7–60.1 (7)	8.0–42.6 (12)	5.1–21.6 (11)	5.3–16.6 (4)	5.7–18.5 (7)
17	Food supplements	–	15.7–88.0 (6)	11.6–77.1 (6)	15.6–84.1 (11)	6.3–87.2 (10)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 8, as some countries submitted more than one survey for a specific population.

Table 12: Main food categories contributing to exposure to polysorbates (E 432–E 436) following the non-brand-loyal exposure scenario (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

FCS category no	Foods	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]				
1.8	Dairy analogues, including beverage whiteners	5.4 (1)	–	–	–	–
03	Edible ices	5.6–20.6 (4)	5.7–32.3 (14)	5.3–18.2 (11)	5.3–12.4 (5)	7.4–13.1 (2)
05.2	Other confectionery with added sugar (only sugar confectionery considered)	5.1 (1)	10.8–28.5 (2)	12.3–42.7 (2)	6.3–8.6 (2)	–
07.2	Fine bakery wares	25.3–96.4 (10)	27.7–91.4 (17)	28.5–83.9 (16)	33.0–84.8 (17)	28.6–93.3 (14)
12.5	Soups and broths (only soups considered)	6.8–40.7 (6)	5.9–42.7 (10)	7.3–42.5 (9)	6.7–54.6 (9)	5.5–48.6 (9)
12.6	Sauces (only emulsified sauces considered)	9.9 (1)	5.5–24.2 (5)	5.5–29.9 (11)	5.8–33.2 (13)	5.9–26.4 (10)
16	Desserts, excluding products covered in categories 01, 03 and 04	5.2–62.7 (8)	5.5–48.3 (13)	5.7–32.7 (12)	5.4–29.1 (12)	5.9–35.6 (10)
17	Food supplements	–	5.1–25.3 (2)	15.7 (1)	10.4–17.3 (4)	5.4–20.5 (5)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 8, as some countries submitted more than one survey for a specific population.

Owing to lack of usage levels, 3 out of 17 food categories for which polysorbates (E 432–E 436) are authorised as a food additive could not be included in the refined exposure scenarios. In order to estimate a possible contribution of those food categories to overall exposure to polysorbates (E 432–E 436), the Panel assessed an additional MPLs scenario considering only food categories for which usage levels were available. This assessment should be considered as indicative, since the interpretation for refined scenario is based on MPLs. The exposure levels based on an additional MPLs scenario considering only food categories for which usage levels were available were, on average, 14 % lower than exposure levels based on the MPL scenario considering all food categories, ranging between from 2 % in the elderly to 32 % in toddlers. It was assumed that in cases where polysorbates (E 432–E 436) are not used in these food categories, the refined scenario could be considered as the most realistic. On the other hand, if polysorbates (E 432–E 436) are nevertheless used in these food categories, the refined scenario exposure might be underestimated.

2.8.5. Exposure to impurities

Two impurities in the polysorbates are of toxicological relevance because of their carcinogenic properties: ethylene oxide and 1,4-dioxane (see section 2.2). Ethylene oxide has been classified as 'carcinogenic to humans (Category 1)' and 1,4-dioxane has been classified as 'possibly carcinogenic to humans (Category 2B)' (see SCF, 2002a, b). These impurities in polysorbates and other food additives have been evaluated and discussed by the SCF (2002a, b), which concluded that there is no toxicological concern for 1,4-dioxane as an impurity in polysorbates with the existing maximum limit of 5 mg/kg additive (SCF, 2002b). For ethylene oxide, the SCF (2002a) recommended a revision of the existing limit of 1 mg/kg additive in the specifications to restrict ethylene oxide as an impurity to a value below its current limit of detection. This was achieved by the current specification giving a limit of ≤ 0.2 mg/kg (compare with section 2.2).

Table 13 summarises the estimated exposure to ethylene oxide in polysorbates from their use as food additives for all five population groups using MPLs when the EU specification for this impurity is met.

Table 13: Summary of anticipated exposure to ethylene oxide in polysorbates (E 432–E 436) from their use as food additives using MPLs in five population groups (ng/kg bw/day)

	Toddlers (12–35 months)	**Children (3–9 years)**	**Adolescents (10–17 years)**	**Adults (18–64 years)**	**The elderly (> 65 years)**
Mean	1.0–5.0	0.8–4.7	0.4–2.4	0.1–3.5	0.1–3.7
High level	3.9–11.6	2.2–12.7	1.0–6.2	0.5–8.3	0.4–9.5

The highest exposure to polysorbates using the MPL scenario, which was found in children (64 mg/kg bw/day) will lead to an exposure to ethylene oxide of 12.7 ng/kg bw/day when the EU specification of 0.2 mg ethylene oxide/kg polysorbate is met.

2.8.6. Uncertainty analysis

Uncertainties in the exposure assessment of polysorbates (E 432–E 436) have been discussed above. According to the guidance provided in the EFSA opinion related to uncertainties in the dietary exposure assessment (EFSA, 2007), sources of uncertainties have been considered and are summarised in Table 14.

Table 14: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate to polysorbates (E 432–E 436) to polysorbates (E 432–E 436)

Sources of uncertainties	**Direction** [(a)]
Consumption data: different methodologies/representativeness/under reporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey of few days to estimate long-term (chronic) exposure	+
Correspondence of reported use levels to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties on which precise types of food the levels refer to	+/–
Uncertainty in possible national differences in use levels of food categories, concentration data not fully representative of foods on the EU market	+/–
Food categories selected for the exposure assessment: exclusion of food categories because of missing FoodEx linkage	–
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception	+
Use levels: no data for some food categories (3/17 food categories)	–
Use levels: levels considered applicable for all items within the entire food category	+/–
Exposure assessment from food supplements: assessment done without considering brand and/or form of food supplement products	–
Regulatory maximum level scenario: exposure calculations based on the MPLs/maximum use level	+
Brand-loyal exposure model: exposure calculations based on the maximum reported use levels for one food category and mean reported use levels for the remaining food categories	+/–
Non-brand-loyal exposure model: exposure calculations based on the mean reported use levels	+/–
Use as carriers in antifoaming agents, in colours and fat-soluble antioxidants, and in glazing agents for fruit, as well as additives other than carriers, in preparations of colours, contrast enhancers, fat soluble antioxidants and glazing agents for fruit not considered in exposure assessment	–

Sources of uncertainties	Direction [a]
Authorisation as carriers/additives in flavourings not considered	–
Use as additives/carriers for some nutrient preparations not considered	–
Impurity exposure calculations: exposure calculations based on the MPLs/maximum use level and maximum level of impurity as given in specifications	+

(a): +, uncertainty with potential to cause over-estimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Overall, the Panel considered that the uncertainties identified would tend to an overestimation of the real exposure to polysorbates (E 432–E 436) as food additives in European countries by the MPL scenario but might underestimate real exposure by the refined scenarios.

3. Biological and toxicological data

New data were found in the literature search in the databases Toxline, Medline and SciFinder. Details are presented in the following sub-sections.

Polysorbates are all mixtures (see section 2.2); in the study reports, details of the composition or analytical data for the test substance were usually not provided. An exception is the long-term feeding study in rats and mice by NTP (1992a; details in section 3.2.4) with polysorbate 80. The impurities of toxicological relevance and their maximum concentrations in the polysorbates are documented in section 2.2 and are evaluated in section 2.10.5.

3.1. Absorption, distribution, metabolism and excretion

The studies cited in this section were already reported/included in the evaluation of JECFA (1974a). No new data were found in the literature search in the Toxline, Medline and SciFinder databases.

Studies on metabolic fate and elimination after oral administration are available for radio-labelled polysorbate 20 (Nelson et al., 1966; Treon et al., 1967), polysorbate 80 (Treon et al., 1967) and polysorbate 60 (Wick and Joseph, 1956) (Table 15). Studies on the other polysorbates were not available but the Panel considered that similar absorption, distribution, metabolic fate and elimination can be expected from polysorbates, as the molecules differ in only their fatty acid chains.

Labelling was performed at different parts of the structure of polysorbates, i.e. in the fatty acid moiety, polyoxyethylene and sorbitan moiety. The position of the label corresponded with clear differences in absorption from the gastro-intestinal tract and elimination pattern. An overview on the elimination of the radioactivity following oral dosing is presented in Table 15.

Table 15: Elimination of radioactivity as a percentage of applied dose in rats after oral exposure to ^{14}C-polysorbates

Substance	Dose (g/kg bw) (number of rats) [a]	Observation period (hours)	Elimination as a percentage of the applied dose					Reference
			CO_2	Urine	Faeces	Carcass	Total	
^{14}C-labelling of the fatty acid moiety								
Polysorbate 20	1 (1m and 1f, fasted)	12	80	3	5	16	104	Nelson et al. (1966) [b]
	1 (1m and 1f, fasted)	24	82	3	4	13	102	Nelson et al. (1966) [b]
	1 (1m, non-fasted)	12	81	2	3	13	99	Nelson et al. (1966) [b]
	1 (1f, non-fasted)	12	64	3	5	25	97	Nelson et al. (1966) [b]
	1 (1m, non-fasted)	24	81	3	3	13	100	Nelson et al. (1966) [b]

Substance	Dose (g/kg bw) (number of rats) [(a)]	Observation period (hours)	Elimination as a percentage of the applied dose					Reference
			CO_2	Urine	Faeces	Carcass	Total	
	1 (1f, non-fasted)	24	56	2	4	31	93	Nelson et al. (1966) [(b)]
Polysorbate 20	1 (2m and 2f, fasted)	24	81	3	4	15	103	Treon et al. (1967) [(c)]
^{14}C-labelling of the polyoxyethylene moiety								
Polysorbate 20	1 (1m and 1f, fasted)	12	nd	11	82	2	95	Nelson et al. (1966) [(b)]
	1 (1m and 1f, fasted)	24	nd	9	92	nd	101	Nelson et al. (1966) [(b)]
	1 (1m and 1f, non-fasted)	12	nd	8	85	3	96	Nelson et al. (1966) [(b)]
	1 (1m, non-fasted)	24	nd	8	91	nd	99	Nelson et al. (1966) [(b)]
Polysorbate 20	1 (2m and 2f, fasted)	24	nd	10	87	1	98	Treon et al. (1967) [(c)]
^{14}C-labelling of the sorbitan moiety								
Polysorbate 80	0.5 (2m and 2f, fasted)	24	nd	2	91	2	95	Treon et al. (1967)
Polysorbate 60	0.25 (1)	48	7	9	67	nm	83	Wick and Joseph (1956)
Polysorbate 60	0.5 (1)	48	2	7	93	nm	102	Wick and Joseph (1956)
Polysorbate 60	1 (1)	48	3	6	73	nm	82	Wick and Joseph (1956)
Polysorbate 60	1 (2)	24	nd	9	82	nm	91	Wick and Joseph (1956)

nd: not detected in corresponding measurements.
nm: not measured.
(a): m: male; f: female plus data on fasting if available.
(b): In these experiments, radioactivity in the faeces also included the content of the gastro-intestinal tract after sacrifice and radioactivity in the urine also included the content of the bladder; radioactivity in the carcass included the liver.
(c): Radioactivity in the carcass included the liver.

Twenty fasted and non-fasted rats were administered by gavage a 40 % aqueous solution of ^{14}C-labelled polysorbate and placed for 12 or 24 hours in metabolism cages (Nelson et al., 1966) (see also Table 15). Fasting had no pronounced effect in this study. The radioactivity was completely absorbed using the fatty acid labelling. Only small amounts (3–5 % of the applied radioactivity) were found in the faeces. In non-fasted females, but not in non-fasted males, there was a slight decrease in exhalation of radioactivity, as $^{14}CO_2$ was detected, suggesting some deceleration of the fatty acid metabolism. In contrast, studies with the polyoxyethylene-labelled polysorbate 20 found excretion mainly via the faeces (82–92 % of applied radioactivity). The authors concluded that the ester bond in polysorbate 20 is easily hydrolysed in the rat intestine and the fatty acid moiety is absorbed and metabolised. As regards the polyoxyethylene moiety, in contrast, major amounts passed through the gastro-intestinal tract and only minor amounts were absorbed (no data are available about metabolism in the intestinal tract). Studies in rats after intravenous (i.v.) injection of labelled polysorbate 20 showed excretion of small amounts via the bile (see below; Nelson et al., 1966). The Panel noted the small number of animals tested (one or two per dose), which does not allow any estimation of the variability in absorption or elimination.

These results were supported by those of Treon et al. (1967) using the same experimental design. Data on fasted rats and non-fasted rats were similar to a certain extent: the labelled lauric acid moiety of polysorbate 20 was rapidly absorbed and oxidised by rats. After 24 hours, 81 % of the radioactivity was expired as $^{14}CO_2$ derived from ^{14}C-labelled polysorbate; only 4 % was not absorbed from the

alimentary tract. In contrast, when the polyoxyethylene moiety of polysorbate 20 was labelled, no radioactivity was found in exhaled air but 87 % was found in faeces; the absorbed radioactivity was excreted via urine (10 %; see Table 15).

Treon et al. (1967) also studied the elimination of radioactivity after gavage administration of ^{14}C-polysorbate 80 labelled at the sorbitan moiety (see Table 15). The elimination pattern is similar to that of polysorbate 20 labelled at the polyoxyethylene moiety. Minor amounts of radioactivity were absorbed (2 % in urine and 2 % in carcass); no radioactivity was exhaled or found in the liver, kidney, spleen, adrenals, brain, gonads or fat, but 91 % of the radioactivity was recovered in the faeces. Studies with polysorbate 80 labelled via the oleic acid revealed similarities with polysorbate 20 also labelled via the fatty acid (no details provided, Treon et al., 1967).

In an earlier study by Wick and Joseph (1956), the sorbitan moiety of polysorbate 60 was ^{14}C-labelled. Like the study of Treon et al. (1967), radioactivity was found mainly in the faeces and minor amounts were found in the urine (see Table 15). Unexpectedly, 2–7 % of applied radioactivity was exhaled as $^{14}CO_2$. Nelson et al. (1966) suggested that, in the study performed by Wick and Joseph (1956), unreacted labelled sorbitan monostearate could have undergone intestinal hydrolysis and metabolism to produce some $^{14}CO_2$.

Nelson et al. (1966) investigated in rats the elimination of the ^{14}C-radioactive label after i.v. injection of 0.5 g/kg bw ^{14}C-labelled polysorbate 20. The results were discussed by the authors as an indication that the ester bond is hydrolysed by blood lipases. When polysorbate 20 labelled at the fatty acid moiety was injected, the labelled lauric acid was metabolised and appeared mostly as expired CO_2 (61–70 % of applied radioactivity). In contrast, using polysorbate 20 with labelling of the polyoxyethylene moiety, radioactivity was mainly in urine (80–87 %), with none recovered as CO_2. The presence of radioactivity in the faeces (about 2 % with fatty acid-labelled polysorbate and 11 % with polyoxyethylene-labelled) suggested excretion via bile. Similar results were presented by Treon et al. (1967) using the same experimental design.

3.1.1. Human studies

Clinical studies with polysorbate 80 in four hospitalised volunteers showed a similar elimination pattern to the rat (Culver et al., 1951). Faeces and urine were quantitatively sampled during a 12-day treatment period and a 6-day post-exposure observation period. The polyoxyethylene moiety in urine and faeces was analysed by measurement of its oxyethylene value. Each volunteer received 4.5 g of unlabelled polysorbate 80 per day in capsules. The measured data were corrected for blank values determined in urine collected from each volunteer six days prior to medication. The total recovery in urine and faeces was 93–99 %. Only 2.3 to 3.1 % was found in urine and 90–97 % in faeces. No fatty acids containing the polyoxyethylene moiety were detected in the urine; the authors concluded that the polyoxyethylene moiety in the urine represented polyoxyethylene sorbitan rather than the parent ester. The authors suggested that polysorbate 80 could be hydrolysed by pancreatic lipase with the oleic acid liberated following the normal metabolic pathways for fatty acids. Minor amounts of polyoxyethylene were absorbed in the intestinal tract after hydrolysis and excreted in the urine. It was not possible with the methodology used to identify the polyoxyethylene moiety in the faeces. The elimination of ingested polysorbate 80 was complete, indicating no storage of the polyoxyethylene sorbitan moiety in the body (Culver et al., 1951). The method could not distinguish between the free polyoxyethylene moiety and the unhydrolysed parent ester.

From the animal studies using labelling at different parts of the structure of polysorbates, the following conclusions for rats and humans could be derived:

- The ester bond between polyoxyethylene and fatty acids can be hydrolysed in the gastro-intestinal tract; based on i.v. data, similar hydrolysis can occur in the blood.
- Fatty acids released from polysorbates are absorbed, metabolised and excreted in the same way as dietary fatty acids.

- Based on the similarity of the excretion in urine between compounds labelled in the polyoxyethylene and sorbitan moiety, cleavage of the polyoxyethylene and sorbitan bond does not occur.
- Only small amounts of polyoxyethylene sorbitans are absorbed.
- Similar toxicokinetics would be expected for all polysorbates.

3.2. Toxicological data

3.2.1. Acute oral toxicity

The studies cited in this section were already presented in the evaluation of JECFA (1974a,b), with the exception of the studies of Bartsch et al. (1976) and Varma et al. (1985), which were identified in literature searches using Toxline, Medline and SciFinder databases.

The lethal dose, 50 % (LD_{50}), for polysorbate 20 was found to be > 20 g/kg bw in mice (Hopper et al., 1949; Bartsch et al., 1976), > 30 mg/kg bw in rats (Eagle and Poling, 1956; Bartsch et al., 1976; Brandner, 1973, unpublished report cited in JECFA, 1974a) and 19.8 mg/kg bw in hamsters (Eagle and Poling, 1956). The LD_{50} for polysorbate 80 was found to be > 11 g/kg bw (Varma et al., 1985) and > 25 g/kg bw in mice (Hopper et al., 1949) and 59.6 mg/kg bw (Eagle and Poling, 1956) and > 11 g/kg bw (Varma et al., 1985) in rats. The LD_{50} for polysorbates 40, 60 and 65 was found to be > 38 g/kg bw in rats (Brandner, 1973, unpublished report cited in JECFA, 1974a).

