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SCIENTIFIC OPINION

Re-evaluation of sucralose (E 955) as a food additive and evaluation of a new application on extension of use of sucralose (E 955) in fine bakery wares

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

The present opinion deals with the re-evaluation of sucralose (E 955) as food additive and with the safety of a proposed extension of use in food category (FC) 7.2 'Fine bakery wares'. Based on the available data, no safety concerns arose for genotoxicity of sucralose (E 955) and its impurities and degradation products. Based on the weight of evidence (WoE), the Panel considered the decrease in body weight observed in rats as the relevant endpoint for the derivation of a reference point (RP). The Panel performed a benchmark dose (BMD) analysis on the data from the longest study (combined chronic and carcinogenicity study) with a modified benchmark dose response to account for the poor palatability of sucralose. The resulting RP was 55 mg/kg bw per day (benchmark dose lower confidence limit; BMDL). The Panel considered it appropriate to derive chemical-specific assessment factor for sucralose and concluded that there is no need to revise the current ADI of 15 mg/kg bw per day of sucralose (E 955) previously established by the Scientific Committee on Food. The exposure estimates considering the currently authorised uses did not exceed the ADI. Therefore, the Panel concluded that there is no safety concern at the reported uses and use levels of sucralose (E 955). The overall exposure did not increase substantially when considering the proposed extension of use. However, based on the available data and the identified uncertainties regarding the potential formation of chlorinated compounds under the wide range of baking processes that may be applicable for FC 7.2, the Panel could not conclude on the safety of the proposed extension of use of E 955 in this FC. The Panel issued recommendations to the European Commission, primarily to consider a revision of the EU specifications for sucralose.

KEYWORDS

E 955, food additive, sucralose, sweetener

CONTENTS

1 | INTRODUCTION

The present opinion deals with the re-evaluation of sucralose (E 955) when used as a food additive and with the evaluation of an application for the extension of use for sucralose (E 955) in energy-reduced or without added sugar fine bakery wares (food category, FC 7.2).

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

1.1.1 | Background

1.1.1.1 | *Re-evaluation of sucralose (E 955) as a food additive under Commission (EU) No 257/2010*

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

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

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee 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 of 2001. The report "Food additives in Europe 2000" 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 a highest priority.

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

1.1.1.2 | *Safety of the proposed extension of use for sucralose (E 955) in energy-reduced or without added sugar fine bakery wares*

The use of food additives is regulated under the European Parliament and Council Regulation (EC) No 1333/2008 on food additives. Only food additives that are included in the Union list, in particular in Annex II to that Regulation, may be placed on the market and used in foods under the conditions of use specified therein. Moreover, food additives shall comply with the specifications as referred to in Article 14 of that Regulation and laid down in Commission Regulation (EU) No 231/2012.[3]

Sucralose (E 955) is a sweetener, which is authorised for use as a food additive in the Union. Since sucralose was permitted as a sweetener in the Union before 20 January 2009, it belongs to the group of food additives that are subject to a new risk assessment by EFSA.

The European Commission received an application from the company Aegis Holding NV for a modification of the conditions of use of sucralose. In particular, the applicant requests an extension of use in energy-reduced or without added sugar fine bakery wares (i.e. the food category 7.2 of part E of Annex II to Regulation (EC) No 1333/2008). The applicant requests the maximum use level of 700 mg/kg. According to the applicant, the use of sucralose in fine bakery wares will make available products with reduced energy or no added sugar, which would be possible without increasing the fat content of the product.

Taking into account that sucralose (E 955) is subject to a new risk assessment by the European Food Safety Authority (EFSA), in accordance with the re-evaluation programme laid down in Regulation (EU) No 257/2010, it is appropriate to address the safety of the proposed extension of use within the scientific opinion re-evaluating the safety of that food additive.

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

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

[3]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 Text with EEA relevance. OJ L 83, 22.3.2012, p. 1–295.

1.1.2 | Terms of Reference

1.1.2.1 | *Re-evaluation of sucralose (E 955) as a food additive under Commission (EU) No 257/2010*

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 the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

1.1.2.2 | *Safety of the proposed extension of use for sucralose (E 955) in energy-reduced or without added sugar fine bakery wares*

The European Commission requests the European Food Safety Authority to provide a scientific opinion on the safety of the proposed extension of use of sucralose (E 955) in energy-reduced or without added sugar fine bakery wares (food category 7.2 of part E of Annex II to Regulation (EC) No 1333/2008), with a maximum use level of 700 mg/kg, in accordance with Regulation (EC) No. 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.[4]

1.1.3 | Information on existing authorisations and evaluations

Sucralose (E 955) is authorised as a food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives and specifications have been defined in the Commission Regulation (EU) No 231/2012.

Sucralose (named also trichlorogalactosucrose or TGS)[5] was evaluated by JECFA (1989, 1991). In its latest evaluation JECFA set an acceptable daily intake (ADI) of 15 mg/kg bw per day (JECFA, 1991). The ADI was based on a no observed effect level (NOEL) of 1500 mg/kg bw per day in a long-term study (2 years) in rats, which also included a period of exposure in utero. An uncertainty factor of 100 was applied to derive the ADI. JECFA also recommended further immunotoxicity studies to assess the significance of observed weight changes in the spleen and thymus and changes in lymphocyte counts in rats, given that the Committee had been unable to rule out a causal relationship between these findings and high levels of exposure to sucralose.

The Scientific Committee on Food (SCF) published its first opinion on sucralose in 1989. At the time the Committee was unable to establish an ADI due to some unresolved questions concerning some treatment-related effects observed on body weight, organ weight and haematological parameters. While some of these concerns could partially be explained by the decreased food intake due to poor palatability of the diet, the Committee could not completely exclude the possibility of a direct toxic effect of sucralose (SCF, 1989). The Committee raised some concerns also regarding adverse findings relating to the immune system, particularly, changes in thymus and spleen weights, as well as a 'sporadic but not entirely random statistically significant' reductions in peripheral white blood cells and lymphocytes counts. Reference was also made to the weak mutagenic activity of 1,6-dichloro-1,6-dideoxyfructose (1,6-DCF), one of the degradation products of sucralose (SCF, 1989).