In conclusion, the acute oral toxicity of all polysorbates is low: 10–60 g/kg bw. No mortality occurred in different rodent species at high dose levels. Although the available data have limitations, the database is sufficient for the evaluation of this endpoint.

3.2.2. Short-term and subchronic toxicity

Several studies in different species are available. Most earlier studies were already documented in the evaluation of JECFA (1974a), with some described as unpublished reports (original reports not available). The literature searches using Toxline, Medline and SciFinder databases resulted in further studies with new information. A summary of these studies is given in Table 16.

Table 16: Summary of subacute and subchronic oral toxicity in experimental animals exposed to polysorbates

Substance (species, strain, sex [(a)], n [(b)])	Duration	Dosing information	NOAEL [(c)]	Investigated parameters/effects	Reference
Polysorbate 20 (mouse, C57BL/6J, m&f, 6) (results combined for m&f)	4 weeks	0 or 1.6 g/kg bw/day; gavage; preliminary high-fat diet for 4 weeks	na	**1.6 g/kg bw/day:** total cholesterol ↓, HDL and triglyceride ↓	Li et al. (2011)
Polysorbate 20 (rat, nd, nd, nd)	nd	0 or 10 % in a 20 % casein high-sucrose diet	10 %	**10 %:** diarrhoea, growth retardation, sucrase activity in jejunum ↓. All effects inhibited by the addition of dietary fibre	Kimura and Yoshida (1982)
Polysorbate 20 (rat, Wistar, m)	7 days	0 or 10 % in the diet (with or without dietary fibre)	10 %	**10 %:** diarrhoea, body weight gain ↓, jejunum sucrase activity ↓; no such effects after diet with 10 % dietary fibre	Nakata and Kimura (1994)

Substance (species, strain, sex [a], n [b])	Duration	Dosing information	NOAEL [c]	Investigated parameters/effects	Reference
Polysorbate 20 (rat, nd, nd, nd)	8 weeks	0, 3 or 5 % in the diet	na	**≥ 3 %:** weight gain ↓, diarrhoea **≤ 5 %:** no effects in gross and microscopic pathology	Krantz (1943a) [d], cited in JECFA (1974a)
Polysorbate 20 (rat, Sprague–Dawley, m, 13–14)	59 days	0 or 25 % in the diet	na	**25 %:** severe diarrhoea, mortality, but diet without dietary fibre	Harris et al. (1951a)
Polysorbate 20 (rat, nd, nd, 8–10/group)	21 weeks	0 or 25 % in the diet (mainly bread)	na	**25 %:** severe diarrhoea; histopathology: stones in bladder and kidney, hypertrophy of kidney, enlarged caecum, atrophy of testis	Eagle and Poling (1956)
Polysorbate 20 (rat, Holtzmann, m, 10)	21 weeks	0 or 25 % in the diet (2 % agar)	na	**25 %:** diarrhoea, body weight ↓, water consumption ↑, no effect on mortality	Poling et al. (1956)
Polysorbate 20 (hamster, m&f, 13–23)	68 days	0, 5 or 15 % in the diet	na	**≥ 5 %:** severe diarrhoea, mortality, but high-sucrose diet containing 3 % dietary fibre	Harris et al. (1951b)
Polysorbate 40 (mouse, C57BL/6J, m&f, 6) (results combined for m&f)	4 weeks	0 or 1.6 g/kg bw/day; gavage; preliminary high-fat diet for 4 weeks	na	**1.6 g/kg bw/day:** total cholesterol ↓, HDL and triglyceride ↓	Li et al. (2011)
Polysorbate 60 (mouse, C57BL/6J, m&f, 6) (results combined for m&f)	4 weeks	0 or 1.6 g/kg bw/day; gavage; preliminary high-fat diet for 4 weeks	na	**1.6 g/kg bw/day:** total cholesterol ↓, HDL and LDL ↓, triglyceride ↓	Li et al. (2011)
Polysorbate 60 (mouse, nd, m&f, 6–13 mice/group) (sex not specified)	3–4 months	0, 2.5, 5 or 10 % in the diet	5 %	**10 %:** diarrhoea **≤ 10 %:** no effects on body and organ weights and in histopathology (but colony infection)	Brush et al. (1957)
Polysorbate 60 (rat, nd, nd, n.d.)	nd	0 or 10 % in a 20 % casein high-sucrose diet	10 %	**10 %:** diarrhoea, growth retardation, sucrase activity in jejunum ↓. All effects inhibited by the addition of dietary fibre	Kimura and Yoshida (1982)
Polysorbate 60 (rat, Sprague–Dawley, m, 6)	14 days	0 or 15 % in the diet	15 %	**15 %:** no effects on body weight gain using a diet with dietary fibre but diarrhoea and mortality without dietary fibre	Ershoff and Marshall (1975)

Substance (species, strain, sex [a], n [b])	Duration	Dosing information	NOAEL [c]	Investigated parameters/effects	Reference
Polysorbate 60 (rat, Sprague–Dawley, m, 12)	14 days	0 or 4 % in the diet	4 %	**4 %:** no effects on body weight gain using a diet with dietary fibre but diarrhoea and decreased body weight without dietary fibre	Ershoff (1976)
Polysorbate 60 (rat, nd, m, nd)	8 weeks	2 or 5 % in the diet, no data about control	na	**≤ 5 %:** no toxic symptoms	Krantz (1943b) [d], cited in JECFA (1974a)
Polysorbate 60 (rat, nd, m&f, 12)	10 weeks	0, 5 or 15 % in the diet	15 %	**≤ 15 %:** no effects on body, no clinical signs; no effects at necropsy and histopathology (normal diet)	Chow et al. (1951, 1953)
Polysorbate 60 (rat, nd, m&f, 12)	10 weeks	0 or 5 % in the diet	na	**5 %:** diarrhoea and body weight ↓, but related to basal casein diet	Chow et al. (1951, 1953)
Polysorbate 60 (rat, nd, m&f, 12)	12 weeks	0 or 25 % in the diet (standard diet)	na	**25 %:** body weight gain in m ↓ (not in f), no effects on food consumption or efficiency	Fitzhugh et al. (1959)
Polysorbate 60 (rat, Sprague–Dawley, m&f, 48 control, 24 test groups)	13 weeks	0, 1.0, 2.0 or 5.0 % in the diet	2 % (m, 1 355 mg/kg bw/day; f, 1 565 mg/kg bw/day; m/f, 1 460 mg/kg bw/day)	**5 %:** diarrhoea, increased water intake, enlarged caecum, haemoglobin slightly decreased	BIBRA (1981) [e]
Polysorbate 60 (rat, nd, m&f, 12)	14 weeks	0 or 5 % in the diet	na	**5 %:** diarrhoea and body weight ↓, but related to basal casein diet	Chow et al. (1953)
Polysorbate 60 (rat, nd, m&f, 12)	14 –16 weeks	0, 5 or 15 % in the diet (with dietary fibre)	15 %	**15 %:** no effects on body weight and food consumption, no clinical signs; no effects at necropsy and histopathology (normal soybean diet); similar results in old rats given 5 % in the diet	Chow et al. (1953)
Polysorbate 60 (rat, nd, nd, nd)	15 weeks	0 or 25 % in the diet	na	**25 %:** diarrhoea and body weight ↓, no effects in haematology, necropsy or histopathology of 'important viscera'	Krantz (1949) [d], cited in JECFA (1974a)
Polysorbate 80 (mouse, B6C3F1, m&f, 5)	14 days	0, 0.3, 0.6, 1.25, 2.5 or 5 % in the diet	5 %	**≤ 5 %:** no clinical signs, no effects on body weight and survival or organ weights; necropsy negative	NTP (1992a)

Substance (species, strain, sex [a], n [b])	Duration	Dosing information	NOAEL [c]	Investigated parameters/effects	Reference
Polysorbate 80 (mouse, C57BL/6J, m&f, 6) (results combined for m&f)	4 weeks	0, 0.4, 1.6 or 6.4 g/kg bw/day; gavage; preliminary high-fat diet for 4 weeks	na	**≥ 0.4 g/kg bw/day:** total cholesterol ↓, HDL and LDL ↓; histopathology of preliminary high-fat diet in liver ameliorated	Li et al. (2011)
Polysorbate 80 (mouse, Swiss, nd, nd)	Up to 10 weeks	0 or 0.35 % in liquid diet	na	**0.35 %:** hepatotoxic effects in light and electron microscopy; insufficient documentation	Reyniers et al. (1985)
Polysorbate 80 (mouse, B6C3F1, m&f, 10)	13 weeks	0, 0.31, 0.62, 1.25, 2.5 or 5 % in the diet	5 % (10 000 mg/kg bw/day)	**≤ 5 %** [f]**:** no clinical signs, no effects on body weight and food consumption (body weight gain in 2.5 % males ↓), survival or organ weights; necropsy and histopathology negative	NTP (1992a)
Polysorbate 80 (rat, F344, m&f, 5)	14 days	0, 0.3, 0.6, 1.25, 2.5 or 5 % in the diet	2.5 %	**5 %:** body weight in m ↓ **≤ 5 %:** no clinical signs, no effects on survival or organ weights; necropsy negative	NTP (1992a)

nd, no data; na, not applicable; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
(a): m, male; f, female.
(b): Number of animals/sex/dose.
(c): The NOAEL was derived by the Panel (EFSA, 2012), except for the study of BIBRA (1981), in which the values for the NOAELs were given by the authors and the Panel agreed with this NOAEL. In this study, feed intake was measured and the intake was calculated by the authors.
(d): Unpublished report (not available to the Panel).
(e): Clinical signs, body weight, food and water intake, haematology and clinical chemistry and urine analysis.
Organ weights of adrenals, brain, caecum, heart, kidneys, liver, gonads, spleen (including histology).
In addition, histopathology of controls and high-dose animals: adipose tissue, adrenals, aorta, urinary bladder, brain, caecum, colon, diaphragm, epididymis, Harderian gland, lung, lymph nodes (axillary, cervical and mesenteric), mammary gland, sciatic nerve, oesophagus, pancreas, pituitary, prostate, rectum, salivary gland, seminal vesicles, skeletal muscle, skin, small intestine, spinal cord, stomach, thymus, thyroid, trachea, uterus, vagina and vein.
(f): Histopathology of controls and high-dose animals: trachea, urinary bladder, uterus, stomach, testes, thymus, thyroid gland, salivary gland, small intestine, spleen, clitoral or preputial gland, pituitary gland, prostate gland, turbinates, ovary, pancreas, parathyroid, mandibular or mesenteric lymph node, nasal cavity, bronchi, mammary gland, heart, kidneys, liver, lung, regional lymph nodes, gall bladder (mice), gross lesions, colon, oesophagus, bone marrow (femur), brain, adrenal gland; organ weights of brain, heart, right kidney, liver, lung, testes, thymus.

There was no indication of toxicological differences between the five polysorbates in the studies summarised in Table 16. However, studies conducted in accordance with current or comparable guidelines were not available; the documentation of the presented studies was not sufficient for evaluation (e.g. data from secondary literature), only limited parameters were investigated or specialised methodological approaches were used.

The most valid studies were performed by BIBRA (1981) and NTP (1992a). From the subchronic study by the British Industrial Biological Research Association (BIBRA) in rats with polysorbate 60, a NOAEL of 2 % in the diet equivalent to 1 460 mg/kg bw/day in rats could be derived. At a concentration of 5 % in the diet, diarrhoea, increased water intake, enlarged caecum and slightly decreased haemoglobin concentrations were observed. From the National Toxicology Program (NTP)

subchronic studies in mice and rats with polysorbate 80, a NOAEL of 5 % in the diet equivalent to 10 000 mg/kg bw/day in mice and 4 500 mg/kg bw/day in rats was derived by the Panel. It should be noted that neither haematology nor clinical chemistry analysis was performed in the latter study.

Diarrhoea was the only effect observed at concentrations ≥ 5 % polysorbates in feed. However, this effect was related to the composition of the diet. The first evidence for this hypothesis came from Chow et al. (1951, 1953; see Table 16). The protective effects of dietary fibre were studied by Ershoff and Marshall (1975) and Ershoff (1976) using polysorbate 60. Kimura and co-workers investigated the mechanism of these protective effects (Kimura et al., 1982; Nakata and Kimura, 1994). Their results suggested that polysorbates in diets without dietary fibre exfoliated or damaged the brush border membrane of the small intestine, inducing diarrhoea and reduced body weight (Kimura et al., 1982; see section 3.2.6). The addition of dietary fibre reduced these effects.

Adverse effects in hamsters observed at a dose level of 5 % polysorbate 20 in the diet (equivalent to approximately 5 000 mg/kg bw/day according to Gold et al. (1984) and Langkilde et al. (2012)) in a subchronic feeding study (Harris et al., 1951b) are of questionable toxicological relevance owing to the use of a high-sucrose diet (61 %).

In conclusion, numerous subacute and subchronic oral studies were available for the polysorbates. Although these studies were not performed in accordance with current or comparable guidelines, the results support one another by indicating diarrhoea as the only observed effect. This effect can be avoided by changing the composition of the diet by adding extra fibre. Most valid studies for the evaluation of subchronic toxicity were available for polysorbate 80 and polysorbate 60. Subchronic studies with polysorbate 80 suggested NOAELs of 5 % in the diet for mice (equivalent to 10 000 mg/kg bw/day) and rats (equivalent to 4 500 mg/kg bw/day) (NTP, 1992a). In the most valid subchronic dietary study in rats, there is evidence for a NOAEL of 2 % polysorbate 60 in the diet, equivalent to 1 460 mg/kg bw/day (BIBRA, 1981). In addition, when these NOAELs were compared with those of the various other studies (Table 16), a similar order of magnitude was obtained for all NOAELs.

3.2.3. Genotoxicity

The literature search in Toxline, Medline and SciFinder resulted in several studies on genotoxicity *in vitro* which were published after the evaluation of JECFA (1974a).

3.2.3.1. Genotoxicity *in vitro*

A summary of all the available studies on genotoxicity *in vitro* is given in Table 17.

Table 17: Genotoxicity of polysorbates *in vitro*

Substance and test system	Tested organisms	Tested concentrations vehicle[(a)]	Cytotoxic concentration	Results –MA	Results +MA	Validity	Reference
Gene mutation in bacteria							
Polysorbate 60 Ames test	*Salmonella typhimurium* TA98, TA100	0.01–2 mg/plate nd	nd	Negative	Negative	Limited[(b)]	Inoue et al. (1980)
Polysorbate 60 Ames test	*S. typhimurium* TA98, TA100	0.01–1 mg/plate water	nd	Negative	Negative	Limited[(b)]	Morita et al. (1981)

Substance and test system	Tested organisms	Tested concentrations vehicle[a]	Cytotoxic concentration	Results –MA	Results +MA	Validity	Reference
Polysorbate 60 Ames test	*S. typhimurium* TA98, TA100	0.01–5 mg/plate water	Tested up to 5 mg/plate	nd	Negative[c]	Limited[b]	Sunakawa et al. (1981)
Polysorbate 80 Ames test	*S. typhimurium* TA100, TA1535, TA1537, TA98	0.1–10 mg/plate buffer	Tested up to 10 mg/plate	Negative	Negative	Limited Sufficient [d]	NTP (1992a)
Polysorbate 80 Ames test	*S. typhimurium* TA98, TA100	0.01–1 mg/plate DMSO	nd	Negative	Negative	Limited [b]	Morita et al. (1981)
Polysorbate 80 Ames test	*S. typhimurium* TA98, TA100	nd	nd	Negative	Negative	Limited [b,e]	Zhong et al. (1997)
Gene mutation in mammalian cells							
Polysorbate 20 mouse lymphoma	L5178Y TK+/– cells	nd	Yes, but no details	Negative	Negative	Limited (abstract only)	Coppinger et al. (1981)
Chromosome mutation							
Polysorbate 60 chromosome aberration test	Chinese hamster fibroblast cells	Up to 0.2 mg/mL PS	Tested up to cytotoxic dose levels	Negative	nd	Sufficient Limited [f]	Ishidate and Odashima (1977)
Polysorbate 80 chromosome aberration test	Chinese hamster fibroblast cells	Up to 0.1 mg/mL PS	Tested up to cytotoxic dose levels	Negative	nd	Sufficient Limited [f]	Ishidate and Odashima (1977)
Transformation in mammalian cells							
Polysorbate 60 cell transformation	Primary golden hamster embryo cells	0.01–0.3 mg/mL DMSO	Cytotoxic effects at 0.3 mg/mL	Negative	Negative	Sufficient	Inoue et al. (1980)
DNA damage							
Polysorbate 20 SOS chromotest	*Escherichia coli* PQ37	Up to 2 mg/assay DMSO	> 2 mg/assay but data not shown	Positive	Positive	Limited [g]	Odunola et al. (1998)
Polysorbate 60 Rec assay	*Bacillus subtilis*	0, 0.05, 0.5, or 5 mg/disc DMSO	nd	Negative	nd	Limited	Morita et al. (1981)
Polysorbate 80 Rec assay	*B. subtilis*	0, 0.05, 0.5, or 5 mg/disc DMSO	nd	Negative	nd	Limited	Morita et al. (1981)

nd, no data available; MA, metabolic activation system.
(a): DMSO, dimethylsulphoxide; PS, physiological saline.
(b): Not tested in all strains recommended in current guidelines.
(c): Various MA systems and norharman used.

(d): Not tested in *E. coli* WP2 or TA102, but no cross-linking or oxidising activity expected.
(e): Polysorbate 80 used as a negative control, no concurrent untreated control.
(f): Related to results without MA.
(g): No data about galactosidase activity in controls; no data about range in triplicate assay.

All *in vitro* genotoxicity studies gave negative results with the exception of a DNA damage assay in bacteria by Odunola et al. (1998) with polysorbate 20. However, in this SOS chromotest, the information on the negative control is insufficient for the assessment of the treatment group. Furthermore, the number of replications that were tested is not indicated and the interpretation of results in this publication cannot be followed easily. Moreover, the Panel noted that the SOS chromotest is not validated and is not considered by current guidelines.

Valid data on gene mutation in bacteria were presented in the study of NTP (1992a), although not all strains were tested.

Coppinger et al. (1981) reported negative results for gene mutations in mammalian cells. However, the information on the study is limited, with only one abstract available. Data presented on a chromosome aberration test by Ishidate and Odashima (1977) are limited for evaluation because no trials were performed with a metabolic activation system. Polysorbate 60 did not induce cell transformation (Inoue et al., 1980).

3.2.3.2. Genotoxicity *in vivo*

In a very limited bone marrow micronucleus test (Jenssen and Ramel, 1980), three male CBA mice per group received 0 or 75 mg/kg bw/day polysorbate 80; however, the exposure route was not given. Negative results were obtained when micronuclei in polychromatic erythrocytes were analysed 30 hours after treatment.

In conclusion, no mutagenic activity was reported in a limited gene mutation assay in bacteria with polysorbate 80 (NTP, 1992a). This test was limited owing to the absence of testing in *S. typhimurium* TA 102 or *E. coli* WP2 strains. However,the evaluation of structural alerts for genotoxicity in polysorbates with the QSAR Toolbox 3.2 did not highlight alerts for DNA reactivity (profilers '*DNA binding by OECD*' and '*DNA binding by OASIS*'), *in vitro* genotoxicity (profilers '*Alerts for Ames, chromosomal aberrations and micronuclei by Oasis 1.2*' and '*in vitro mutagenicity by ISS*') and carcinogenicity (profiler '*Carcinogenicity (genotoxic and nongenotoxic) by ISS*').

Two alerts were detected by the profiler '*in vivo mutagenicity (micronucleus) alerts by ISS*', namely the 'Hacceptor-path3-Hacceptor' and 'Oxolane'. The 'Hacceptor-path3-Hacceptor' refers to the possibility of non-covalent binding to DNA or proteins as a result of the presence of two bonded atoms connecting two hydrogen bond acceptors. However, the Panel noted that the positive predictivity of such alerts for *in vivo* genotoxicity was quite low, ranging from 'none' (34 %) to just 63 % depending on the database, with a high incidence of false positives (Benigni et al., 2010, 2012).

Concerning the 'Oxolane' alert, the Panel noted that the oxolane (tetrahydrofuran) moiety represents the chemical skeleton of biological important aldopentoses, including cyclic sorbitol, and that the alleged positive of this structure for the *in vivo* micronucleus test is secondary to the presence of the oxolane moiety in the nucleoside-analogue drugs included in the ISSMIC database. Substances bearing the oxolane moiety and that were positive in the *in vivo* micronucleus were in fact nucleoside analogues able to inhibit DNA polymerase function and/or to be incorporated into DNA as fraudulent nucleosides (i.e. azidothymidine, 8-chloroadenosine monophosphate, 2,3 dideoxyadenosine, 5-azacytidine, ribavirin, cytarabine hydrochloride, 2,3-dideoxycytidine). On the other hand, such activity is not associated with simple oxolanes, e.g. ribose, and not mechanistically plausible for polysorbates, which are structurally unrelated to nucleosides.

Taking into account the overall information on structure–activity relationships, the Panel concluded that, despite the limited database, polysorbates do not give rise to concerns for genotoxicity.

3.2.4. Chronic toxicity and carcinogenicity

Studies on different polysorbates were performed in rats, mice, hamsters, monkeys and dogs. Data are available for all polysorbates except polysorbate 40. Most of the earlier studies were already documented in the evaluation of JECFA (1974a), with some being unpublished reports (original reports not available). The literature search in Toxline, Medline and SciFinder resulted in further studies presenting new information, which are summarised in Table 18. Data on tumour promotion and co-carcinogenic effects in rats and mice after oral exposure are also presented in the following section.

Studies on carcinogenic effects after dermal exposure are summarised in CIR (1984). It is concluded that the polysorbates are not carcinogenic when applied to the skin. Several studies are also available on tumour promotion and co-carcinogenicity after dermal exposure; an overview is given in CIR (1984). However, this route of exposure is not relevant for food additives. This is particularly relevant for the induction of tumours after subcutaneous injection (Walpole, 1962).

Table 18: Summary of chronic toxicity studies in experimental animals exposed via the oral route to polysorbates

Substance, species, strain, sex [(a)], n [(b)]	Duration	Dosing information	NOAEL [(c)]	Investigated parameters/effects	Reference
Polysorbate 20, hamster, nd, nd, 10/group	28–39 weeks	0, 5, 10 or 15 % in the diet (bread)	na	**≥ 5 %:** diarrhoea and mortality ↑. Histopathology: liver cirrhosis and nephropathy in survivors	Eagle and Poling (1956)
Polysorbate 20, hamster, nd, m, 10	39 weeks	0, 5, 10 or 15 % in the diet (presumably no dietary fibre)	na	**≥ 5 %:** diarrhoea, body weight ↓, unthrifty appearance **≥ 10 %:** mortality ↑	Poling et al. (1956)
Polysorbate 20, monkey, nd, nd, 6/group	Up to 17 months	Each monkey received 1 g/day; no data about control	1 g/day	**1 g/day/animal:** weight gain not affected, no effects on histopathology	Krantz (1943a) [(d)], cited in JECFA (1974a)
Polysorbate 60, rat, Wistar, m, 24 (control 10)	12 months	0 or 1 % in drinking water	1 %	**1 %:** no effects on body weight gain	Shirai et al. (1982)
Polysorbate 60, rat, Wistar, 12 m & 20–21 f per group	104 weeks	0, 5, 10 or 20 % in the diet (including dietary fibre)	5 %	**≤ 10 %:** no effects on body weight or food efficiency **≤ 20 %:** haematology and histopathology negative **≥ 10 %:** diarrhoea in m&f; albumin in urine ↑ **20 %** [(e)]**:** body weight and food efficiency in m ↓; mortality ↑ in f	Oser and Oser (1956a, 1957a, b)

Substance, species, strain, sex [a], n [b]	Duration	Dosing information	NOAEL [c]	Investigated parameters/effects	Reference
Polysorbate 60, rat, Osborne–Mendel, m&f, 12	24 months	0, 2, 5, 10 or 25 % in the diet ('rat biscuits')	2 %	**2 %:** no effects **5 %:** slight diarrhoea, no other effects **10 %:** moderate diarrhoea, no other effects **25 %** [f]**:** severe diarrhoea, body weight gain in m ↓; no effects on survival, food intake and efficiency; haematology negative; liver weight ↑, but no pathological changes; caecal enlargement (histopathology negative)	Fitzhugh et al. (1959)
Polysorbate 60, hamster, nd, nd, 9–12 animals/group in each of 3 independent trials	12–13 months	0, 1 or 5 % in the diet (high amounts of sugar, lard and casein; 3.3 % agar)	1 %	**≤ 5 %:** no effects on mortality, body or organ weights [e,f,g,h] and on food efficiency **5 %** [g]**:** diarrhoea, casts and chronic interstitial nephritis ↑ in histopathology (colony infection)	Brush et al. (1957)
Polysorbate 60, dog, Beagle, m&f, 1–4 (no f at 5 % in the diet)	12 months	0, 5 or 10 % in the diet	10 %	**≤ 10 %:** no effects on general health condition or body weight gain (measured first 14 weeks)	Brush et al, (1957)
Polysorbate 65, rat, Wistar, 12 m & 20–21 f per group	104 weeks	0, 5, 10 or 20 % in the diet (including dietary fibre)	5 %	**≤ 10 %:** no effects on body weight **≤ 20 %** [e]**:** no effects on food efficiency; haematology and histopathology negative **≥ 10 %:** diarrhoea in m **20 %:** body weight in m ↓; mortality ↑ in f	Oser and Oser (1956a, 1957a, b)
Polysorbate 80, mouse, C57BL, m, 23–28	51 weeks	0 or 3.3–5 g/kg bw/day via the diet	3.3 g/kg bw/day	**3.3 g/kg bw/day:** no effects on body weight gain or mortality; no effects in histopathology (colony infection with *Salmonella*)	Wong et al. (1959)
Polysorbate 80, mouse, B6C3F1, m&f, 60 (7–10 animals for interim sacrifice after 15 months)	103 weeks	0, 2.5 or 5 % in the diet (NIH-07 with dietary fibre); analytical control	2.5 % (3 750 mg/kg bw/day)	**2.5 %:** no effects **5 %** [g]**:** no clinical signs, no effect on survival or food consumption, body weight in f ↓ (not in m). Forestomach: hyperplasia and chronic inflammation in m&f ↑ and ulcer in f ↑; non-neoplastic lesions	NTP (1992a)

Substance, species, strain, sex [(a)], n [(b)]	Duration	Dosing information	NOAEL [(c)]	Investigated parameters/effects	Reference
Polysorbate 80, rat, F344/N, m&f, 60 (7–10 animals for interim sacrifice after 15 months)	103 weeks (~ 24 months)	0, 2.5 or 5 % in the diet (NIH-07 with dietary fibre); analytical control	5 % 2 500 mg/kg bw/day)	**≤ 5 %:** no clinical signs, no effects on body weight and food consumption **5 %** [(g)]**:** survival in m ↓ (not in f) after week 93 owing to common neoplasm; in m phaeochromocytoma ↑	NTP (1992a)
Polysorbate 80, rat, Wistar, 12 m & 20–21 f per group	104 weeks	0, 5, 10 or 20 % in the diet (including dietary fibre)	5 %	**≤ 10 %:** no effects on body weight **≤ 20 %** [(e)]**:** no effects on food efficiency; histopathology and haematology negative **≥ 10 %:** diarrhoea in f; albumin in urine ↑ **20 %:** body weight in m ↓; mortality ↑ in f; diarrhoea in m	Oser and Oser (1956a, 1957a, b)
Polysorbate 80, monkey, nd, nd, 6/group	Up to 17 months	Each monkey received 1 g/day; no data about control	1 g/day	**1 g/day/animal:** weight gain not affected, no effects in histopathology	Krantz (1947) [(c)], cited in JECFA (1974a)

nd, no data; na, not applicable.
(a): m, male; f, female.
(b): Number of animals/sex/dose.
(c): Intake of the key studies was calculated by the Panel using the proposed calculation factors for chronic studies of 0.05 for rats and 0.15 for mice (EFSA, 2012).
(d): Unpublished report (not available to the Panel).
(e): Haematology: blood samples collected in males and females of F0 generation at weeks 12, 52, 78 and 104 (at least three rats per sex per dose) and in succeeding generations at week 12; determinations of haemoglobin, sugar and non-protein nitrogen levels, and plasma cholesterol, and of the erythrocyte, leucocyte and differential leucocyte counts. Urinalysis: e.g. albumin, reducing sugars and oxalate; in F0 generation including microscopic examination of the sediment; performed half-yearly in three rats per dose and in all survivors at termination. Necropsy: performed on all F0 rats that died during the study or that were sacrificed at termination; liver and kidney weight measured. Histopathology: complete histopathology performed in only two rats per sex at the high dose level; liver and kidney in all groups examined but restricted to 8–10 organs per dose level (sexes combined).
(f): Haematology twice during exposure: red and white blood cell counts, differential blood cell counts, haemoglobin; no details about organ weights. Histopathology: lung, heart, liver, spleen, pancreas, stomach, small intestine, colon, kidneys, adrenal, thyroid, leg muscles, leg bones with included marrow, testes (or uterus and ovary), and any lesion.
(g): Histopathology: adrenal, bone and bone marrow, brain, clitoral or preputial gland (rats), lesions, oesophagus, gallbladder (mice), heart, kidneys, caecum, colon, rectum, lymph nodes, liver, lungs, bronchus, small intestine, mammary gland, nasal cavity, ovary, pancreas, parathyroid, pituitary, prostate gland, salivary gland, seminal vesicles, spleen, stomach, testes, thymus, thyroid, trachea, bladder and uterus.
(h): Organ weights of heart, liver, kidneys and spleen; histopathology of lymph node, thyroid, lung, heart, kidneys, liver, adrenal, spleen, stomach, jejunum, duodenum, ileum, colon, caecum, bladder and testes.

Several chronic oral studies were available for the polysorbates but none of these studies fulfilled the requirements of the current guidelines for chronic toxicity. Nevertheless, the feeding studies by Oser and Oser (1956a, 1957a, b) on polysorbate 80, polysorbate 60 and polysorbate 65, the study of Fitzhugh et al. (1959) on polysorbate 60 and the carcinogenicity studies published by NTP (1992a) on polysorbate 80 were sufficient for the evaluation of these endpoints; these studies are described in more detail in the following paragraphs. Other studies were suffering from limitations on detailed study information (data from secondary literature), only investigated limited parameters or used a diet that was not appropriate. Therefore, these studies have only a low validity.

The most reliable chronic study was the carcinogenicity study in mice and rats with polysorbate 80 (NTP, 1992a). The composition of polysorbate 80 was consistent with the specifications given in section 2 (85 % sorbitan polyethylene glycols fatty acid esters and 15 % sorbitan polyethylene glycols). This study design is comparable to that described in the Organisation for Economic Co-operation and Development (OECD) Guideline 451.[19] The option described in this guideline to study, like in chronic studies, the following recommended parameters and haematology—clinical chemistry, urinalysis and organ weights at necropsy—was not performed. Organ weights of the heart, right kidney, liver, lungs, thymus and brain were recorded only in the satellite group at interim sacrifice at 15 months and no effects were detected regarding this parameter. In this study, clinical observations were made twice daily and body weights were recorded weekly for 14 weeks and then monthly. Food consumption was measured once monthly. Necropsy was performed on moribund animals and survivors, and histopathology was performed on all animals (details in Table 18).

In the mice study with polysorbate 80, no effects were detected at the low dose of 2.5 % in the diet (equivalent to 3 750 mg/kg bw/day). At 5 % (equivalent to 7 500 mg/kg bw/day), there was no effect on clinical signs, survival or food consumption. The final body weights of high dose females were decreased (11 %) but not those of males. No treatment-related neoplastic lesions were found in any group. However, the high dose resulted in non-neoplastic lesions of the forestomach with incidences of squamous hyperplasia and chronic inflammation significantly increased in both sexes, and incidence of forestomach ulcer increased only in females (NTP, 1992a). No NOAEL was derived by the authors of the study. However, on the basis of the available results the Panel concluded on a NOAEL of 2.5 % in the diet equivalent to 3 750 mg/kg bw/day.

In a similar study in rats with polysorbate 80 at doses of 2.5 and 5 % in the diet (equivalent to 1 250 and 2 500 mg/kg bw/ day), a significantly reduced survival rate was found in males at the highest dose level. This effect was related to neoplasms, which are common in ageing rats, and occurred after week 93; it was, therefore, not considered by the Panel as a treatment-related effect. Histopathology revealed no treatment-related non-neoplastic effects in male or female rats. In high-dose males, a slight but significant increase in benign phaeochromocytoma as in adrenal medulla was found (incidence 21 out of 50 in controls, 19 out of 50 at the low dose and 29 out of 50 at the high dose). This incidence of 58 % at the high dose is above the historical control data (22–48 %). However, the incidence of adrenal medulla hyperplasia in high-dose males was not different from controls but was increased at the low dose level (11, 22 and 12 out of 50 in the control, low dose level and high dose level, respectively). No neoplastic effects were reported in females. The authors concluded that there was equivocal evidence for carcinogenic activity in male rats (NTP, 1992a). No NOAEL was allocated by the authors of the study. The Panel considered 5 % in the diet as a NOAEL for this study (equivalent to 2 500 mg/kg bw/day). Oser and Oser (1956a, b, 1957a, b) performed screening studies in the rat on seven different emulsifiers that included polysorbate 80, polysorbate 60 and polysorbate 65, which were mixed with basal diet (also containing dietary fibre: 1 % celluflour and 2 % lucerne) at concentrations of 0, 5, 10 and 20 % (equivalent to 0, 2 500, 5 000 and 20 000 mg/kg bw/day). The study was designed as a multigeneration study; groups of 12 male and 20–21 female Wistar rats (initial body weight 50–70 g) were fed *ad libitum* for two years. Matings of the F0 generation within the same dose group started five weeks after initiation of exposure. The first litter was discarded at weaning and the second matings of F0 for F1 generation started after 12 weeks. In total, four generations were examined: F0–F3 (Oser and Oser, 1956b; see details in section 3.2.5). The body weights of the F0 animals were measured weekly for the first 12 weeks, and thereafter biweekly. Food consumption (n = 5 per sex per dose) was measured during the first 12 weeks, and after 6, 12, 18 and 24 months for a two-week period. During the course of the study, observations were made of physical appearance, behaviour, reproduction and lactation through three successive generations (see section 3.2.5), and gross and histological evaluations of F0 were made at termination. The results of this study

[19] OECD Guidelines for the Testing of Chemicals, Section 4. Test No. 451: Carcinogenicity Studies. doi:10.1787/20745788. Available online: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788

are summarised in Table 18. The validity of the studies by Oser and Oser is restricted because of limited documentation, the low number of animals, limited histopathology and organ weight data, only partial statistical analysis and infection of the respiratory tract in all groups. Although this long-term feeding study has several limitations, the results suggested treatment-related laxative effects of all three tested polysorbates at a dose level of ≥ 10 % in the diet (equivalent to ≥ 5 000 mg/kg bw/day) (effects at the 10 % dose level were found in females only for polysorbate 80, in males and females for polysorbate 60 and in males only for polysorbate 65). The Panel concluded from the results of this study that a NOAEL of 5 % equivalent to 2 500 mg/kg bw/day could be derived for female rats treated with polysorbate 80, for male and female rats treated with polysorbate 60 and for male rats treated with polysorbate 65; this NOAEL is based on the laxative effects.