In the latest evaluation by SCF (2000), the Committee considered the new studies sufficient to rule out the concerns previously raised. The Committee concluded that for the effects on the immune system there was a clear NOEL of 3000 mg/kg bw per day. A lower NOEL of 350 mg/kg bw per day was set for maternal gastrointestinal effects from a reproductive toxicity study in rabbits. However, after further consideration of the data including comparative toxicokinetic studies in rabbits and rats, this effect was considered an effect not relevant to humans. The pivotal effect on which the ADI was based was the consistent reduction in body weight gain observed in several rats' studies.

Effects on body weight gain were observed at 0.3% in the diet (reported by the SCF as equivalent to around 150 mg/kg bw per day) in the chronic toxicity/carcinogenicity study and in the parental animals and offspring in the reproductive toxicity study. Reductions in food consumption around 10% or less compared with controls, not dose-related, were observed in both studies. Taking into account this and the results of a pair feeding/dietary restriction study, the Committee identified a no observed adverse effect level (NOAEL) of 1500 mg/kg bw from a 26-week gavage study and derived an ADI of 15 mg/kg body per day applying an assessment factor of 100 (SCF, 2000).

In 2016, the EFSA ANS Panel evaluated the request for an extension of use of sucralose in food for special medical purposes in young children, aged 1–3 years. The Panel concluded that the request for the extension of use at the proposed level of 400 mg/kg was of no safety concern (EFSA ANS Panel, 2016).

[4]Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, p. 1.

[5]It is noted that E 955 was also named trichlorogalactosucrose or TGS in the context of its previous evaluations, for clarity in the present opinion the name of E 955 as in Commission Regulation (EU) No 231/2012 i.e. sucralose will be used.

A year later, upon request of the European Commission, the EFSA ANS Panel (2017) issued a statement on the validity of the conclusions of a mouse study on the carcinogenic potential of sucralose (E 955) performed by the Ramazzini Institute. Taking into consideration the publication from Soffritti et al. (2016) and additional information provided by the Ramazzini Institute, the ANS Panel concluded that the available data did not support the conclusions of the authors that sucralose induced haematopoietic neoplasia in male Swiss mice (EFSA ANS Panel, 2017).

In ECHA (online)[6] it is stated that this substance is used in the following products: pharmaceuticals, photo-chemicals and cosmetics and personal care products. This substance is used in the following areas: health services.

Furthermore, sucralose (CAS No 56038-13-2) is permitted as skin conditioning in cosmetics products (European Commission-CosIng[7]).

2 | DATA AND METHODOLOGIES

The current risk assessment was carried out by the EFSA Panel on Food Additives and Flavourings (FAF Panel) in the context of Regulation (EC) No 257/2010. Structured protocols on hazard identification and characterisation (EFSA, 2020a; EFSA FAF Panel, 2023) and on exposure assessment (EFSA, 2020b; EFSA FAF Panel, 2024b) were developed in line with the principles of the EFSA PROMETHEUS project (PROmoting METHods for Evidence Use in Scientific assessments; EFSA, 2015). These protocols define the strategy to be applied for collecting and selecting data, appraising the relevant evidence and analysing and integrating the evidence to draw conclusions that will form the basis for the scientific opinions of sweeteners.

The draft protocol for the hazard identification and characterisation of sweeteners was published on EFSA's website for comments, and the online public consultation was made available until 19 September 2019. A technical report on the outcome of this public consultation with the overview of the comments received and the general responses from EFSA was published (EFSA, 2020a). During the implementation phase, some amendments and further elaborations to the original protocol were introduced. The changes introduced are documented in the revised version published in April 2023 (EFSA FAF Panel, 2023) and were followed for the preparation of the present opinion.

The draft protocol for assessing dietary exposure to sweeteners was published on EFSA's website for comments, and the online public consultation was made available until 22 November 2019. A technical report on the outcome of this public consultation with the overview of the comments received and the general responses from EFSA was published (EFSA, 2020b). This protocol was further revised, and the changes introduced are documented in the revised version published in December 2024 (EFSA FAF Panel, 2024a).

2.1 | Data

The FAF Panel was not provided with a newly submitted dossier for the re-evaluation of sucralose (E 955). In accordance with Regulation (EU) No. 257/2010, EFSA launched public calls for data[8,9,10,11,12] and contacted interested parties that had replied to the call for data to collect additional clarification or supplemental information (Documentation provided to EFSA n. 1–45).

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature, up to 4 March 2025. In line with the established methodology, literature published outside this timeframe will not be considered in the assessment. Clarifications on the data submitted by the IBOs were also provided in technical hearings held on: 22 February 2024 during the 53rd meeting of the FAF Panel Working Group on Re-evaluation of Sweeteners; on 6 March 2023 during the 45th meeting of the FAF Panel Working Group on Re-evaluation of Sweetener; on 5 May 2022 during the 38th meeting of the FAF Panel Working Group on Re-evaluation of Sweetener, or in a clarification teleconference held on 10 August 2021. The steps followed for the acquisition of data and their selection are documented in Appendix A.

The Panel is aware of an ongoing complaint[13] in relation to a publication from Schiffman et al. (2023) which was part of the data available on absorption, distribution, metabolism and excretion (ADME) and genotoxicity. EFSA contacted the Journal and its Editorial office[14] to ascertain whether any actions have been taken in response to the ongoing complaint.