The study of Fitzhugh et al. (1959) revealed local laxative effects in rats after exposure to polysorbate 60 at a dose level of ≥ 5 % in the diet (equivalent to 2 500 mg/kg bw/day). Body weight gain, survival and haematological parameters remained unaffected. No effects were found in histopathology. No diarrhoea was observed at the low dose level of 2 % in the diet. However, due to several limitations of this study (sex and constituents in the diet not specified) the basis for a NOAEL derivation (NOAEL 2 % in the diet equivalent to 1 000 mg/kg bw/day) is less reliable and should be considered only as supporting information for the study of Oser and Oser (1956a,b; 1957a,b).

In 1992, NTP concluded that there was equivocal evidence for carcinogenic activity of polysorbate 80 based on increased incidences of benign phaeochromocytomas in the adrenal gland of male rats at a dose of 2 500 mg/kg bw/day (NTP, 1992a). The SCF (1995) concluded that lesions such as phaeochromocytomas in the rat study have no relevance in humans. The Panel agreed with this conclusion. Furthermore, there is no evidence for genotoxicity (see section 3.2.3) or for malignant tumour formation. Polysorbates are non-oxidising agents and there is no structural alert for DNA cross-linking. Thus, based on the given information, a NOAEL of 5 % in the diet (equivalent to 2 500 mg/kg bw/day) was derived. In the same study in mice (males and females), forestomach lesions were induced by polysorbate 80 at a dose of 5 % in the diet (equivalent to 7 500 mg/kg bw/day); the NOAEL would be 3 750 mg/kg bw/day. The results of limited long-term feeding studies (Oser and Oser, 1956a, b, 1957a, b) with polysorbate 80, polysorbate 60 or polysorbate 65 suggested treatment-related laxative effects of all three tested polysorbates at a dose level of ≥ 10 % in the diet (equivalent to ≥ 5 000 mg/kg bw/day). Thus, the NOAELs derived from different studies are in the same range. The available data are considered sufficient for the evaluation of this endpoint. Due to the similarities in structure and metabolic fate the available data on chronic toxicity are considered to be valid for all polysorbates.

3.2.5. Reproductive and developmental toxicity

3.2.5.1. Reproductive toxicity studies

The studies cited in this section were already presented in the evaluation of JECFA (1974a). New data were found in the literature search in the databases Toxline, Medline and SciFinder. However, the relevance of these new studies (Paschall, 1964; Enright et al., 2010) is limited.

In the feeding study by Paschall (1964), three generations of male and female C57BL/6 mice (number of animals unknown) were given, continuously, a diet containing 0 or 10 % polysorbate 20 or 10 % polysorbate 60 (equivalent to 0 or 5 g/kg bw/day; higher doses expected in pregnant females and in the post-weaning period of 10 g/kg bw/day). Four matings were performed in the F0 and F1 generations and three matings in the F2 generation. Pup weight at weaning was significantly reduced after exposure to both polysorbates. The number of live pups born, the viability index and the lactation index decreased. No effects on body weight gain of pregnant mice were found, and no effects on organ weights of gonads or sperm motility were found (no data on clinical signs). The Panel had no access to the study and therefore the validity of this study is limited due to insufficient documentation.

In a long-term feeding study (Oser and Oser, 1956a, b, 1957a, b; see also section 3.2.4), polysorbate 80, polysorbate 60 or polysorbate 65 were fed *ad libitum* at concentrations of 0, 5, 10 and 20 % in the basal diet (equivalent to 0, 2 500, 5 000 and 10 000 g/kg bw/day; higher doses in females expected at pregnancy) to groups of 12 male and 20 female Wistar rats for two years. In the Oser and Oser (1956b) study, effects on reproduction and lactation were reported. Matings were continued in the F0 generation throughout the entire two-year period (seven-week intervals). The first litters of F0 were discarded at weaning. From the second litters, 10 rats per sex were selected for the F1 generation. These F1 generation animals were raised to maturity and mated like the F0 generation. The second litters of the F2 generation were carried through the same breeding programme. Similarly, F3 rats were raised to maturity for growth observations but were not mated because the entire study was terminated when the F0 rats reached two years on test. During the reproduction phase, body weights were recorded biweekly; females were also weighed during pregnancy (no details available). Pups were weighed at days 4, 12 and 21 postnatally. Further parameters were examined: the fertility and gestation index, the viability index and the lactation index. No data were available on maternal body weight gain during pregnancy and lactation period. Clinical signs, except diarrhoea, were not observed in F0 and presumably not in succeeding generations (limited information). Generally, the effects on reproduction occurred at dose levels also inducing diarrhoea in dams (≥ 10 % in the diet). Some effects in F1 and F2 offspring were recorded and tabulated but not evaluated by the authors (e.g. pup weight). The reliability of the study is limited due to discrepancies between methodological documentation and reported results, and missing statistical analysis. Overall, the study is not sufficient for the evaluation of reproductive toxicity.

Enright et al. (2010) conducted a reproductive toxicology study in rats with polysorbate 80. The test substance did not exhibit toxicity. However, only a low dose level of 10 mg/kg bw/day was tested, which is three orders of magnitude or more lower than that used by Oser and Oser (1956b).

In conclusion, studies on reproductive toxicity are not sufficient for comprehensive evaluation of this endpoint. However, there is no indication of reproductive effects of polysorbates at dose levels inducing no laxative effects in the parental generation.

3.2.5.2. Developmental toxicity studies

The literature search in Toxline, Medline and SciFinder resulted in studies of sufficient validity for evaluation of this endpoint. Data are available in rats for polysorbate 20, polysorbate 80 and polysorbate 60. Limited studies are available for the two other species: mouse and rabbit. All these studies were published after the JECFA evaluation (1974a). A summary is given in Table 19.

Table 19: Developmental toxicity in animals exposed via the oral route to polysorbates

Substance, species, strain, n [(a)]	Duration [(b)]	Dosing information	Investigated parameters/effects	Reference
Polysorbate 20, rat, Sprague–Dawley, 22–24	GD 6–15 T: GD 20	0, 0.5 or 5 g/kg bw/day gavage	**NOAEL [(c)]: 0.5 g/kg bw/day** for maternal toxicity **NOAEL: 5 g/kg bw/day** for developmental toxicity **5 g/kg bw/day:** maternal body weight gain ↓ and water consumption ↑	NTP (1992b, c), Price et al. (1994)
Polysorbate 60, mouse, CD-1, 50	GD 6–13 T: PND 3	0 or 5.2 g/kg bw/day gavage	**5.2 g/kg bw/day:** no effects on maternal weight gain and survival; no effects on live pups/litter, pup survival and birth weight, but decreased postnatal pup weight gain Screening test; limited parameters	Hardin et al. (1987)

Substance, species, strain, n [(a)]	Duration [(b)]	Dosing information	Investigated parameters/effects	Reference
Polysorbate 60, rat, Wistar, 10–12	GD 7–14 T: GD 20	0, 0.1, 1 or 10 % in the diet (0, 0.1, 1 or 7.7 g/kg bw/day)	**NOAEL [(c)]: 7.7 g/kg bw/day** for maternal and developmental toxicity **≤ 7.7 g/kg bw/day [(d)]:** no treatment-related maternal and developmental toxicity	Ema et al. (1988)
Polysorbate 80, mouse, CD-1, 22–33	GD 8–12 T: nd	0 or 2.5 g/kg bw/day gavage	**2.5 g/kg bw/day:** no effects on maternal weight and survival; no effects on number of live pups or pup weight at PND 1 and 3, and pup weight gain Screening test; limited parameters	Kavlock et al. (1987)
Polysorbate 80, rat, Sprague–Dawley, 19–23	GD 6–15 T: GD 20	0, 0.5 or 5 g/kg bw/day gavage	**LOAEL [(c)]: 0.5 g/kg bw/day** for maternal toxicity (liver weight ↑, absolute 9 %, relative 6 %) **NOAEL: 5 g/kg bw/day** for developmental toxicity **5 g/kg bw/day:** maternal food intake↓ (liver weight ↑, absolute 5 %, relative 5 %)	NTP (1992d, e), Price et al. (1994)
Polysorbate 80, rat, Crl:CD(SD), 20–22	GD 0–PND 21 T: PND 21	0, 0.018, 0.13, 1.0 or 7.5 % in the diet (0, 0.04, 0.27, 2.1 or 18.5 g/kg bw/day)	**NOAEL: 2.1 g/kg bw/day** for maternal or developmental toxicity **18.5 g/kg bw/day [(e)]:** in dams body weight ↓, water and food consumption ↓, dilatation of caecum at necropsy; no effects on fecundity and gestation index and length of gestation; number of pups ↓, pups body weight ↓, pups rate of avoidance responses ↓	Ema et al. (2008)

NOAELs are defined by the authors of the corresponding reference.
(a): Number of pregnant animals.
(b): Duration of exposure period. GD, gestation day; PND, postnatal day; T, termination (sacrifice of dams); nd, no data.
(c): Study comparable to OECD Guideline 414[20] (but limited exposure period). Maternal toxicity: clinical signs, body weight gain, food and water consumption; organ weights of gravid uterine, heart, liver and kidney at necropsy. Developmental toxicity: number of corpora lutea, implantations, early and late resorptions, live and dead fetuses, fetal weight and sex; external, visceral and skeletal variations and malformations.
(d): No treatment-related effects on maternal body weight gain but no data about clinical signs were presented. Developmental toxicity: number of implantations, resorptions, dead and live fetuses; sex ratio, fetal body weight for males and females. Fetal pathology: external examinations, skeletal and visceral variations and malformation.
(e): Developmental toxicity parameters: numbers of implantations, pups born alive and dead pups, delivery index, sex ratio, viability index of pups before weaning, pinna unfolding, fur appearance, incisor eruption, eye opening; preputial separation, vaginal opening; reflex ontogeny; pain response, locomotor activity, conditioned avoidance response; necropsy and histopathology of cerebrum, cerebellum, medulla oblongata, pons, spinal cord in the thoracic and lumbar regions, and sciatic nerve; organ weights of brain, liver, spleen, adrenal and kidney.
(e): Enright et al. (2010) studied polysorbate 80 at a very low dose level of 10 mg/kg bw/day from GD 6 to 17 in rats and from GD 7 to 19 in rabbits and observed no treatment-related developmental effects.

NTP published gavage studies comparable to the current OECD Test Guideline 414 for polysorbate 20 (NTP, 1992b, c) and polysorbate 80 (NTP, 1992d, e). In both studies, rats were exposed at GD 6–15 only, which is a restricted period compared with the current guidelines (GD 6–20). However, the validity is sufficient for evaluation. The authors of the studies suggested a NOAEL of 5 000 mg/kg bw/day for both polysorbates for the developmental toxicity endpoints. The authors suggested

[20] http://www.oecd.org/chemicalsafety/risk-assessment/1948482.pdf

maternal toxicity, since an effect on liver weight was shown at this or lower dose levels. Absolute liver weights were increased by 9 and 5 % in the 500 and 5 000 mg/kg bw groups, respectively, and relative liver weights were increased by 6 and 5 % in these groups, respectively. The Panel noted that this effect on liver weight should not be considered as maternal toxicity as no dose–effect relationship was observed and there was only a small increase in liver weight. The study with polysorbate 80 conducted by Ema et al. (2008) supported these results; other studies with polysorbate 80 are of limited validity (Kavlock et al., 1987; Enright et al., 2010).

In the study presented by Ema et al. (2008), the function of the nervous system in the offspring of rats given polysorbate 80 via the drinking water was examined. No treatment-related changes were found in reflex responses. A decrease in the rate of avoidance responses was noted on postnatal days 23–27 in male and female offspring at the high dose level of 7.5 %. However, no treatment-related effects were found in performance in the conditioned avoidance response on postnatal days 60–67. Histopathological examinations of the brain revealed no toxicological effects.

Polysorbate 60 was tested in a feeding study in a comparable way to the current guidelines (Ema et al., 1988) in rats. The exposure period was restricted to GD 7–14 and the information was partly limited (e.g. no data on clinical signs in dams). The authors of the study proposed a NOAEL of 7 700 mg/kg bw/day for maternal and developmental toxicity. The limited study of Hardin et al. (1987) in mice supported these results.

In addition, several studies using the parenteral application route are available (Kocher-Becker et al., 1981; Ockenfels et al., 1982; Gajdova et al., 1993; Naya et al., 1999); however, this route has low relevance to human exposure and therefore the studies are not presented in detail.

In conclusion, the database on developmental toxicity is sufficient for evaluation. In oral studies in rats performed in accordance with current guidelines with acceptable restrictions, no developmental effects were reported even at the highest dose levels tested. The NOAEL for polysorbate 20 and polysorbate 80 is 5 000 mg/kg bw/day. For polysorbate 60, the NOAEL for maternal and developmental toxicity is 7 700 mg/kg bw/day. No data were available for polysorbate 40 or polysorbate 65.

3.2.6. Other studies

In this section, all studies were published before 1974 and are cited in JECFA (1974a). In addition, studies found in a literature search in Toxline, Medline and SciFinder are included in this section.

3.2.6.1. Human studies

Observations in humans

The indication from animal studies of low toxicity of polysorbates was supported by studies in human volunteers or patients including special subgroups such as children and elderly patients.

A placebo-controlled study by Waldstein et al. (1954) was performed in infirmary patients (aged 35–70 years) and a second study was performed in hospital staff (‘normal’ healthy subjects; aged 25–40 years). Nineteen patients were fed 6 g/day polysorbate 60 (twice daily 3 g/patient) and nine were given placebo. Ten normal subjects were fed the same dose of polysorbate 60, and 10 received a placebo. The treatment period lasted 28 days. Laboratory examinations were done before initiation of treatment, after 14 days of treatment and at the conclusion of the 28-day programme. The examinations included clinical observations, urinalysis, haematology and clinical chemistry plus a bromsulphophthalein (BSP) retention test. In both studies, no untoward clinical reactions occurred and testing revealed no significant changes except abnormal results in the BSP retention test in infirmary patients (but not in normal subjects). The authors performed a second trial for BSP testing in infirmary patients (15 patients received polysorbate 60 and 16 received placebo) and hospital staff (10 subjects treated with polysorbate 60 and 10 given placebo). The BSP abnormalities were not reproducible suggesting false-positive reactions not related to the treatment with polysorbate 60. The authors

concluded that there were no observable effects after ingestion of 6 g/day of polysorbate 60 for 28 days.

Krantz et al. (1951) reported a study in 100 human subjects who ingested 4.5–6 g of polysorbate 80 daily. The exposure period varied: 10 subjects were treated for three to four years, 17 were treated for two to three years, 19 were treated for one to two years and 54 were treated for less than one year. No effects could be demonstrated on metabolic rates, clinical chemistry parameters or haematology. No change in excretion of water-soluble vitamins or any evidence for liver and kidney damage were found. Data on controls were not reported.

In a placebo-controlled study in 12 volunteers (5 women and 7 men; 16–53 years old) the effects of polysorbate 80 on gastro-intestinal motility were studied. The subjects received 9 g/day (divided into three applications with meals) of polysorbate 80 for 13 days or the placebo, followed by one week without treatment (follow-up). Clinical signs and body weight were recorded and gastro-intestinal motility was investigated via transit time, number of stools and stool character. No treatment-related effects were reported. Using the same experimental design, similar results were obtained after treatment with polysorbate 65 (Janowitz et al., 1953).

McCorriston (1952) used polysorbate 80 as an oral therapeutic agent for the treatment of atopic dermatitis, psoriasis and other dermatoses. Each patient served as his or her own control. Daily doses of 6 g polysorbate 80 were administered to a total of 85 patients (37 men and 48 women; 6 g/day divided into three doses with meals) for up to three months in an attempt to alter lipid metabolism after a 'moderate-fat diet'. A 'softening' of the skin was noted. There was an increase in serum total lipid levels (measured in only nine patients), but there was no evidence of toxic effects such as diarrhoea.

Jones et al. (1948) showed effects of treatment with polysorbate 80 for several months on the absorption of fat in patients with malnutrition. Ingestion of doses of up to 15 g/day did not show evidence of toxicity.

Snyderman et al. (1953) studied the effects of polysorbate 80 on the absorption of fat and vitamin A in nine healthy premature infants. The dose was related to the applied fat in the diet. The infants were treated with 40 mg/g fat. The treatment with polysorbate 80 resulted in a slight increase in absorption of fat and vitamin A. The effect was reversible. Data on toxic effects were not presented.

The accidental oral administration of a dose of 19.2 g/kg bw of polysorbate 80 on two successive days to a four-month old baby boy was followed by loose stool but no further ill effects (Chusid and Diamond, 1955).

In the study of Steigmann et al. (1953), 10 elderly patients were given oral doses of 6 g polysorbate 60 per day (twice daily 3 g/subject) for 28 days. No significant effects on the physiological activity of the gastro-intestinal tract were found, which was measured by changes in the gas pattern of the bowel, by gastric emptying time, by passage of barium through the gastro-intestinal tract and by gallbladder visualisation studies.

Preston et al. (1953) studied toxic effects in four children (6–11 months old, one child with mucoviscidosis) ingesting polysorbate 60 at a dose level of 1 g/day for 11–34 days (no data on body weight). Clinical observations, haematology, body weight measurements, examinations of stool, gastro-intestinal radiographic studies and urinalysis were performed. No harmful effects were noted.

Jeans and Stearn (1970, unpublished report; cited in CIR, 1984) reported no adverse effects as a result of adding emulsifier mixtures containing polysorbate 60 and polysorbate 80 to the daily diets of nine infants ranging in age from one week to seven months. The infants received daily 0.2 g polysorbate 60 with 0.04 g polysorbate 80 for periods of 1.5 to 5 months, with three of these infants receiving 0.4 g polysorbate 60 with 0.04 g polysorbate 80 per day for an additional 1 to 2 months. Clinical signs were

recorded, including comparative growth curves and nutritional balance studies. No adverse effects were detected.

In conclusion, studies on toxicity in humans are available for polysorbate 80, polysorbate 60 and polysorbate 65. The most valid study was performed by Waldstein et al. (1954). In this placebo-controlled study, the ingestion of 6 g/day of polysorbate 60 for 28 days produced no adverse effects in humans. The results of Waldstein et al. are supported by further studies with different polysorbates. Assuming a body weight of 60 kg, the dose in the Waldstein study corresponds to 100 mg/kg bw/day. In addition, no harmful effects were observed on the health of children or elderly patients.