[6] https://echa.europa.eu/it/substance-information/-/substanceinfo/100.054.484?_disssubsinfo_WAR_disssubsinfoportlet_substanceId=100.054.484

[7] Available online: https://ec.europa.eu/growth/tools-databases/cosing/details/59924

[8] Available online: https://www.efsa.europa.eu/en/data/call/170621

[9] Available online: https://www.efsa.europa.eu/en/consultations/call/call-technical-data-sweeteners-authorised-food-additives-eu

[10] Available online: https://www.efsa.europa.eu/en/call/call-data-genotoxicity-data-sweeteners

[11] Available online: https://www.efsa.europa.eu/en/call/open-call-food-additive-occurrence-data-food-and-beverages-intended-human-consumption-2

[12] Available online: https://www.efsa.europa.eu/en/call/call-technical-data-sucralose-e-955

[13] *TC HEARTLAND LLC v. SCHIFFMAN, 1:23-cv-00665 – CourtListener.com*

[14] https://www.tandfonline.com/journals/uteb20

At the time of the finalisation of this scientific opinion the reply was still pending. The status of the publication on the Journal website[15] was verified on the date of the adoption of the opinion (10/12/2025) and no actions were publicly communicated in relation to this publication. In consideration of this, the data reported in the publication were included in the assessment. By contrast, the Panel decided to exclude from its assessment a second publication co-authored by the defendant to the complaint, in light of the Editor's expression of concern accompanying the paper (Editor and Publisher of *Journal of Toxicology and Environmental Health, Part A, 2024*[16]). In any event, the data reported in the mentioned publication of 2023 are not regarded as decisive for the genotoxicity assessment of this scientific opinion (see Section 3.5.3 and Annex A).

Food consumption data used to estimate the dietary exposure to sucralose (E 955) were derived from the EFSA Comprehensive European Food Consumption Database[17] (Comprehensive Database). The Mintel's Global New Products Database (GNPD)[18] was checked to identify the uses of sucralose (E 955) in food and beverage products and food supplements. The Mintel's GNPD is an online database that contains the compulsory ingredient information present on the label of numerous products.

Mintel's GNPD is not a database managed by EFSA, data retrieved are presented in their original form and are not further scrutinised by the Panel.

For the proposed extension of use for sucralose (E 955) in energy-reduced or without added sugar fine bakery wares, the FAF Panel was provided with a newly submitted dossier (Documentation provided to EFSA n. 24).

2.2 | Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee. In line with these principles, this risk assessment was carried out based on structured protocols on hazard identification and characterisation of sweeteners (EFSA, 2020a; EFSA FAF Panel, 2023) and on exposure assessment (EFSA, 2020b; EFSA FAF Panel 2024b).

The FAF Panel assessed the safety of sucralose (E 955) as a food additive in line with the principles laid down in Regulation (EU) No. 257/2010 and in the relevant guidance documents: Guidance on submission for food additive evaluations by the SCF (SCF, 2001) and the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

In animal studies, when the test substance is administered in the feed or in the drinking water, but doses are not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake is calculated by the Panel using the relevant default values. In case of rodents, the values indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012) are applied. The Panel considered it appropriate to use the generic default values from the EFSA Scientific Committee Guidance document (2012) for studies in which both males and females were treated, while for studies in which only males or females were treated the respective sex specific default values were used. As regard to the duration of the studies, the Panel used the default values for sub-acute studies for all studies of duration lower than 8 weeks, the default values for sub-chronic studies for studies of duration equal or longer than 8 weeks up to 1 year and the default value for chronic studies for studies of duration equal or longer than 1 year. In the case of other animal species than rodents, the default values used by JECFA (2000) are used. In these cases, the dose is expressed as 'equivalent to mg/kg bw per day'. If a concentration in feed or drinking water was reported and the dose in mg/kg bw per day was calculated (by the authors of the study report or by the Panel) based on these reported concentrations and on reported consumption data for feed or drinking water, the dose is expressed as 'equal to mg/kg bw per day'. When in adult human studies (aged above 18 years) the dose of the test substance administered was reported in mg/person per day, the dose in mg/kg bw per day is calculated by the Panel using a body weight of 70 kg as default for the adult population as described in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012).

Animal studies retrieved in the literature search and performed with commercial mixtures containing sucralose (Barrios-Correa et al., 2018; Contreras-Chavez et al., 2021; de-la-Cruz et al., 2018; Ghasemi & Kahnamooi, 2020; Hernandez Garcia et al., 2018; Kahnamoei & Ghaleh, 2016; Malbert et al., 2019; Martinez-Carrillo et al., 2019; Mendoza-Pérez, García-Gómez, et al., 2021; Mendoza-Pérez, Guzmán-Gómez, et al., 2021; Olivier-Van Stichelen et al., 2019; Ramos-Garcia et al., 2021; Risdon et al., 2020; Rodriguez-Palacios et al., 2018; Saada et al., 2013; Shi et al., 2018) were not considered further in the assessment because of the low or in some cases unknown percentage of sucralose in the mixture.

Epidemiological studies in which the exposure could not be estimated were excluded from the assessment (Ajami et al., 2020; Fernandes et al., 2013; Memon et al., 2011, 2014; Mohan et al., 2024; Young et al., 2019). Epidemiological studies on commercial mixtures were considered only if the exposure could be estimated e.g. if the composition of the commercial mixture was clearly reported in the study.