Allergenicity

No data are available on intolerance reactions or allergenicity after oral exposure to polysorbates.

A case report describes anaphylactoid reactions, which were induced in one pregnant female patient immediately after an i.v. injection with a multivitamin preparation containing polysorbate 80. Further testing revealed that the polysorbate was the causative agent and that the reaction was non-immunological (Coors et al., 2005).

In another case report (Steele et al., 2005), hypersensitivity reactions to polysorbate 80 were reported after subcutaneous injection. Intradermal prick testing was positive.

Data on skin sensitisation are summarised in CIR (1984). In a review (Hannuksela and Haahtela, 1987), the authors stated that immunological contact urticaria was induced by polysorbate 80 in a few cases. In a study on 1 206 patients with eczema, allergy was induced in only two patients tested with polysorbate 40 and polysorbate 80 (Hannuksela et al., 1976). In 5 out of 737 patients with contact dermatitis, positive results were obtained in patch tests with polysorbate 40, and in 4 out of 737 with polysorbate 80 (Tosti et al., 1990).

3.2.6.2. *In vivo*, *ex vivo* and *in vitro* studies

Neurotoxicity

Varma et al. (1985) treated Swiss mice via gavage with doses up to 11 000 mg/kg bw of polysorbate 80. No effects on general behaviour were observed. The high dose resulted in no hypnotic activity but slight paralytic activity and a 54 % reduction in locomotor activity; slight effects on locomotor activity were also seen at 2.2 g/kg bw.

Oestrogenic activity

Williams et al. (1997) reported no effects on uterine growth in immature female rats (20–22 days old) of polysorbate 80 after oral gavage up to doses of 5 000 mg/kg bw/day for three consecutive days. Oestradiol benzoate administered subcutaneously was used as a positive control and significantly increased uterine weight in this age and strain of female rat (21–23 days, Alpk:APfSD Wistar derived) by up to 4.5-fold above vehicle control values.

Effects of polysorbates on intestinal mucosa

Kimura and co-workers investigated the protective effects of dietary fibre at high dose levels of polysorbate 20 in feeding studies (see section 3.2.2; Kimura and Yoshida, 1982, Nakata and Kimura, 1994). Kimura et al. (1982) investigated, in rats, the mechanisms of this effect using a jejunum perfusion *in vivo*. A Ringer bicarbonate solution was used for perfusion. Polysorbate 20 and/or polysorbate 60 was added to the solution; the authors used a concentration of 2 %. The sucrase activity in the solution increased and reached threefold the control value after 90 minutes of perfusion. The addition of 0.04 % dietary fibre inhibited this effect. The results suggested that polysorbates in diets without dietary fibre exfoliated or damaged the brush border membrane of the small intestine, inducing diarrhoea and reduced body weight. The addition of dietary fibre ameliorated these effects.

Tagesson and Edling (1984) studied the influence of polysorbate 80 and polysorbate 60 on the integrity and permeability of rat ileal mucosa. They determined the activity of *N*-acetyl-fl-glucosaminidase, a lysosomal enzyme, in the rat intestinal lumen in a section of ligated, cannulated gut. An increase in the activity of *N*-acetyl-fl-glucosaminidase was found at a high concentration (10 mg/mL) of either polysorbate 15 minutes after application of the solution. Further *in vitro* experiments suggested the release of lysosomal enzymes from the intestinal mucosal cells and damage of the intestinal mucosa, together with an increase in permeability.

Roberts (2010, 2013) suggested that the consumption of polysorbate 80 and less polysorbate 60 in processed foods may promote Crohn's disease by increasing the translocation of Crohn's disease mucosal bacteria across intestinal cells. This hypothesis has been tested only in *in vitro* experiments with human epithelia colorectal carcinoma cells (Caco2) and specialised microfold epithelial cells (M cells)[21] and thus does not take into consideration gut lumen physiological conditions. However, as this suggestion was based solely on *in vitro* results and *ex vivo* results, and its relevance to the *in vivo* situation remains unclear in the absence of relevant clinical studies, the Panel could not use the results of these studies for risk assessment.

A recent study (Chassaing et al., 2015) reported that exposing C57Bl6 mice, of either four weeks or four months of age, to drinking water containing an emulsifier, either carboxymethylcellulose or polysorbate 80 at a concentration of 1 % w/v or 1 % v/v, respectively (equivalent to 1 500 mg/kg bw/day), for three months resulted in reduced gut mucus thickness, increased bacteria proximity to gut epithelium, alterations in gut microbiota composition, increased faecal levels of lipopolysaccharides (LPS) and flagellin, low-grade gut inflammation, increases in food consumption, body weights and adiposity and impaired glycaemic control (increased fasting blood glucose and reduced glucose and insulin tolerances). These effects were also reproduced when the emulsifiers were incorporated into the diet and the inflammatory effects (faecal lipocalin 2, colonic tissue myeloperoxidase activity, colon length and by histopathology) were reported to be exacerbated in mice null for Il10 or Tlr5 genes, leading to colitis. The effect of the emulsifiers on the gut microbiota appears to have played a pivotal role in causing these adverse effects in the study, as treating germ-free mice with the emulsifiers did not lead to the effects seen in normally housed C57Bl6 mice, whereas the effects were observed in germ-free mice if gut microbiota were transferred from emulsifier pre-treated C57Bl6 mice.

The Panel noted that, even though some of these endpoints are not systematically included in toxicity studies performed according to toxicity testing guidelines, they would be investigated on a case by case basis if indicated by the results of the general toxicity testing as recommended in the guidance of the ANS Panel on food additive evaluation (EFSA, 2012).

However, effects such an increases in body weight, blood glucose, histopathology observations, etc., in subchronic and chronic toxicity and carcinogenicity studies have not been observed with polysorbates. Furthermore, the Panel noted that studies with mice at doses up to 3 750 mg/kg bw/day did not result in increased body weights but were more likely to lead to (a trend of) body weight loss (7 500 mg/kg bw/day).

According to the authors, additional studies will be needed to show the relevance of the effects seen in mice for human health. The Panel agreed with this conclusion.

Effects on absorption of lipids

Rats were fed polysorbate 80 for one week in amounts of 0.1 % and 1 % of the diet (Sergiel et al., 1971). This diet contained different amounts of fat. The concurrent exposure to polysorbate 80

[21] M (microfold) cells are found in the follicle (Peyer's patches)-associated epithelium of the gastro-intestinal tract. In the Roberts (2010) study, monolayers of M cells were generated by co-culture of Caco2 and Raji B cells, together with human Peyer's patches.

augmented the absorption of fats when these were present in high concentrations of 10 to 33 % in the diet, but not at concentrations ≤ 7 %.

Biochemical effects

The inhibitory activity of polysorbate 80 on cytochrome P450-mediated metabolism has been shown *in vitro*. The IC_{50} of the 6-beta-hydroxylation of testosterone was 0.40 mM (Christiansen et al., 2011).

Ren et al. (2009) examined the effects of polysorbate 20 on the 1′-hydroxylation of midazolam *in vitro*. Polysorbate 20 induced concentration-dependent inhibition of the hydroxylation; an IC_{50} of 2.06 and 0.39 mg/mL was measured in the liver and intestinal microsomes, respectively. *In vivo* results in rats were not consistent. The authors concluded that polysorbate 20 has a potential inhibitory effect on cytochrome P450 3A.

The induction of proliferin gene expression in mouse fibroblast C3H/10T1/2 cells was shown *in vitro* at a concentration of 0.1 mM polysorbate 60. The author discussed proliferin induction as a marker to predict chemically induced promotion of cell transformation (Parfett, 1992).

4. Discussion

The present draft opinion concerns the re-evaluation of the safety of polyoxyethylene sorbitan monolaurate (polysorbate 20, E 432), polyoxyethylene sorbitan monooleate (polysorbate 80, E 433), polyoxyethylene sorbitan monopalmitate (polysorbate 40, E 434), polyoxyethylene sorbitan monostearate (polysorbate 60, E 435) and polyoxyethylene sorbitan tristearate (polysorbate 65, E 436) used as food additives. Polysorbates (E 432–E 436) are authorised as food additives in the EU.

The SCF evaluated these additives in 1983 (SCF, 1985) and re-evaluated them in 1993 (SCF, 1995). JECFA evaluated polyoxyethylene sorbates in 1973 (JECFA, 1974a,b).

The SCF (1985) allocated a group ADI of 10 mg/kg bw/day for polysorbates 20, 40, 60, 65 and 80. The basis was a NOEL of 2 % (equivalent to 1 460 mg/kg bw/day) in the diet in the 90-day study in rats with polyoxyethylene sorbitan monostearate (polysorbate 60) (BIBRA, 1981; cited in SCF, 1985). The safety factor was not specified. No details from that study were presented in this first evaluation. A re-evaluation of polysorbate 80 (SCF, 1995) was performed in view of the data published by NTP (1992a) without changes to the group ADI. A higher group ADI value of 0–25 mg/kg bw/day was allocated by JECFA (1974a, b).

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

Specifications for the polysorbates have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (JECFA, 2006a). The Panel considered that the maximum limits for the impurities of toxic elements (arsenic, lead, cadmium and mercury) in the EC specification for polysorbates (E 432–E 436) should be revised in order to ascertain that polysorbates (E 432–E 436) as food additives will not be a significant source of exposure to those toxic elements in food.

The Panel considered the evaluation of polysorbates (E 432–E 436) as a group in one opinion because of their similarities in structure and metabolic fate. These additives are hydrolysed to oxyethylene sorbitans and the relevant fatty acids, the latter being normal constituents of the diet. From the toxicological data as described in this opinion, there is no indication of any relevant difference between the single polysorbates. Data on absorption and metabolic fate suggested hydrolysis of the ester bond between polyoxyethylene and the fatty acid of polysorbates in the gastro-intestinal tract after oral application. Intravenous data show that a similar hydrolysis can occur in blood. Fatty acids are absorbed, metabolised and excreted in the same way as dietary fatty acids. Based on the similarity

of the excretion in urine between compounds labelled in the polyoxyethylene and sorbitan moiety, cleavage of the polyoxyethylene and sorbitan bond does not occur. Only small amounts of polyoxyethylene sorbitans are absorbed. Similar toxicokinetics would be expected for all polysorbates.

The acute oral toxicity of all polysorbates was low. No mortality occurred in different species at high dose levels. Although the available data have limitations, the database was sufficient for the evaluation of this endpoint.

Subacute and subchronic oral studies were available for the polysorbates, but no studies performed in accordance or in line with current guidelines were published. Generally, the available studies were not sufficient for evaluating these endpoints. Adverse effects described in hamsters receiving a dose level of 5 % in the diet (equivalent to approximately 5 000 mg/kg bw/day, according to Gold et al. (1984) and Langkilde et al. (2012)) were related to the composition of the diet. Subchronic studies with polysorbate 80 in rats (NTP, 1992a) suggested NOAELs of 5 % in the diet (equivalent to 4 500 mg/kg bw/day as calculated according to EFSA (2012)). In the most valid dietary subchronic study in rats (BIBRA, 1981), a NOAEL of 2 % polysorbate 60 in the diet (equivalent to 1 460 mg/kg bw/day) was identified. This NOAEL was based on increased caecum weight and slightly increased haemoglobin levels, abnormalities which were not seen in rats exposed to up to 5 % polysorbate 80 (equivalent to 2 500 mg/kg bw/day as calculated according to EFSA (2012)) for 24 months (NTP, 1992a). Increased caecum weight is a common observation in rodents consuming low-digestible carbohydrates. In addition, these NOAELs were compared with those of various other studies (Table 16) and a similar order of magnitude was obtained for all NOAELs.

The available data on genotoxicity *in vitro* did not show mutagenic potential as reported in a limited gene mutation assay in bacteria with polysorbate 80 (NTP, 1992a) because of the absence of *S. typhimurium* TA 102 or *E. coli* WP2 tester strains but they were not sufficient for evaluation of the endpoints of gene and chromosome mutations in mammalian cells (Ishidate and Odashima, 1977; Coppinger et al., 1981). However, the evaluation of structural alerts for genotoxicity in polysorbates with the OECD QSAR Toolbox 3.2, did not highlight alerts for DNA reactivity (profilers '*DNA binding by OECD*' and '*DNA binding by OASIS*'), *in vitro* genotoxicity (profilers '*Alerts for Ames, chromosomal aberrations and micronuclei by Oasis 1.2*' and '*in vitro mutagenicity by ISS*') and carcinogenicity (profiler '*Carcinogenicity (genotoxic and non-genotoxic) by ISS*'. This outcome is considered valid for all polysorbates owing to the similarities in chemical structures and metabolic fate.

Taking into account the overall information on structure–activity relationships, the Panel concluded that, despite the limited database, polysorbates do not give rise to concerns for genotoxicity. The available long-term oral studies did not fulfil the requirements of current standards but these data were sufficient for evaluation. In male and female mice, forestomach squamous hyperplasia and inflammation and, in females, forestomach ulcers were induced by polysorbate 80 in the NTP (1992a) study at a dose of 5 % in the diet (equivalent to 7 500 mg/kg bw/day as calculated according to EFSA (2012)); the NOAEL was calculated to be 3 750 mg/kg bw/day. The carcinogenicity study in rats by NTP (1992a) indicated equivocal evidence for carcinogenic activity of polysorbate 80 based on increased incidences of benign phaeochromocytomas in the adrenal gland of males at a dose of 5 % in the diet equivalent to 2 500 mg/kg bw/day as calculated according to EFSA (2012). However, considering that (1) there was no evidence for *in vitro* genotoxicity (see section 3.2.3) or (2) for malignant tumour formation, and (3) that phaeochromocytomas were associated with exposure to poorly metabolised food additives at high doses and therefore are of no biological significance for humans (SCF 1995), a NOAEL of 5 % in the diet was considered by the Panel. The results of a limited long-term feeding study in rats (Oser and Oser, 1956a, 1957a, b) with polysorbate 80, polysorbate 60 or polysorbate 65 suggested treatment-related effects of all three tested polysorbates at a dose level of ≥ 10 % in the diet. Haematological parameters were not affected at concentrations up to 20 % in the diet. Overall, the Panel considered that the long-term studies in rats indicated a NOAEL of 5 % in the diet (approximately 2 500 mg/kg bw/day as calculated according to EFSA (2012)).

Studies on reproductive toxicity are not sufficient for comprehensive evaluation of this endpoint. However, there is no indication of reproductive effects of polysorbates at dose levels inducing no laxative effects in the parental generation (< 10 % of the diet).

In contrast, the database on developmental toxicity was sufficient for evaluation. Oral studies in rats performed in accordance with current guidelines were available. No developmental effects were reported even at the highest dose levels tested. The NOAEL for polysorbate 20 and polysorbate 80 was 5 000 mg/kg bw/day. For polysorbate 60, the NOAEL for maternal and developmental toxicity was 7 700 mg/kg bw/day.

Data on toxic effects in humans were published; however, clinical studies performed in accordance with current standards are not available. The most valid study was performed by Waldstein et al. (1954). In this placebo-controlled study, the ingestion of 6 000 mg/day of polysorbate 60 for 28 days (equivalent to 100 mg/kg bw/day) produced no deleterious effects in humans.

In a recent study (Chassaing et al., 2015) the effects of emulsifiers, including polysorbate 80, have been discussed. The Panel considered that if such effects occurred with polysorbates, then an increase in body weights would have been expected in subchronic, chronic toxicity and carcinogenicity studies. No such increase has been observed, and therefore the relevance of the observed effects remains unclear. According to the authors, additional studies will be needed to show the relevance of the effects seen in mice for human health. The Panel agreed with this conclusion.

The Panel considered the long-term toxicity and carcinogenicity studies in rats with a NOAEL at the level of 5 % (equivalent to 2 500 mg/kg bw/day) as the pivotal study for allocation of the ADI in consistence with the NOAEL defined in subchronic studies.

Exposure assessment for food additives under re-evaluation was carried out by the ANS Panel based on (1) MPLs set out in EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) the availability of adequate usage or analytical data (defined as the refined exposure assessment scenario).

To date, the ANS Panel has used the maximum concentration value (maximum reported use level or maximum value from the analytical results) available for each authorised food category. However, given the extensive range of data that have been made available through the most recent calls, the ANS Panel considered that this should also be used in additional scenarios of the exposure assessment approach intended to provide more realistic exposure estimates.

Analytical data on the content of polysorbates in food were not available; therefore, the refined exposure scenarios were based on use data only. The Panel calculated two estimates based on different assumptions: a brand-loyal consumer scenario, where it was assumed that the population is exposed long-term to the food additive present at the maximum reported use levels for one food category; and a non-brand-loyal scenario, where it was assumed that the population is exposed long-term to the food additive present at the mean reported use levels in the food.

Overall, the Panel considered the regulatory maximum level exposure assessment scenario as conservative, as it assumes that in all processed foods and beverages polysorbates (E 432 – E 436) are used as the food additives at the level of MPLs. The Panel considered that the refined exposure assessment approach was a more realistic scenario, as it was based on the range of use level data and assumed that the processed foods and beverages contain the additive at the mean level for all products (non-brand-loyal consumer scenario) and considers one product containing polysorbates at the maximum level (brand-loyal consumer scenario). For this exposure assessment scenario, food categories for which no or inadequate reported use levels were available were not considered in the exposure assessment. Therefore, the Panel noted that if polysorbates are nevertheless used in those food categories that are not considered in the exposure estimate, the calculated refined exposure

assessment might result in underestimation of exposure to polysorbates. The Panel also noted that the refined exposure estimates will not cover future changes in the level of use of polysorbates.

It should be mentioned that a high variability of use levels of food supplements, which may be dependent on the form (solid to be diluted, liquid, etc.) or by the specific brand of the product, could not be taken into consideration for the exposure assessment because of the lack of information and FoodEx linkage. As a consequence, exposure to polysorbates of consumers with a long term use of food supplements with high polysorbate levels might be underestimated by the calculated exposure ranges.

Using the *regulatory maximum level exposure assessment scenario*, mean exposure to polysorbates from its use as a food additive ranged from 0.7 mg/kg bw/day in adults and the elderly to 25.0 mg/kg bw/day in toddlers. The high exposure to polysorbates using this scenario ranged from 2.1 mg/kg bw/day in the elderly to 63.7 mg/kg bw/day in children. The Panel noted that exposure estimates of polysorbates (E 432–E 436) did not exceed the ADI at the mean exposure level but did exceed the ADI for all age groups at the high level (95th percentile). The main contributing food categories to the total mean exposure estimates for all population age groups in this scenario were fine bakery wares and flavoured fermented milk products in toddlers, and fine bakery wares and food supplements in other all population groups. Using the refined brand-loyal assessment exposure scenario, mean exposure to polysorbates from its use as a food additive ranged from 0.6 mg/kg bw/day in adults and the elderly to 18.1 mg/kg bw/day in children. The high exposure to polysorbates using this scenario ranged from 1.8 mg/kg bw/day in the elderly to 57.5 mg/kg bw/day in children. The Panel noted that exposure estimates of polysorbates (E 432–E 436) did not exceed the ADI for all age groups at the mean exposure level but did exceed the ADI for all age groups, except for adolescents, at the high level (95th percentile). The main contributing food categories were fine bakery wares and food supplements, except for toddlers, for which, besides fine bakery wares, desserts were also a very important contributor to the total mean exposure to polysorbates.

Using the refined non-brand-loyal assessment exposure scenario, mean exposure to polysorbates from its use as a food additive ranged from 0.3 mg/kg bw/day in adults and the elderly to 9.6 mg/kg bw/day in toddlers. The high exposure to polysorbates using this scenario ranged from 1.1 mg/kg bw/day in the elderly to 24.5 mg/kg bw/day in toddlers. The Panel noted that exposure estimates of polysorbates (E 432–E 436) did not exceed the ADI for all age groups at both the mean exposure level and the high level (95th percentile) but that the highest exposure of toddlers remains very close to the ADI. The main contributing foods were fine bakery wares, soups and desserts.

Considering only use levels reported by industry and assuming that polysorbates (E 432–E 436) are not used in the food categories where no use level was reported (refined exposure scenarios) resulted in lower exposure estimates for all age groups. According to this scenario, the food category 'flavoured fermented milk products', identified as one of the main contributing food categories in the MPL scenario (particularly in toddlers), is no longer a contributor to the total mean exposure to polysorbates. It has to be clarified whether lack of reported use levels can be interpreted as non-use of polysorbates in this food category, especially taking into account the usage of these additives was reported in the comparable food category of desserts (FCS 16).

The Panel noted that the exposure estimates of polysorbates (E 432–E 436) based on the MPL scenario could be considered as being conservative as it was assumed that all foods in which their use is authorised contain polysorbates (E 432–E 436) at the MPL. This was also true considering a possible small underestimation of exposure because of the exclusion of food categories FCS 02.2.2 'Fat and oil emulsions mainly of type water-in-oil' and FCS 05.4 'Decorations, coatings and fillings', in the exposure assessment. Exposure resulting from the use of polysorbates (E 432–E 436) in these food categories was assumed to be already considered in categories 'Fine bakery wares' and 'Other confectionery, including breath freshening microsweets, only sugar confectionery'. The requested extension of use of polysorbate 80 (E 433) of 700 mg/kg in FCS 07.2 'Fine bakery wares' is assumed

to be covered by the already existing regulation permitting up to 3 000 mg/kg for the group of polysorbates (E 432–E 436).

Exposure due to permitted uses under Annex III to Regulation No 1333/2008 on additives to be used in other additives or flavourings and nutrients could not be considered. Exposure to polysorbates may result from other sources, such as via their use as cosmetic ingredients, in personal care products, textiles and pharmaceuticals.

Ethylene oxide is an impurity in polysorbates which is classified as 'carcinogenic to humans (Category 1)'. The highest exposure to polysorbates using MPL scenario, which was found in children (64 mg/kg bw/day), will lead to an exposure to ethylene oxide of 12.7 ng/kg bw/day when the EU specification of 0.2 mg ethylene oxide/kg polysorbate is met.

For comparison, Benchmark Dose (Lower Confidence Limits; BMDLs) were calculated from the most sensitive animal studies using inhalation and were converted to the oral equivalents of 18.7 mg/kg bw/day for mice and 14.4 mg/kg bw/day for rats (Appendix D). From the rat BMDL, a Margin of Exposure (MOE) for ethylene oxide of at least 1.1×10^6 could be calculated, which would be considered a low risk. As, at other ages, the amounts are lower, this is an underestimate of the true MOE.

In reaching the conclusion that this route to extrapolation was valid, the Panel noted this was based on available data on the distribution of ethylene oxide and the patterns of tumours observed following oral and inhalation exposures. The Panel recognised that there was endogenous production of ethylene oxide, although data on tissue levels were limited. The Panel further agreed with the comment in the SCF opinion that it '*...is likely to be significant loss of ethylene oxide from foods during cooking*' (SCF, 2002b).

Regarding ethylene glycol impurities, the Tolerable Daily Intake (TDI) set by the SCF (2002b) is unlikely to be exceeded when the EU specification of 0.25 % ethylene glycols are met, taking into consideration the highest estimated exposures to polysorbates calculated in this opinion.

CONCLUSION

The Panel concluded that, based on the NOAEL of 2 500 mg/kg bw/day, identified from an oral carcinogenicity study with polysorbate 80 in rats, and applying an uncertainty factor of 100, a group ADI of 25 mg/kg bw/day for polysorbates 20, 80, 40, 60 and 65 (E 432, E 433, E 434, E 435 and E 436, respectively) can be established.

The non-brand-loyal scenario shows that the highest exposure of toddlers to polysorbates as a food additive remains very close to the ADI. Overall, the Panel concluded that the uncertainties identified would tend to an overestimation of the real exposure to polysorbates (E 432–E 436) as food additives in European countries by the MPL scenario but might underestimate real exposure by the refined scenarios. The Panel is aware that for three food categories no reported uses have been obtained and that other dietary sources of exposure to polysorbates have not been considered in this opinion and therefore more data (usage and analytical data) are needed to decrease uncertainties and to exclude the risk of underestimation in the refined exposure assessment scenario.

As regards the request for extension of use of polyoxyethylene sorbitan monooleate (E 433) as a whipping agent added to emulsifiers intended for fine bakery wares to a level of 700 mg/kg in the final food, it was assumed that no additional exposure to E 433 will result from this use, further to the exposure from its currently authorised use in fine bakery wares.

RECOMMENDATION

The Panel recommended that the maximum limits for the impurities of toxic elements (arsenic, lead, cadmium and mercury) in the EC specification for polysorbates (E 432–E 436) should be revised in

order to ascertain that polysorbates (E 432–E 436) as food additives will not be a significant source of exposure to those toxic elements in food.

DOCUMENTATION PROVIDED TO EFSA

1. ARBEITSANWEISUNG – Nr.:AAM-SA-012. 06.02.03. Submitted by EFEMA.
2. Tumor promoting and co-carcinogenic effects of some non-ionic lipophilic-hydrophilic agents. 1956. Submitted by EFEMA
3. PAFA, FDA, 2011. Polysorbate 20
4. PAFA, FDA, 2011. Polysorbate 40
5. PAFA, FDA, 2011. Polysorbate 60
6. PAFA, FDA, 2011. Polysorbate 65
7. PAFA, FDA, 2011. Polysorbate 80

REFERENCES

Abrar S and Trathnigg B, 2010. Separation of nonionic surfactants according to functionality by hydrophilic interaction chromatography and comprehensive two-dimensional liquid chromatography. Journal of Chromatography A, 1217, 8222–8229.

AOAC (American Organization of Analytical Chemists), 2000. AOAC Official Method 974.11. In: Official Methods of Analysis of AOAC International, 17th edition, Vol. II. Ed. Horwitz W.

Aoki M, Kamada A, Yata N, Kishimoto K, Mugino C, Sakaguchi H, Tanabe K and Mimura I, 1969. Studies on absorption of drugs. IV Effects of surface active agents on intestinal absorption of drugs. Chemical and Pharmaceutical Bulletin, 17, 1109–1114.

Ayorinde FO, Gelain SV, Johnson JH and Wan LW, 2000. Analysis of some commercial polysorbate formulations using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Communications in Mass Spectrometry, 14, 2116–2124.

Barcklow P, 1967. Gravimetric procedure for the quantitation and identification of polysorbate 80 in pickle products. Journal of the Association of Official Analytical Chemists, 50, 1265–1268.

Bartsch W, Sponer G, Dietmann K and Fuchs G, 1976. Acute toxicity of various solvents in the mouse and rat. Arzneimittelforschung, 26, 1581–1583.

Bates TR, Nightingale CH and Dixon E, 1973. Kinetics of hydrolysis of polyoxyethylene (20) sorbitan fatty acid ester surfactants. Journal of Pharmacy and Pharmacology, 25, 470–477.

Benigni R, Bossa C and Worth A, 2010. Structural analysis and predictive value of the rodent in vivo micronucleus assay results. Mutagenesis, 25, 335–341.

Benigni R, Bossa C, Tcheremenskaia O, Battistelli CL and Crettaz P, 2012. The new ISSMIC database on in vivo micronucleus and its role in assessing genotoxicity testing strategies. Mutagenesis, 27, 87–92.

Benigni R, Bossa C, Battistelli CL and Tcheremenskaia O, 2013a. IARC classes 1 and 2 carcinogens are successfully identified by an alternative strategy that detects DNA-reactivity and cell transformation ability of chemicals. Mutation Research, 758, 56–61. doi: 10.1016/j.mrgentox.2013.09.006

Benigni R, Bossa C and Tcheremenskaia O, 2013b. In vitro cell transformation assays for an integrated, alternative assessment of carcinogenicity: a data-based analysis. Mutagenesis, 28, 107–116.

BIBRA (British Industrial Biological Research Association), 1981. A short-term (13 week) study in rats with polyoxyethyLene (20) sorbitan monostearate. BIBRA Report No. 356/2/81 (also cited in SCF, 1985).

Borrego E, Sicilia D, Rubio S and Perez-Bendito D, 1999. Determination of polysorbates in foods by formation of mixed micelles. Analytica Chimica Acta, 384, 175–183.

Brandner JD, 1973. Unpublished report submitted by ICI America (cited in JECFA, 1974a).

Brueschweiler H and Hautfenne A, 1990. Determination of ester-emulsifiers components content after hydrolysis and silylation by gas chromatography: results of a collaborative study and the standardised method. Pure and Applied Chemistry, 62, 781–793.

Brush MK, McCoy JR, Rosenthal HL, Stauber LA and Allison JB, 1957. The addition of non-ionic surface-active agents of the polyoxyethylene type to the diet of the hamster, the mouse and the dog. Journal of Nutrition, 62, 601–619.

Burch R, Topping J and Haines J, 2007. Final report for food standards agency: review of literature relating to the extraction of additives from complex food matrices. Project number A01058. 86 pp. Available online: http://www.foodbase.org.uk/admintools/reportdocuments/599-1-1018_A01058.pdf

Chassaing B, Koren O, Goodrich J, Poole A, Srivivasan S, Ley R,and Gewirtz A. 2015. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome.Nature 14232/doi:10.1038

Chow B, Ling C Barrows L and Burnett JM, 1951. Effect of basal diets on the response of rats to certain dietary non-ionic surface active agents. Federation Proceedings, 10, 378.

Chow BF, Burnett JM, Ling CT and Barrows L, 1953. Effects of basal diets on the response of rats to certain dietary non-ionic surface-active agents. Journal of Nutrition, 49, 563–577.

Christiansen A, Backensfeld T, Denner K and Weitschies W, 2011. Effects of non-ionic surfactants on cytochrome P450-mediated metabolism *in vitro*. European Journal of Pharmaceutics and Biopharmaceutics, 78, 166–172.

Chusid E and Diamond J, 1955. Accidental massive overdose of monitan in an infant. Journal of Pediatrics, 46, 222–223.

CIR (Cosmetic Ingredient Review), 1984. Final Report on the Safety Assessment of Polysorbates 20, 21, 40, 60, 61, 65, 80, 81, and 85. International Journal of Toxicology, 3, 1–82.

Coors EA, Seybold H, Merk HF and Mahler V, 2005. Polysorbate 80 in medical products and nonimmunologic anaphylactoid reactions. Annals of Allergy, Asthma & Immunology, 95, 593–599.

Coppinger WJ, Brennan SA and Thompson ED, 1981. Testing of surfactants for mutagenic potential in the L5178Y TK+/- mouse lymphoma assay. Environmental Mutagenesis, 1, 320.

Cottrell T and van Peij J, 2004. Sorbitan esters and polysorbates. In: Emulsifiers in food technology. Ed. Whitehurst RJ. Blackwell Publishing Ltd, Oxford, UK, 162–185.

Culver PJ, Wilcox CS, Jones CM and Rose RSJ, 1951. Intermediary metabolism of certain polyoxyethylene derivatives in man. I. Recovery of the polyoxyethylene moiety from urine and faces following ingestion of polyoxyethylene (40) mono-stearate. Journal of Pharmacology and Experimental Therapeutics, 103, 377–381.

Gold LA, Sawyer CB, Magaw R, Backman GM, de Veciana M, Levinson R, Hooper NK, Havender WR, Bernstein L, Peto R, Pike MC, Ames B. 1984. A carcinogenic potency database of the standardised results of animal bioassays. Environmental Health Perspectives, 58, pp 9-319

Donbrow M, Azaz E and Pillersdorf A, 1978. Autoxidation of polysorbates. Journal of Pharmaceutical Sciences, 67, 1676–1681.

Eagle E and Poling CE, 1956. The oral toxicity and pathology of polyoxyethylene derivatives in rats and hamsters. Journal of Food Science, 21, 348–361.

EC (European Commission), 1997. SCOOP TASK 4.2 – Report on methodologies for the monitoring of food additive intake across the European Union.

EC (European Commission), 2001. Report from the Commission on Dietary Food Additive Intake in the European Union, Brussels, 01.10.2001, COM(2001) 542 final.

EFEMA (European Food Emulsifiers Manufacturers Association), 2009. Index of food emulsifiers, September 2009, 5th edition. Available online: http://www.emulsifiers.org/ViewDocument.asp?ItemId=21andTitle=E-numbers

EFSA (European Food Safety Authority), 2007. Guidance of the Scientific Committee on a request from EFSA related to Uncertainties in Dietary Exposure Assessment EFSA Journal 2006; 438, 2-54.

EFSA (European Food Safety Authority), 2009. Scientific opinion of the Panel on Contaminants in the Food Chain (CONTAM) on cadmium in food. The EFSA Journal 2009, 980, 1–139

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009a. Scientific opinion on arsenic in food. EFSA Journal 2009;7(10):1351, 199 pp. doi:10.2903/j.efsa.2009.1351

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2010. Scientific opinion on lead in food. EFSA Journal 2010;8(4):1570, 151 pp. doi:10.2903/j.efsa.2010.1570

EFSA (European Food Safety Authority), 2011a. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. doi:10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal 2011;9(3):1970, 27 pp. doi:10.2903/j.efsa.2011.1970

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579. [32 pp.]doi:10.2903/j.efsa.2012.2579.

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012a. Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 241 pp. doi:10.2903/j.efsa.2012.2985

Ema M, Hara H, Matsumoto M, Hirata-Koizumi M, Hirose A and Kamata E, 2008. Evaluation of developmental neurotoxicity of polysorbate 80 in rats. Reproductive Toxicology, 25, 89–99.

Ema M, Itami T, Kawasaki H and Kanoh S, 1988. Teratology study of Tween 60 in rats. Drug and Chemical Toxicology, 11, 249–260.

Enright B, Mclntyre B, Thackaberry E, Treinen K and Kopytek A, 2010. Assessment of hydroxypropyl methylcellulose, propylene glycol, polysorbate 80 and hydroxypropyl-β-cyclodextrin for use in developmental and reproductive toxicology studies. Birth Defects Research Part B: Developmental and Reproductive Toxicology, 89, 504–516.

Ershoff BH, 1976. Synergistic toxicity of food additives in rats fed a diet low in dietary fiber. Journal of Food Science, 41, 949–951.

Ershoff BH and Marshall WE, 1975. Protective effects of dietary fiber in rats fed toxic doses of sodium cyclamate and polyoxyethylene sorbitan monostearate (Tween 60). Journal of Food Science, 40, 357–361.

FCC (Food Chemicals Codex), 1996. Polysorbate 20, Polysorbate 60, Polysorbate 65, Polysorbate 80. In: Food Chemicals Codex, Committee on food chemicals codex (4th). Ed. Institute of Medicine. National Academies Press, Washington, DC, USA, 306–309.

Fitzhugh OG, Bourke AR, Nelson AA and Frawley JP, 1959. Chronic oral toxicities of four stearic acid emulsifiers. Toxicology and Applied Pharmacology, 1, 315–331.

Food Safety Commission of Japan, 2007. Evaluation report of food additives. Polysorbates (polysorbates 20, 60, 65 and 80. Available online: http://www.fsc.go.jp/english/evaluationreports/foodadditive/polysorbate_report.pdf

Gajdova M., Jakubovsky J, and Valky J. 1993. Delayed effects of neonatal exposure to tween 80 on emale reproductive organs in rats. Fd Chem. Toxic. 31, 183-190.

Gold et al., 1984.

GSFA (Codex General Standard for Food Additives), 2011. Online database: Polysorbates Updated up to the 34th Session of the Codex Alimentarius Commission. Available online: http://www.codexalimentarius.net/gsfaonline/groups/details.html?id=99

Hall MJ, 1964. Determination of polysorbate 80 in bakery products and frozen desserts. Journal of the Association of Official Agricultural Chemists, 47, 685–688.

Hannuksela M and Haahtela T, 1987. Hypersensitivity reactions to food additives. Allergy, 42, 561–575.

Hannuksela M, Kousa M and Pirila V, 1976. Contact sensitivity to emulsifiers. Contact Dermatitis, 2, 201–204.