In the case of genotoxicity, studies were evaluated according to the approach outlined in the revised protocol (EFSA FAF Panel, 2023). For the other toxicological endpoints, the methods for hazard identification, including the assessment

[15] https://doi.org/10.1080/10937404.2023.2213903, checked on the 10 December 2025

[16] Full article: Expression of Concern: Splenda Alters Gut Microflora and Increases Intestinal P-Glycoprotein and Cytochrome P-450 in Male Rats

[17] Available online: https://www.efsa.europa.eu/en/data-report/food-consumption

[18] Available online: http://www.gnpd.com/sinatra/home/

of internal validity for individual studies (risk of bias, RoB) and the assessment of the body of evidence across all health outcomes, are described in the revised protocol and detailed in Appendix A. In brief, following data retrieval and screening for relevance, a RoB evaluation was performed and the studies were classified into tier from 1 to 3. In the current opinion, relevant studies retrieved from the literature with moderate to low RoB (tier 1 and 2) were considered and included in the weight of evidence (WoE) evaluation. In accordance with the revised protocol, studies previously evaluated by the SCF and considered for setting an ADI were also subjected to a RoB evaluation.

During the WoE evaluation, ratings of initial confidence (expressed as 'high', 'moderate', 'low' or 'very low') were assigned to all studies based on study design for each relevant, reported outcome. For each outcome across studies, the initial confidence rating could be downgraded based on either a concern for bias across studies, unexplained inconsistency, relevance of studies and/or imprecision; similarly, it could be upgraded based on the magnitude of effect, dose response, consideration of residual confounding (human studies only) and consistency across study designs and experimental model systems (NTP-OHAT, 2019). The confidence ratings for an outcome or group of outcomes were translated into levels of evidence for adverse effects or no adverse effects on health. The following descriptors were used to rate the level of evidence for the presence of adverse effects on health: 'high', 'moderate', 'low' or 'inadequate when the confidence in the body of evidence is 'high', 'moderate', 'low' or 'very low', respectively. When no adverse effects on health are identified, the following descriptors for level of evidence were used: 'high', 'moderate' and 'inadequate' corresponding to confidence in the body of evidence ratings of 'high', 'moderate' and 'low/very low', respectively. More details on the WoE procedure are outlined in step 1.14 of the revised protocol on hazard identification and characterisation and the US National Toxicology Program (NTP) Handbook for conducting a literature-based health assessment (NTP-OHAT, 2019), with some modifications. The integration of animal and human data was based on the highest level of evidence rating for an adverse or no adverse effect on health. Hazard identification conclusions i.e. expressions of likelihood of an association between intake of sucralose (E 955) and adverse effect on health, were reached on groups of toxicological outcomes following a guidance developed by the FAF Panel (EFSA FAF Panel, 2023).

Dietary exposure to sucralose (E 955) from its use as a food additive was estimated by combining food consumption data available within the EFSA Comprehensive Database with the maximum levels according to Annex II to Regulation (EC) No 1333/2008 and with reported use levels and analytical data submitted to EFSA following public calls for data. The exposure was calculated according to different scenarios (see Section 3.4).

Finally, uncertainties in the hazard identification, characterisation and exposure assessment were identified and discussed.

3 | ASSESSMENT

3.1 | Technical data

3.1.1 | Identity of the substance and specifications

Sucralose is 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside.

Specifications for sucralose (E 955), as laid down in the Commission Regulation (EU) No 231/20123 and JECFA (2006), are listed in Table 1.

TABLE 1 Specifications for sucralose (E 955) according to Commission Regulation (EU) No 231/2012 and the JECFA (2006).

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Synonyms	4,1′,6′-Trichlorogalactosucrose	4,1′,6′-Trichlorogalactosucrose; INS No. 955
Definition		
CAS no		56038-13-2
Einecs	259-952-2	
Chemical name	1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside	1,6-Dichloro-1,6-dideoxy-ß-D-fructofuranosyl-4-chloro-4-deoxy-alpha-D-galactopyranoside
Chemical formula	$C_{12}H_{19}Cl_3O_8$	$C_{12}H_{19}Cl_3O_8$
Molecular weight	397.64	397.64
Assay	Content not less than 98% and not more than 102% $C_{12}H_{19}Cl_3O_8$ calculated on an anhydrous basis	Not less than 98% and not more than 102% calculated on an anhydrous basis
Description	White to off-white, practically odourless, crystalline powder	White to off-white, practically odourless crystalline powder

TABLE 1 (Continued)

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Identification		
Solubility	Freely soluble in water, methanol and ethanol. Slightly soluble in ethyl acetate	Freely soluble in water, methanol and ethanol; slightly soluble in ethyl acetate (Vol. 4)
Infrared absorption spectrum	The infrared spectrum of a potassium bromide dispersion of the sample exhibits relative maxima at similar wave numbers as those shown in the reference spectrum obtained using a sucralose reference standard	The infrared spectrum of a potassium bromide dispersion of the sample corresponds with the reference infrared spectrum in the Appendix
Thin layer chromatography	The main spot in the test solution has the same Rf-value as that of the main spot of standard solution A referred to in the test for other chlorinated disaccharides. This standard solution is obtained by dissolving 1 g of sucralose reference standard in 10 mL of methanol	The main spot in the test solution has the same Rf-value as that of the main spot of Standard Solution A obtained in the test for Other chlorinated disaccharides
Specific rotation	$[\alpha]_D^{20}$ +84° to +87.5° calculated on the anhydrous basis (10% w/v solution)	$[alpha]^{20}$, D: Between +84° and +87.5° (10% w/v solution)
Purity		
Water content	NMT 2% (Karl Fischer method)	NMT 2% (Karl Fischer method)
Sulphated ash	NMT 0.7%	NMT 0.7%
Other chlorinated disaccharides	NMT 0.5%	Passes test
Chlorinated monosaccharides	NMT 0.1%	Passes test
Triphenylphosphine oxide	NMT 150 mg/kg	NMT 150 mg/kg
Methanol	NMT 0.1%	NMT 0.1%
Lead	NMT 1 mg/kg	NMT 1 mg/kg

Abbreviation: NMT, Not more than.

An additional identification number for E 955, currently not reported in the Commission Regulation (EU) No 231/2012, is the CAS number 56038-13-2 (JECFA, 2006).

The structural formula of E 955 is given in Figure 1.

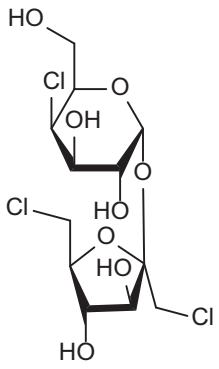


FIGURE 1 Chemical structure of E 955.

An IBO provided information on the identity and physicochemical characteristics of sucralose (E 955) as follows: Fourier transform infrared (FTIR) spectrum, ultraviolet (UV) absorbance spectrum, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectrum, mass spectrum, octanol/water partition coefficient, surface tension, specific gravimetry, melting point, refractive index (RI), specific optical rotation (aqueous sucralose solutions) and elemental analysis (Documentation provided to EFSA n. 1). Upon request from EFSA, the IBO submitted additional analytical results for several parameters, including those reported in the EU specifications, for 10 samples of E 955, i.e. five samples of micronised sucralose and five samples of granular sucralose, manufactured in 2017. According to the information provided by the IBO, granular and micronised sucralose are used as E 955. Micronised sucralose is produced by jet milling sucralose crystals into fine particles, while granular sucralose is obtained by drying wet sucralose crystals using a fluid bed dryer. However, according to the IBO, these two types of E 955 share identical composition and internal specifications (Documentation provided to EFSA n. 1,2). In all tested

samples, the purity of E 955, analysed using reverse phase high-performance liquid chromatography with refractive index detection (RP-HPLC/RID), ranged from 99.6% to 100.6%. The water content, assessed according to the Karl Fischer method (General Chapters and NF Monograph for Sucralose, United States Pharmacopeia–National Formulary (USP-NF)[19]), was determined at a maximum level of 0.2%. The specific rotation (method 781S) ranged from 86.2° to 86.6°. The levels of sulphated ash, determined according to the General Chapters and NF Monograph for Sucralose (USP-NF), were reported as below 0.7% (except in two samples of micronised sucralose, where sulfated ash was not detected). The levels of residual methanol, determined by headspace gas chromatography with flame ionisation detection (HSGC–FID), were reported as below 0.1%.

Analytical data analysing solvents employed in five commercial samples of E 955 were submitted and all tested solvents, analysed by headspace gas chromatography–mass spectrometry (HSGC–MS) (either following Environmental Protection Agency (EPA) 5021 method or modified USP-NF 467 method), were found below their respective limit of quantification (LOQ) (Documentation provided to EFSA No 2). Another IBO submitted the results of the analysis of solvents employed in the manufacturing of sucralose for three commercial samples of E 955 and all of them were reported as below the corresponding LOQs (Documentation provided to EFSA No 3). Considering the various steps of the production processes described by the IBOs (see Section 3.1.2), the Panel did not consider it necessary to recommend inclusion of limits for additional solvents in the EU specifications for E 955.

Inorganic impurities

Regarding toxic elements, two IBOs provided analytical data on commercial samples of E 955. Details of the analytical data provided are available in Appendix F (Documentation provided to EFSA No 1, 3, 4, 5).

Since tin-containing reagents are used in the manufacturing processes described by the IBOs, analytical data on tin in 19 samples of sucralose (E 955) were submitted and reported below the LOQs (0.005–0.05 mg/kg) of the methods used (see Appendix F). These levels in the food additive are of no toxicological concern, considering that the maximum limits for inorganic tin in different categories of canned foods and beverages range from 50 to 200 mg/kg, according to Commission Regulation (EU) No 2023/915 on maximum levels for certain contaminants in food.[20] Considering the purification steps applied during the production processes described by the IBOs (see Section 3.1.2), as well as the analytical data submitted, the Panel did not consider necessary to recommend inclusion of a limit for tin in the EU specifications for E 955.

The EU specifications for E 955 have a limit value only for lead of not more than 1 mg/kg (Table 1). As described in the manufacturing processes provided by the IBO (see Section 3.1.2), the food additive E 955 is manufactured by chemical synthesis from sucrose, involving several separation and purification steps. Taking this into account and the analytical data provided by the IBOs (see Appendix F), the Panel did not consider necessary to recommend additional specification limits for other toxic elements, i.e. arsenic, mercury, aluminium and cadmium.

The Panel calculated the potential exposure to lead from the use of E 955 considering that it is present in the food additive at: (i) the existing limit in the EU specifications (i.e. 1 mg/kg); (ii) the reported limit of 0.02 mg/kg and applying a modulation factor of 10. The exposure calculation to lead is presented and discussed in Appendix F. Taking into account the calculations performed by the Panel (Table F.3), and that the maximum limit should be established based on actual levels in the commercial food additive, the Panel recommended to lower the specification limit for lead. If the European Commission decides to revise the current limit for lead in the EU specifications, the estimates of exposure could be considered.

Organic impurities

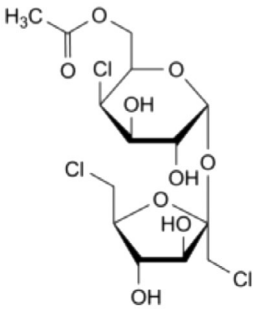
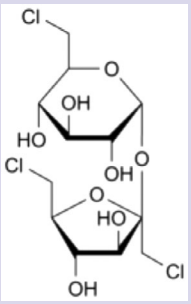
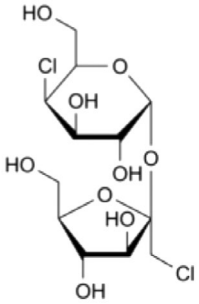
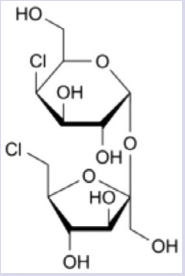
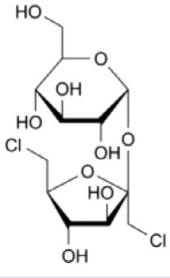
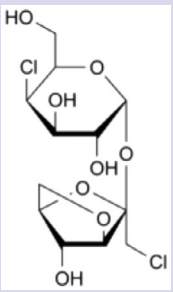
According to the sucralose monograph of the European Pharmacopoeia (Ph. Eur. 11.8),[21] 8 organic impurities can be present in sucralose (Table 2): impurities A, B, D, E, F and G, at the limit of not more than 0.5% (thin layer chromatography (TLC) method), and impurities H and I, at the limit of not more than 0.1% (TLC method). The Panel noted that in the EU specifications of sucralose (E 955) impurities A, B, D, E, F, G would be included within 'other chlorinated disaccharides' (limit: 0.5%), while impurities H and I in 'chlorinated monosaccharides' (limit: 0.1%).