Hardin BD, Schuler RL, Burg JR, Booth GM, Hazelden KP, MacKenzie KM, Piccirillo VJ and Smith KN, 1987. Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratogenesis, Carcinogenesis, and Mutagenesis, 7, 29–48.

Harris RS, Sherman H and Jetter WW, 1951a. Nutritional and pathological effects of sorbitan monolaurate, polyoxyethylene sorbitan monolaurate, polyoxyethylene monolaurate, and polyoxyethylene monostearate when fed to rats. Archives of Biochemistry and Biophysics, 34, 249–258.

Harris RS, Sherman H and Jetter WW, 1951b. Nutritional and pathological effects of sorbitan monolaurate, polyoxyethylene sorbitan monolaurate and polyoxyethylene monolaurate when fed to hamsters. Archives of Biochemistry and Biophysics, 34, 259–265.

Hewitt D, Alvarez M, Robinson K, Ji J, Wang Y, Kao Y and Zhang T, 2011. Mixed-mode and reversed-phase liquid chromatography-tandem mass spectrometry methodologies to study composition and base hydrolysis of polysorbate 20 and 80. Journal of Chromatography A, 1218, 2138–2145.

Hopper S, Hulpieu H and Cole V, 1949. Some toxicological properties of surface-active agents. Journal of the American Pharmaceutical Association. Scientific Edition, 38, 428–432.

Inoue K, Sunakawa T and Takayama S, 1980. Studies of *in vitro* cell transformation and mutagenicity by surfactants and other compounds. Food and Cosmetics Toxicology, 18, 289–296.

Ishidate M and Odashima S, 1977. Chromosome tests with 134 compounds on Chinese hamster cells *in vitro* - a screening for chemical carcinogens. Mutation Research, 48, 337–354.

Janowitz HD, Hollander F and Marshak RH, 1953. The effect of tween-65 and tween-80 on gastrointestinal motility in man. Gastroenterology, 24, 510–516.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1974a. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. WHO Food Additive Series No 5.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1974b. Toxicological evaluation of certain food additives with a review of general principles and of specifications (17th report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53; WHO Technical Report Series, No. 539, 1974, and corrigendum.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006a. Combined Compendium of Food Additive Specifications – all Specifications Monographs from the 1st to the 65th Meeting (1956–2005) Food Additives Vols 1–3, 10 pp.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2014. Combined Compendium of Food Additive Specifications – all Specifications Revised specifications prepared at the 79th JECFA (2014) published in FAO JECFA Monographs 16 (2014)

Jenssen D and Ramel C, 1980. The micronucleus test as part of a short-term mutagenicity test program for the prediction of carcinogenicity evaluated by 143 agents tested. Mutation Research, 75, 191–202.

Jones CM, Culver PJ, Drummey GD and Ryan AE, 1948. Modification of fat absorption in the digestive tract by the use of an emulsifying agent. Annals of Internal Medicine, 29, 1–10.

Kato H, Nagai Y, Yamamoto K and Sakabe Y, 1989. Determination of polysorbates in foods by colorimetry with confirmation by infrared spectrophotometry, thin-layer chromatography, and gas chromatography. Journal of the Association of Official Analytical Chemists, 72, 27–29.

Kavlock R, Short R and Chernoff N, 1987. Further evaluation of an *in vitro* teratology screen. Techn. Sci. Méthod, 7, 7–16.

Kerwin B, 2008. Polysorbates 20 and 80 used in the formulation of protein biotherapeutics: structure and degradation pathways. Journal of Pharmaceutical Sciences, 97, 2924–2935.

Kimura T and Yoshida A, 1982. Toxicity of detergent feeding and effect of the concurrent feeding of dietary fiber in rats. Nutrition Reports International, 26, 271–279.

Kimura T, Imamura H, Hasegawa K and Yoshida A, 1982. Mechanisms of toxicities of some detergents added to a diet and of the ameliorating effect of dietary fiber in the rat. Journal of Nutritional Science and Vitaminology, 28, 483–489.

Kishore RS, Pappenberger A, Dauphin IB, Ross A, Buergi B, Staempfli A and Mahler HC, 2011. Degradation of polysorbates 20 and 80: studies on thermal autoxidation and hydrolysis. Journal of Pharmaceutical Sciences, 100, 721–731.

Kocher-Becker U, Kocher W and Ockenfels H, 1981. Thalidomide-like malformations caused by a tween surfactant in mice. Zeitschrift für Naturforschung C, 36, 904–906.

Krantz JC, Culver PJ, Carr J and Jones CM, 1951. Sugar alcohols, XXVIII. Toxicologic, pharmacodynamic and clinical observations on Tween 80. Bulletin of the School of Medicine, 36, 48–56.

Langkilde S1, Schrøder M, Frank T, Shepherd LV, Conner S, Davies HV, Meyer O, Danier J, Rychlik M, Belknap WR, McCue KF, Engel KH, Stewart D, Knudsen I, Poulsen M 2012. Compositional and toxicological analysis of a GM potato line with reduced α-solanine content--a 90-day feeding study in the Syrian Golden hamster. Regul Toxicol Pharmacol. 2012, 64, 177-85. doi: 10.1016/j.yrtph.2012.06.018. Epub 2012 Jul 13

Li X, Wang L, Li Y, Ho Y, Yang D, Chen Y, Hu X and Xue M, 2011. Polysorbates as novel lipid-modulating candidates for reducing serum total cholesterol and low-density lipoprotein levels in hyperlipidemic C57BL/6J mice and rats. European Journal of Pharmacology, 660, 468–475.

Lundquist G and Meloan CE, 1971. Determination of polysorbates in food products by reaction gas chromatography. Analytical Chemistry, 43, 1122–1123.

McCorriston LR, 1952. Effect of an oral emulsifying agent on serum lipids in patients with chronic dermatoses. AMA Archives of Dermatology and Syphilology, 65, 224–227.

McKean D, Pesce A and Koo W, 1987. Analysis of polysorbate and its polyoxyethylated metabolite. Analytical Biochemistry, 161, 348–351.

Matsukura N, Kawachi T, Sano T, Sasajima K and Sugimura T, 1979. Promoting action of croton oil on gastrocarcinogenesis by N-methyl-N'-nitro-N-nitrosoguanidine in rats. Journal of Cancer Research and Clinical Oncology, 93, 323–327.

Mittal K, 1972. Determination of CMC of polysorbate 20 in aqueous solution by surface tension method. Journal of Pharmaceutical Sciences, 61, 1334–1345.

Morita K, Ishigaki M and Abe T, 1981. Mutagenicity of materials related with cosmetics. Journal of the Society of Cosmetic Chemists of Japan, 15, 243–253.

Nakata S and Kimura T, 1994. Mechanism involved in the ameliorating effect of dietary fiber on the toxicity of a non-ionic surface-active agent and amaranth added to the diet for rats. Bioscience, Biotechnology, and Biochemistry, 58, 998–1001.

Naya M, Imoto H, Sanai I, Mutoh H, Iida S and Kojima N, 1999. Effects of polysorbate 80 on dams and fetuses in rabbits. The Journal of Toxicological Science, 24, 353.

Nelson M, Poulos T, Gongwer L and Kirschman J, 1966. Preparations of Carbon-14-Labelled Polyoxyethylene (20) Sorbitan Monolaurate and their metabolic fate in rats. Journal of Food Science, 31, 253–258.

NTP (National Toxicology Program), 1992a. Toxicology and carcinogenesis studies of polysorbate 80 (CAS No 9005-65-6) in F344/N rats and B6C3F1 mice (Feed studies). Research Triangle Park, NTP. (Technical Report Series, NTP TR No. 415). 225 pp.

NTP (National Toxicology Program), 1992b. Final report on the developmental toxicity of polyoxyethylene sorbitan monolaurate (CAS No 9005-64-5) in Sprague–Dawley (CD (TradeName)) rats on gestational days 6 through 15 (includes Appendix): PB 93-123800. National Toxicology Program, Research Triangle Park, NC, USA, 128 pp.

NTP (National Toxicology Program), 1992c. Final report on the developmental toxicity of polyoxyethylene sorbitan monolaurate (CAS No 9005-64-5) in Sprague–Dawley (CD (TradeName)) rats on gestational days 6 through 15: laboratory supplement, sections 1–7A: PB 93-123818. National Toxicology Program, Research Triangle Park, NC, USA,178 pp.

NTP (National Toxicology Program), 1992d. Final report on the developmental toxicity of polyoxyethylene sorbitan monooleate (CAS No 9005-65-6) in Sprague–Dawley (CD (TradeName)) rats on gestational days 6 through 15. PB93-123826, 124 pp.

NTP (National Toxicology Program), 1992e. Final report on the developmental toxicity of polyoxyethylene sorbitan monooleate (CAS No 9005-65-6) in Sprague–Dawley (CD (TradeName)) rats: laboratory supplement, sections 1–7A. PB93-123834. National Toxicology Program, Research Triangle Park, NC, USA, 162 pp.

Ockenfels H, Kocher-Becker U and Kocher W, 1982. Effects of single and combined action of a hydrolysis product of thalidomide and the surfactant Tween 20 in mice. Teratology, 26, 19A.

Odunola O, Uwaifo A and Olorunsogo O, 1998. Comparative genotoxicities of Tween 20 and Tween 80 in *Escherichia coli* PQ37. Biokemistri, 8, 123–129.

Oser BL and Oser M, 1956a. Nutritional studies on rats on diets containing high levels of partial ester emulsifiers. I. General plan and procedures; growth and food utilization. Journal of Nutrition, 60, 367–390.

Oser BL and Oser M, 1956b. Nutritional studies on rats on diets containing high levels of partial ester emulsifiers. II. Reproduction and lactation. Journal of Nutrition, 60, 489–505.

Oser BL and Oser M, 1957a. Nutritional studies on rats on diets containing high levels of partial ester emulsifiers. III. Clinical and metabolic observations. Journal of Nutrition, 61, 149–166.

Oser BL and Oser M, 1957b. Nutritional studies on rats on diets containing high levels of partial ester emulsifiers. IV. Mortality and post-mortem pathology; general conclusions. Journal of Nutrition, 61, 235–252.

Öszi Z and Pethö G, 1998. Quantitative determination of polysorbate 20 in nasal pharmaceutical preparations by high-performance liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis, 18, 715–720.

Parfett CLJ, 1992. Induction of proliferin gene expression by diverse chemical agents that promote morphological transformation in C3H/10T1/2 cultures. Cancer Letters, 64, 1–9.

Paschall H, 1964. Effects of feeding emulsifiers on reproduction of C57BL/6 mice. Dissertation Abstracts International B, 24, 1764–1765.

Poling C, Eagle E and Rice E, 1956. Effects of feeding polyoxyethylene preparations to rats and hamsters. Journal of Food Science, 21, 337–347.

Pollard JM, Shi AJ and Göklen KE, 2006. Solubility and partitioning behavior of surfactants and additives used in bioprocesses. Journal of Chemical & Engineering Data, 51, 230–236.

Preston E, Hunt AD, McNair Scott TF and Spur B, 1953. Short-term feeding studies in infants and children with certain surface-active agents used in food technology: absence of evidence of toxic effects. The American Journal of Clinical Nutrition, 1, 539–550.

Price C, George J, Marr M, Myers C, Heindel J and Schwetz B, 1994. Developmental toxicity evaluation of polyoxyethylene sorbitan monolaurate (TW20) and polyoxyethylene sorbitan monooleate (TW80) in rats. The Toxicologist, 14, 163.

Ren X, Mao X, Cao L, Xue K, Si L, Qiu J, Schimmer A and Li G, 2009. Nonionic surfactants are strong inhibitors of cytochrome P450 3A biotransformation activity *in vitro* and *in vivo*. European Journal of Pharmaceutical Sciences, 36, 401–411.

Reyniers JP, Machado EA and Farkas WR, 1985. Hepatotoxicity of chemically-defined diets containing polysorbate 80 in germfree and conventional mice. In: Germfree research: microflora control and its application to the biomedical sciences. 181 Progress in clinical and biological research. Ed. Worstmann BS. Alan R. Liss, Inc., New York, USA, 91–94.

Roberts CL, Keita AV, Duncan SH, et al. Gut (2010). Translocation of Crohn's disease Escherichia coli across M-cells: contrasting effects of soluble plant fibres and emulsifiers doi:10.1136/gut.2009.195370

Roberts C, Rushworth S, Richman E, Rhodes J. 2013. Hypothesis: Increased consumption of emulsifiers as an explanation for the rising incidence of Crohn's disease. Journal of Crohn's and Colitis 7, 338-341

Rowe RC, 2009. Polyoxyethylene sorbitan fatty acid esters. In: Handbook of Pharmaceutical Excipients (6th ed.). Eds Rowe RC, Shesky PJ and Quinn ME. Pharmaceutical Press, London, UK, 551.

SCF (Scientific Committee for Food), 1985. Reports from the Scientific Committee for Food (15th series). Opinion expressed 8 July 1983. Food science and techniques. Available online: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_15.pdf

SCF (Scientific Committee for Food), 1995. Reports from the Scientific Committee for Food (34th series). Opinion expressed 1993. Food science and techniques. Available online: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_34.pdf

SCF (Scientific Committee on Food), 2002a. Opinion of the Scientific Committee on Food on impurities of ethylene oxide in food additives. Opinion expressed on 17 April 2002. SCF/CS/ADD/EMU/186 final. 6 May 2002. Available online: http://ec.europa.eu/food/fs/sc/scf/out127_en.pdf

SCF (Scientific Committee on Food), 2002b. Opinion of the Scientific Committee on Food on Impurities of 1,4-dioxane, 2-chloroethanol and mono- and diethylene glycol in currently permitted food additives and in proposed use of ethyl hydroxyethyl cellulose in gluten-free bread. Opinion expressed on 4 December 2002. SCF/CS/ADD/EMU/198 Final. 4 December 2002. Available online: http://ec.europa.eu/food/fs/sc/scf/out156_en.pdf

Schiweck H, Bär A, Vogel R, Schwarz E, Kunz M, Dusautois C, Clement A, Lefranc C, Lüssem B, Moser M and fried Peters S, 2012. Sugar alcohols. In: Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH, Weinheim, Germany.

Sergiel J, Paris R and Clement J, 1971. L'emploi des tweens en technologie alimentaire. Cahiers de Nutrition et de Dietetique, 5, 33–43.

Shirai T, Iaida K, Fukushima S, Hasegawa R and Tatematsu M, 1982. Effects of NaCl, Tween 60 and a low dose of N-ethyl-N′-nitro-N-nitrosoguanidine on gastric carcinogenesis of rat given a single dose of N-methyl-N'-nitro-N-nitrosoguanidine. Carcinogenesis, 3, 1419–1422.

Smullin CF, 1978. Quantitative determination of polysorbate to in non standard salad dressings. Journal of the Association of Official Analytical Chemists, 61, 506–507.

Smullin C, Wetterau F and Olsanski V, 1971. The determination of polysorbate 60 in foods. Journal of the American Oil Chemists' Society, 48, 18–20.

Snelling J, Scarff CA, and Scrivens JH, 2012. Characterization of complex polysorbate formulations by means of shape-selective mass spectrometry. Analytical Chemistry, 84, 6521−6529.

Snyderman SE, Morales S, Chung AW, Lewis JM, Messina A and Holt LE, 1953. Absorption of fat and vitamin A in premature infants. III. Effect of surface active agents on the absorption of these substances. Pediatrics, 12, 158–164.

Steele R, Limaye S, Cleland B, Chow J and Suranyi M, 2005. Hypersensitivity reactions to the polysorbate contained in recombinant erythropoietin and darbepoietin. Nephrology, 10, 317–320.

Steigmann F, Goldberg EM and Schoolman HM, 1953. The effect of emulsifying agents (tween 60 and span 60) on the gastrointestinal tract. American Journal of Digestive Diseases, 20, 380–384.

Sunakawa T, Inoue K and Okamoto K, 1981. Studies on the mutagenicity of surfactants. Mutagenicity of surfactants following activation with various liver homogenates (S-9) and mutagenicity in the presence of norharman. Eisei-Kagaku, 27, 204–211.

Tagesson C and Edling C, 1984. Influence of surface-active food additives on the integrity and permeability of rat intestinal mucosa. Food and Chemical Toxicology, 22, 861–864.

Thackaberry EA, Kopytek S, Sherratt P, Trouba K and McIntyre B, 2010. Comprehensive investigation of hydroxypropyl methylcellulose, propylene glycol, polysorbate 80, and hydroxypropyl-beta-cyclodextrin for use in general toxicology studies. Toxicological Sciences, 117, 485–492.

Tosti A, Guerra L, Morelli R and Bardazzi F, 1990. Prevalence and sources of sensitization to emulsifiers: a clinical study. Contact Dermatitis, 23, 68–72.

Treon JF, Gongwer LE, Nelson MF and Kirschman JC, 1967. Physiologic and metabolic patterns of non-ionic surfactants. In: Chemistry, physics and application of surface active substances (4th edition). Ed. Asinger F. Gordon and Breach, London, UK, 381–395.

Varma RK, Kaushal R, Junnarkar AY, Thomas GP and Shridhar DR, 1985. Polysorbate 80: pharmacological study. Arzneimittelforschung, 35, 804–808.

Vu Dang H, Gray A, Watson D, Bates C, Scholes P and Eccleston G, 2006. Composition analysis of two batches of polysorbate 60 using MS and NMR techniques. Journal of Pharmaceutical and Biomedical Analysis, 40, 1155–1165.

Waldstein SS, Schoolman HM and Popper H, 1954. The effect of feeding large amounts of emulsifiers polyoxyethylene (20) sorbitan monostearate (tween 60) and sorbitan monostearate (span 60) to humans. American Journal of Digestive Diseases, 21, 181–185.

Walpole AL, 1962. Observations upon the induction of subcutaneous sarcomata in rats. In: The morphological precursors of cancer. Ed. Severi L. Proceedings of an international conference held at the University of Perugia, 26th to 30th June, 1961, Perugia, pp. 83–88.