[19]USP29-NF24. Available online: http://www.pharmacopeia.cn/v29240/usp29nf24s0_m78575.html.

[20]Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. OJ L 119, 5.5.2023, pp. 103–157.

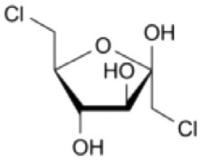
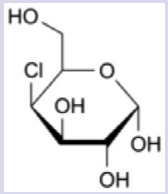
[21]Available online: https://pheur.edqm.eu/app/11-8/search (Accessed November 2025).

TABLE 2 Chemical structures of sucralose (E 955) impurities.

Impurity (list as in the Ph. Eur. 11.6)	Chemical name and synonyms	CAS No.	Structure (Ph. Eur. 11.6)
A	1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl 6-*O*-acetyl-4-chloro-4-deoxy-α-D-galactopyranoside Synonyms: 6-*O*-acetylsucralose; Sucralose-6-acetate	105066-21-5	
B	1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl 6-chloro-6-deoxy-α-D-glucopyranoside Synonyms: 1′,6,6′-trichloro-1′,6,6′-trideoxysucrose; 6,1′,6′-trichlorosucrose	40631-75-2	
D	1-chloro-1-deoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside Synonym: 1′,4-dichloro-1′,4-dideoxygalactosucrose; 4,1′-dichlorogalactosucrose	64644-65-1	
E	6-chloro-6-deoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside Synonyms: 4,6′-dichloro-4,6′-dideoxygalactosucrose; 4,6′-dichlorogalactosucrose	55832-24-1	
F	1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl α-D-glucopyranoside Synonyms: 1′,6′-dichloro-1′,6′-dideoxysucrose; 1′,6′-dichlorosucrose	61854-83-9	
G	3,6-anhydro-1-chloro-1-deoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside Synonym: 3′,6′-anhydro-1′,4-dichloro-1′,4-dideoxygalactosucrose	105066-20-4	

(Continues)

TABLE 2 (Continued)

Impurity (list as in the Ph. Eur. 11.6)	Chemical name and synonyms	CAS No.	Structure (Ph. Eur. 11.6)
H	1,6-dichloro-1,6-dideoxy-β-D-fructofuranose Synonym: 1,6-dichlorofructofuranose (1,6-DCF)	78508-21-1	
I	4-chloro-4-deoxy-α-D-galactopyranose Synonym: 4-chlorogalactopyranose (4-CG)	848642-13-7	

Upon request from EFSA, one IBO submitted analytical results of the analysis of different impurities in 59 samples of E 955 (manufactured between April 2020 and January 2024). Chlorinated monosaccharides: 4-chlorogalactopyranose (4-CG) (impurity I) and 1,6-dichlorofructofuranose (1,6-DCF) (impurity H); and chlorinated disaccharides: 4,6,1′,6′ tetrachlorogalactosucrose; 4,1′,4′,6′-tetrachlorogalactosucrose; sucralose-6-acetate (impurity A); 3′,6′-anhydro 4,1′ dichlorogalactosucrose (impurity G); 4,1′,6′-trichlorosucrose; 4,1′-dichlorogalactosucrose (impurity D); 4,6′-dichlorogalactosucrose (impurity E)) were analysed (Documentation provided to EFSA No 6). Analyses were performed by high-performance liquid chromatography with refractive index detection (HPLC–RID).

A reference standard of 4-CG was used for the quantification of 4-CG and 1,6 DCF in the sucralose samples. In all analysed samples, the levels of 4-CG ranged from 23 to 169 mg/kg, while the levels of 1,6-DCF ranged from 25 to 185 mg/kg. The lowest concentration of both impurities is reported in the same sample, while the highest concentrations are reported in another sample. The highest reported concentration for total chlorinated monosaccharides was 354 mg/kg (0.035%), that is below the current limit of 0.1% in the EU specifications for E 955.

The IBO stated that as there were no standards for chlorinated disaccharides available, the results were reported as percentage of total area (Documentation provided to EFSA No. 7). Results for individual chlorinated disaccharides and the sum of them as % of total peak area which ranged between 0.05% and 0.42% were reported. Assuming the same response factor for all substances in the chromatogram, the Panel considers that the peak area percentages could correspond to the percentage of total weight in relation to sucralose. The Panel considers that the uncertainty associated with the assumption of the same response factor for chlorinated impurities analysed using the HPLC–RID method, is likely to be no greater than the uncertainty associated with the existing JECFA TLC method (JECFA, 2006) for the chlorinated impurities included in the EU specifications (Table 1) which also assumes the same response factor. The Panel noted that the levels of total chlorinated disaccharides were below the current limit of 0.5% in the EU specifications for E955.

Another IBO reported only 'dichlorosucrose' (up to 0.2%) as an impurity in three samples of E 955 (99.8% purity) analysed by HPLC–RI (Documentation provided to EFSA No 8).

The Panel noted that a limit for triphenylphosphine oxide (TPPO) of 150 mg/kg is included in the specifications for E 955. However, this substance is not used in the manufacturing processes reported by the IBOs (Section 3.1.2). Accordingly, the TTPO was reported to be below the LOD (0.0022 mg/kg) in the analysed samples of E955 (Documentation provided to EFSA No 4). However, no specific information on the manufacturing process of E 955 is indicated in the current EU specifications, therefore the use of TPPO in manufacturing processes of E995 cannot be excluded (WIPO, 2010).

Microbiological criteria

The results of the microbiological analysis in 10 samples of E 955 (five samples of micronised sucralose and five samples of granular sucralose manufactured in 2017) were submitted (Documentation provided to EFSA No 2). In all analysed samples, the total aerobic plate count (determined using Bacteriological Analytical Manual (BAM) Chapter 3 method[22]) and yeast and mould (determined by BAM Chapter 18 method[23]) were reported to be below 10 CFU/g. Coliforms and *E. coli* (both determined by BAM Chapter 4 method[24]), as well as *Staphylococcus aureus* and *Salmonella* (determined by USP[25] and Association Of Official Analytical Collaboration (AOAC) Official Method (OM) 2013.01[26] methods, respectively), were not detected in any of the analysed samples. Considering the various steps of the production processes described by the IBOs

[22]Available online: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobic-plate-count (accessed November 2025).
[23]Available online: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-18-yeasts-molds-and-mycotoxins (accessed November 2025).
[24]Available online: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria (accessed November 2025).
[25]Available online: https://www.usp.org/sites/default/files/usp/document/harmonization/gen-method/q05a_pf_ira_34_6_2008.pdf (accessed November 2025).
[26]Available online http://www.aoacofficialmethod.org/index.php?main_page=product_info&products_id=2926 (Accessed November 2025).

(see Section 3.1.2), the Panel considered that a microbiological contamination is unlikely and this is supported by the microbiological data reported above. Hence, the Panel considered that no specifications for microbiological criteria need to be included into the EU specifications for E 955.

Solubility

One IBO reported values on the solubility of sucralose in water, ethanol and corn oil to support the E 955 re-evaluation without further information on the applied method (Documentation provided to EFSA No 1).

Following an additional data request, the IBO submitted the results of a solubility test in water at 20°C of granular and micronised sucralose to be used as E 955 following the OECD TG 105 (OECD, 1995) method (Documentation provided to EFSA No 5). The average solubility for five samples of micronised sucralose was 335 g/L and the average solubility for five samples of granular sucralose was 363 g/L.

The Panel noted that the ultrafiltration step recommended in the EFSA Guidance particle-TR (EFSA Scientific Committee, 2021a) to remove any small particles from the solubilised fraction was not included in these tests for solubility. Given the nature of this substance, the Panel considered nonetheless that the solubility values for both forms of sucralose are substantially higher than the value of 33.3 g/L proposed as a criterion to decide whether an additional assessment for the fraction of small particles is needed according to the EFSA Guidance particle-TR (EFSA Scientific Committee, 2021a).

Considering the water solubility values reported by the IBO for E 955 the Panel noted that E 955 can be considered as fully dissolved when consumed as a food additive. Therefore, the Panel considered that there is no concern regarding the potential presence of small particles, including nanoparticles and sucralose can be assessed following the conventional risk assessment, i.e. EFSA Guidance for submission for food additives evaluations (EFSA ANS Panel, 2012).

3.1.2 | Manufacturing process

According to information provided by two IBOs, sucralose is produced in a three-step process that selectively replaces three hydroxyl groups on the sucrose molecule with chlorine. Sucrose is first converted to sucrose-6-acetate by reaction with a tin-containing catalyst and then chlorinated to form sucralose-6-acetate. In the final step, sucralose-6-acetate is deacylated to produce sucralose. The resulting sucralose is then purified and isolated through processes such as decolorisation, filtration and crystallisation, and then finally dried, sieved and packed (Documentation provided to EFSA No 1, 3, 15).

The Panel noted that in the peer-reviewed literature, other manufacturing processes are described using different chemical reagents (e.g. trityl chloride, phenylphosphonate, tertamethylisocyanide, sodium methylate (Von Rymon Lipinski, 2015), trimethyl orthoacetate, p-toluenesulfonic acid, tert-butylamine (Luo et al., 2008)), a mixture of 4-dimethylaminopyridine and sodium acetate as the catalyst (Bumroongsri, 2024). However, no IBOs have indicated their use and, therefore, they have not been assessed by the Panel.

Similarly, the manufacturing processes of the intermediate sucrose-6-acetate, described in the peer-reviewed literature, use enzyme or microorganism-based methods (e.g. cross-linked enzyme aggregates (CLEAs) of microbial lipase (Yang et al., 2012), *Candida rugosa* lipase (Zhong et al., 2013; Zhong et al., 2014), fructosyltransferase (FTase) from *Aspergillus* sp. GX-0010 (Li et al., 2018), *Bacillus amyloliquefaciens* WZS01 (Sun et al., 2017)). Also, the enzymatic hydrolysis of sucralose-6-acetate to sucralose (e.g. using acyl hydrolase enzyme from *Arthrobacter* sp. (ABL) and *Bacillus subtilis* (RRL 1789) (Chaubey et al., 2013)) is described. However, as no IBOs have indicated the use of these processes, they have not been assessed by the Panel.

3.1.3 | Methods of analysis in food

General methodologies for the analytical determination of sucralose in beverages and different solid food categories were provided by one IBO in response to the 1st EFSA call for data on sweeteners (Documentation provided to EFSA No 1) and are summarised as follows. The determination of sucralose (E 955) in beverages is often performed via HPLC with refractive index detection (RID) or UV absorption detection (at 190 nm) following solid-phase extraction (SPE) techniques. Both detection techniques (RI and UV) give a linear response ranging from 50 to 10,000 mg/kg. In milk-based beverages, proteins are previously removed by precipitation in methanol before SPE and HPLC analysis. The determination of sucralose in solid food products (cereal products, canned products, yogurts, desserts) is carried out by extraction with solvents (such as water or methanol) followed by clean-up procedures and HPLC analysis (with RI or UV detectors). The determination of sucralose in table-top products (e.g. condiments) involves diluting/dissolving the sample with distilled water and then HPLC analysis (with RI or UV detectors).