Wenger M, Bowman A, Thorsteinsson M, Little K, Wang L, Zhong J, Lee A and DePhillips P, 2005. An automated homogeneous method for quantifying polysorbate using fluorescence polarization. Analytical Biochemistry, 337, 48–54.

Wick A and Joseph L, 1956. The fate of ingested polyoxyethylene (20) sorbitan monostearate in rats. Journal of Food Science, 21, 250–253.

Williams J, Odum J, Lewis RW and Brady AM, 1997. The oral administration of polysorbate 80 to the immature female rat does not increase uterine weight. Toxicology Letters, 91, 19–24.

Wong TW, Juras DS and Wissler RW, 1959. Effect of concurrent feeding of tween 80 on the carcinogenicity of orally administered 3-methylcholanthrene. Journal of the National Cancer Institute, 22, 363–399.

Wood R, Foster L, Damant A and Key P, 2004. Analytical methods for food additives. CRC Press, Boca Raton, FL, USA.

Yao J, Dokuru D, Noestheden M, Park S, Kerwin B, Jona J, Ostovic D and Reid D, 2009. A quantitative kinetic study of polysorbate autoxidation: the role of unsaturated fatty acid ester substituents. Pharmaceutical Research, 26, 2303–2313.

Zhong B-Z, Stamm SC, Robbins S, Bryant D, Lan W, Xin W-F, Ma JKH, Whong W-Z and Ong T-M, 1997. Studies on the mutagenicity of mild gasification products of coal and their subfractions by the Salmonella/microsomal assay. Environmental Research, 72, 32–44.

APPENDICES

Appendix A. Summary of reported use levels (mg/kg) of polysorbates (E 432–E 436) provided by industry

FCS Category number	FCS Food category	MPL	Restrictions/ exceptions	Number of data	Reported use levels from industry		Information provided by	Comments
					Typical mean (range)	Highest maximum level		
01.4 [(a)]	Flavoured fermented milk products including heat-treated products	1 000		–	–	–	–	
01.8	Dairy analogues, including beverage whiteners	5 000	Only milk and cream analogues	2	255 (10–500)	5 000	EFEMA, CIAA	
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	10 000	Only fat emulsions for baking	2	2 250 (2 000–2 500)	5 000	EFEMA, CIAA	Not considered for exposure assessment, because authorisation is for fat emulsions for baking and it is assumed that this only is already covered in category 07.2
03	Edible ices	1 000		2	(0–500)	1 000	EFEMA, CIAA	
04.2.4.1 [(a)]	Fruit and vegetable preparations excluding compote	500	Only coconut milk	–	–	–	–	
05.2	Other confectionery including breath-freshening microsweets	1 000	Only sugar confectionery	2	300 (100–500)	1 000	EFEMA, CIAA	
05.3	Chewing gum	5 000		1	–	5 000	EFEMA	
05.4 [(a)]	Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4	1 000		–	–	–	–	It was assumed that this is already covered in category 07.2
07.2	Fine bakery wares	3 000		2	1 300 (600–2 000)	1 000	EFEMA, CIAA	
12.5	Soups and broths	1 000	Only soups	2	650 (500–800)	1 000	EFEMA, CIAA	

FCS Category number	FCS Food category	MPL	Restrictions/ exceptions	Number of data	Reported use levels from industry		Information provided by	Comments
					Typical mean (range)	Highest maximum level		
12.6	Sauces	5 000	Only emulsified sauces	2	(0–3 000)	5 000	EFEMA, CIAA	
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	1 000		1	260	1 300	SNE	
13.3	Dietary foods for weight-control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	1 000		1	-	1 300	CIAA	Values provided in the finished product as sold. Nevertheless, the product is always subject to dilution with water or milk. The doctor, based on age, weight, etc., determines the grade of dilution. These products are provided only under medical supervision and are generally used only in the short term as a high-energy supplement in certain disease states. The products are not used in children under 3 years of age. Typical intake (average) 1 × 85 g (adult); 1 × 42.5 g (child). Highest use 2 × 85 g (adult); 1 × 42.5 g (child)
16	Desserts excluding products covered in categories 01, 03 and 04	3 000		1	(0–2 000)	3 000	CIAA	
17.1/17.2/17.3	Food supplements	*Quantum satis*		227	9 914	425 000	AESGP, FSE, EHPM	

(a): Industries reported no use in this food category.

Appendix B. Concentration levels of polysorbates (E 432–E 436) used in the refined exposure scenarios (mg/kg)

FCS Category number	FCS Food category	MPL	Concentration levels used in the refined exposure assessment		Data source/comments
			Mean	Maximum	
01.4	Flavoured fermented milk products including heat-treated products	1 000	–	–	No data available
01.8	Dairy analogues, including beverage whiteners	5 000	255	5 000	Use levels
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	10 000	–	–	Not considered, assumed to be a covered in category 07.2
03	Edible ices	1 000	500	1 000	Use levels
04.2.4.1	Fruit and vegetable preparations excluding compote	500	–	–	No data available
05.2	Other confectionery including breath-freshening microsweets	1 000	300	1 000	Use levels
05.3	Chewing gum	5 000	–	5 000	Use levels
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4	1 000	–	–	No data available, assumed to be covered in category 07.2
07.2	Fine bakery wares	3 000	1 300	3 000	Use levels
12.5	Soups and broths	1 000	650	1 000	Use levels
12.6	Sauces	5 000			Use levels
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	1 000	260	1 000	Use levels; owing to unclear dilution of use level of 1 300 mg/kg in the final product, the highest possible value at the level of MPL was used
13.3	Dietary foods for weight-control diets intended to replace total daily use levels food intake or an individual use levels meal (the whole or part of the total daily diet)	1 000	1 000	1 000	Use levels; owing to unclear dilution of use level of 1 300 mg/kg in the final product, the highest possible value at the level of MPL was used
16	Desserts excluding products covered in categories 01, 03 and 04	3 000	2 000	3 000	Use levels
17.1/17.2/17.3	Food supplements	*Quantum satis*	9 914	425 000	Use levels

Appendix C. Summary of total estimated exposure to polysorbates (E 432–E 436) from their use as food additives for the MPL scenario and refined exposure scenarios per population group and survey: mean and high level (mg/kg bw/day)

Country	Survey	Number of subjects	MPL scenario		Refined scenario			
					Brand-loyal scenario		Non-brand-loyal scenario	
			Mean	High level[(a)]	Mean	High level[(a)]	Mean	High level[(a)]
Toddlers								
Belgium	Regional_Flanders	36	24.2	-	14.0	-	9.4	-
Bulgaria	NUTRICHILD	428	10.1	27.9	9.5	25.2	4.2	11.3
Denmark	IAT 2006_07	917	6.3	19.4	2.9	8.9	1.8	5.9
Finland	DIPP_2001_2009	500	5.0	19.4	1.4	5.1	0.5	2.3
Germany	VELS	348	13.0	29.5	9.5	23.5	5.5	16.3
Italy	INRAN_SCAI_2005_06	36	8.2	-	5.4	-	2.6	-
Spain	enKid	17	13.5	-	7.2	-	4.6	-
The Netherlands	VCP_kids	322	25.0	58.0	15.0	37.4	9.6	24.5
UK	NDNS-RollingProgrammeYears1-3	185	11.3	26.8	8.6	21.9	5.1	14.7
UK	DNSIYC_2011	1314	9.8	26.6	7.1	22.0	4.4	15.2
Children								
Austria	ASNS_Children	128	9.2	22.5	8.0	20.0	4.1	10.2
Belgium	Regional_Flanders	625	20.9	46.2	12.4	27.9	8.0	18.2
Bulgaria	NUTRICHILD	433	11.4	27.7	10.9	27.5	4.9	12.0
Czech Republic	SISP04	389	11.8	26.9	8.4	21.1	4.3	10.6
Denmark	DANSDA 2005-08	298	3.8	11.1	2.2	5.8	1.3	3.3
Finland	DIPP_2001_2009	750	22.7	63.7	18.1	57.5	1.5	3.7
France	INCA2	482	15.7	32.0	13.0	26.5	7.5	15.9
Germany	EsKiMo	835	11.8	38.3	10.5	36.4	2.7	7.7
Germany	VELS	293	15.2	32.1	11.7	26.5	5.8	13.5
Greece	Regional_Crete	838	10.7	24.6	9.0	21.4	4.6	11.3
Italy	INRAN_SCAI_2005_06	193	7.3	18.9	6.3	16.3	2.8	7.2

Country	Survey	Number of subjects	MPL scenario		Refined scenario			
					Brand-loyal scenario		Non-brand-loyal scenario	
			Mean	High level[(a)]	Mean	High level[(a)]	Mean	High level[(a)]
Latvia	EFSA_TEST	187	12.0	29.2	10.4	25.2	6.3	16.6
Spain	enKid	156	10.6	26.7	6.9	19.8	3.7	11.5
Spain	NUT_INK05	399	9.7	23.1	6.6	17.0	3.7	10.2
Sweden	NFA	1473	19.2	39.1	13.6	27.2	4.6	10.6
The Netherlands	VCP_kids	957	22.0	49.4	13.0	31.5	8.0	20.7
The Netherlands	VCPBasis_AVL2007_2010	447	23.6	56.5	16.5	41.8	6.9	16.3
UK	NDNS-RollingProgrammeYears1-3	651	16.1	46.6	13.4	39.7	4.4	10.3
Adolescents								
Austria	ASNS_Children	237	4.7	13.5	4.2	11.9	2.1	6.0
Belgium	Diet_National_2004	576	6.4	14.8	5.3	12.7	3.1	7.4
Cyprus	Childhealth	303	2.2	6.5	2.1	6.3	1.0	2.9
Czech Republic	SISP04	298	7.5	18	5.8	15.0	2.8	7.2
Denmark	DANSDA 2005-08	377	1.8	5.1	1.1	3.2	0.6	1.8
Finland	NWSSP07_08	306	8.4	22.2	6.5	20.2	0.8	2.2
France	INCA2	973	7.7	17.9	6.5	15.3	3.6	8.5
Germany	National_Nutrition_Survey_II	1011	4.8	14.3	4.0	12.2	2.1	6.9
Germany	EsKiMo	393	7.1	24	6.4	22.8	1.7	5.0
Italy	INRAN_SCAI_2005_06	247	4.2	12.1	3.7	10.5	1.6	4.8
Latvia	EFSA_TEST	453	7.5	19.6	6.5	16.6	4.0	9.7
Spain	AESAN_FIAB	86	4.2	11.0	3.6	9.8	1.8	4.6
Spain	enKid	209	6.1	15.4	4.7	12.5	2.4	6.7
Spain	NUT_INK05	651	5.0	12.7	4.0	10.4	2.1	5.5
Sweden	NFA	1018	8.9	21.8	6.2	15.1	2.5	6.6
The Netherlands	VCPBasis_AVL2007_2010	1142	11.9	31.0	8.5	22.0	4.1	10.1
UK	NDNS-RollingProgrammeYears1-3	666	6.3	16.2	5.2	13.1	2.0	5.6

Country	Survey	Number of subjects	MPL scenario		Refined scenario			
					Brand-loyal scenario		Non-brand-loyal scenario	
			Mean	High level[(a)]	Mean	High level[(a)]	Mean	High level[(a)]
Adults								
Austria	ASNS_Adults	308	8.3	16.0	7.4	14.0	2.7	6.9
Belgium	Diet_National_2004	1292	5.3	13.1	4.3	10.5	2.6	6.3
Czech Republic	SISP04	1666	4.4	10.0	3.7	8.7	1.3	4.1
Denmark	DANSDA 2005-08	1739	1.2	3.2	0.8	2.3	0.5	1.2
Finland	FINDIET2012	1295	14.8	41.6	12.9	38.5	1.7	4.8
France	INCA2	2276	4.5	10.6	3.7	8.9	2.1	5.1
Germany	National_Nutrition_Survey_II	10419	7.8	12.5	7.0	10.6	2.1	6.0
Hungary	National_Repr_Surv	1074	0.7	3.6	0.6	3.0	0.3	1.6
Ireland	NANS_2012	1274	13.4	40.6	12.4	36.5	1.5	3.9
Italy	INRAN_SCAI_2005_06	2313	3.5	7.3	3.1	6.5	0.8	2.4
Latvia	EFSA_TEST	1271	4.5	11.4	4.0	9.8	2.4	5.9
Romania	Dieta_Pilot_Adults	1254	0.8	2.6	0.7	2.2	0.4	1.3
Spain	AESAN	410	4.1	8.6	3.7	7.8	1.2	3.7
Spain	AESAN_FIAB	981	3.3	8.1	3.0	7.3	1.2	3.6
Sweden	Riksmaten 2010	1430	17.7	13.5	16.9	11.3	1.9	5.3
The Netherlands	VCPBasis_AVL2007_2010	2057	9.9	25.1	8.0	20.4	2.7	7.0
UK	NDNS-RollingProgrammeYears1-3	1266	9.0	23.9	8.2	22.0	1.4	3.8
Elderly and very elderly								
Austria	ASNS_Adults	92	6.0	13.8	5.3	11.8	2.6	6.7
Belgium	Diet_National_2004	1215	6.0	12.8	5.0	10.8	2.9	6.9
Denmark	DANSDA 2005-08	286	1.1	3.2	0.7	2.2	0.4	1.2
Finland	FINDIET2012	413	14.6	37.4	13.1	33.8	1.5	4.3
France	INCA2	348	3.5	9.1	3.0	8.0	1.7	4.5
Germany	National_Nutrition_Survey_II	2496	4.8	12.1	4.1	10.7	2.1	5.8

Country	Survey	Number of subjects	MPL scenario		Refined scenario			
					Brand-loyal scenario		Non-brand-loyal scenario	
			Mean	High level[(a)]	Mean	High level[(a)]	Mean	High level[(a)]
Hungary	National_Repr_Surv	286	0.8	3.9	0.8	3.8	0.3	1.7
Ireland	NANS_2012	226	18.5	47.7	17.3	42.9	1.7	4.5
Italy	INRAN_SCAI_2005_06	518	3.3	7.2	3.1	6.4	0.6	1.9
Romania	Dieta_Pilot_Adults	128	0.7	2.1	0.6	1.8	0.4	1.1
Sweden	Riksmaten 2010	367	4.4	10.9	3.6	8.3	1.9	5.0
The Netherlands	VCPBasis_AVL2007_2010	173	10.5	29.7	8.7	24.8	2.8	6.4
The Netherlands	VCP-Elderly	739	11.6	36.6	9.8	32.9	2.8	7.0
UK	NDNS-RollingProgrammeYears1-3	305	10.3	28.5	9.0	26.6	2.0	5.0

(a): The 95th percentile estimates obtained on dietary surveys/age classes with fewer than 60 observations may not be statistically robust (EFSA, 2011a). Those estimates were not included in this table.

Appendix D. BMDLs for ethylene oxide from inhalation studies and calculations of the oral equivalent dose

For ethylene oxide, the most sensitive animal studies were conducted using inhalation and the BMDL10 was calculated from these studies.[22],[23] The dose for cancer excess of 1 in 10^5 (BMDL10/10 000) was calculated. Thereafter, an equivalent oral dose for the cancer risk was estimated. [24] This resulted in a calculated oral equivalent dose for the BMDL itself of 18.7 mg/kg bw/day for mice and 14.4 mg/kg bw/day for rats.

BMDL10 range (mg/m3)	Mouse: 16.05–32.07 Rat: 22.54–66.70
Lowest BMDL10 (mg/m3)	Mouse: 16.05 Rat: 22.54
Model for lowest BMDL10	Mouse: Multi-stage cancer (1st order) Rat: Log logistic
Dose conversion	Mouse: /24*6 (h/d) /7*5 (d/wk) Rat: /24*6 (h/d) /7*5 (d/wk)
Dose for cancer excess of 1 in 10^5 (BMDL10/10 000)	Mouse: 0.29 µg/m^3 Rat: 0.40 µg/m^3
Convert to oral	Mouse: 0.338 µg/kg bw/d (Mouse: 0.035 m^3 air/d, 30 g) Rat: 0.255 µg/kg bw/d (Rat: 0.223 m^3 air/d, 350 g)

[22] http://www.food.gov.uk/science/research/foodcomponentsresearch/t01programme/t01projlist/t01051

[23] http://www.food.gov.uk/sites/default/files/T01051%20Final%20Report%20-%20Annex%206%20-%20Appendix%201%20doc%20BMD%20estimation.pdf

[24] http://www.food.gov.uk/sites/default/files/T01051%20Final%20Report%20-%20Annex%206%20Objective%204%20Interpretation%20of%20MOEs.pdf

ABBREVIATIONS

ADI	Acceptable Daily Intake
ANS	Panel on Food Additives and Nutrient Sources added to Food
AOAC	American Organization of Analytical Chemists
BIBRA	British Industrial Biological Research Association
BMDL	Benchmark Dose (Lower Confidence Limits)
BSP	bromosulphophthalein
bw	body weight
CAS	Chemical Abstracts Service
CIAA	Confederation of Food and Drink Industries of the European Economic Community
CIR	Cosmetic Ingredient Review
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
EC	European Commission
EFEMA	European Food Emulsifiers Manufacturers Association
EINECS	European Inventory of Existing Commercial chemical Substances
ESIS	European chemical Substances Information System
FAO/WHO	Food and Agriculture Organization/World Health Organization
FCC	Food Chemicals Codex
FCS	Food Categorisation System
GD	gestation day
GSFA	Codex General Standard for Food Additives
HDL	high-density lipoprotein
HPLC	high-performance liquid chromatography
IC	inhibitory concentration
i.v.	intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD	lethal dose
LDL	low-density lipoprotein
MA	metabolic activation system
MOE	Margin of Exposure
MPL	Maximum Permitted Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development

PEG	polyethylene glycol
POE	polyoxyethylene
PND	postnatal day
PS	physiological saline
QS	*quantum satis*
SCF	Scientific Committee on Food
SCOOP	Scientific Co-operation
TLC	thin-layer chromatography
TDI	Tolerable Daily Intake
TMDI	Theoretical Maximum Daily Intake