Analytical methods for the determination of sucralose in solid foods and beverages are widely published in the scientific literature. It is beyond the scope of this re-evaluation of E 955 to provide a comprehensive review of these methods. The following examples give an impression of the numerous approaches used (Hanko & Rohrer, 2004; McCourt et al., 2005; Wasik et al., 2007; Buchgraber and Wasik, 2009; Feinberg et al., 2009; George et al., 2010; Ferrer & Thurman, 2010; Kaufmann et al., 2018; Krmela, Kharoshka, Schulzova, Pulkrabova, & Hajslova, 2020; Neves et al., 2021; Ayyappa et al., 2021). The methods involve pre-treatments, such as SPE, silylation or pre-column derivatisation, and consist mainly of chromatography-based

techniques, such as high-performance thin layer chromatography (HPTLC) (George et al., 2010; Morlock & Prabha, 2007; Spangenberg et al., 2003), liquid chromatography/time-of-flight mass spectrometry (LC/TOF–MS) (Ferrer & Thurman, 2010), high-performance liquid chromatography with ultraviolet detection (HPLC–UV) (Nojiri et al., 2000), high-performance liquid chromatography with evaporative light scattering detection (HPLC–ELSD) (Wasik et al., 2007; Buchgraber and Wasik, 2009; Yan et al., 2016, De Sousa et al., 2023), liquid chromatography–mass spectrometry (LC–MS) (Neves et al., 2021), liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Detry et al., 2022; Moldoveanu, 2023; Nicoluci et al., 2023; Nicoluci et al., 2025), high-performance liquid chromatography/electrospray ionisation-mass spectrometry (HPLC/ESI–MS) (Yang & Chen, 2009), reverse phase ultra high-performance liquid chromatography–mass spectrometry (RP-UHPLC/MS) (Krmela, Kharoshka, Schulzova, Pulkrabova, & Hajslova, 2020) or quadrupole ion trap (QTRAP) mass spectrometry (Hu et al., 2023).

3.1.4 | Stability of the substance, and reaction and fate in food

One IBO stated that sucralose (E 955), in dry powder form, is stable for at least 2 years when stored at 25°C and that it can tolerate short periods at higher temperatures (e.g. during shipment) without any significant impact on the shelf life (Documentation provided to EFSA No 1). However, no experimental data were provided.

A study investigating the hydrolytic stability of a sucralose solution (2% w/v) at different pHs (1–9) and temperatures (20–60°C) was submitted (Documentation provided to EFSA No 9). Residual sucralose was analysed by HPLC at different time points. A large number of pH/time/temperature combinations were tested, and only a limited summary is presented below.

At pH 1, 24% sucralose was degraded by week 4 at 30°C, 61% by day 8 at 40°C, while near 100% was degraded by day 4 at 60°C.

At pH 3, only 1% of sucralose was degraded after 12 weeks and 11% after 48 weeks at 30°C, while 27% was degraded after 4 weeks at 60°C.

Sucralose remained stable at pH level between 4 and 7.5, with no detectable loss (less than 0.1%) after 48 weeks at 30°C.

At pH 9, there was 7% degradation after 16 weeks at 30°C and 27% loss after 4 days at 60°C.

In the same study report, there was no detectable loss of sucralose (initial concentration of 260 mg/L) in a carbonated cola beverage stored at 20°C for 12 months and at 35°C for 9 months. In a fruit-based orange drink (initial sucralose concentration of 190 mg/L), an initial 5% loss occurred upon pasteurisation (time and temperature not reported), then an approximately 10% loss of sucralose after 3 months at 20°C (final concentration of 160 mg/L). The pHs of the cola and the orange beverage was not reported.

Sucralose was found to breakdown at pH 1 to 3 by hydrolysis following a first-order reaction kinetics, to the monosaccharides, 1,6-DCF and 4-CG. No other breakdown products were detected using HPLC. The reaction kinetics indicated that for a cola beverage at pH 3 and formulated with sucralose, the loss of sucralose after 6 months would be 0.3% at 20°C and 0.8% at 25°C (Documentation provided to EFSA No 9). Another study indicates a calculated loss after 6 months at 25°C of 1.6% at pH 2.5 and of 0.4% at pH 3. In a similar study, with a range of temperatures from 30°C to 80°C (analysis 1985–1986), it is stated the hydrolysis of sucralose at basic pH, to the 3′,6′-anhydro derivative of sucralose with further breakdown involving 'caramelisation' (Documentation provided to EFSA N 9).

A study investigating the effects of temperature and light on the stability of sucralose in formulated products (carbonated and non-carbonated drinks, dry mix beverages, dry mix desserts, table-top sweeteners and dairy products) was also submitted (Documentation provided to EFSA N 9). The concentration of sucralose was determined by HPLC (RI detector). The formulation levels and the time and temperature storage conditions used were appropriate for the types of products investigated. For all food products tested, the sucralose was stable and close to 100% of the formulation levels were recovered at the end of the storage periods.

The results of a study investigating stability and degradation of sucralose under different conditions and the formation of degradation products were submitted (Documentation provided to EFSA N 8). The analysis and identification were carried out using HPLC–MS and NMR.

Sucralose samples were subjected to thermal, acidic, alkaline and oxidative conditions. For thermal degradation, samples were heated at 80°C for 6.5 h, resulting in a slight increase (from 0.168% to 0.336%) in the sucralose impurity identified as 'dichlorosucrose'. In the acidic degradation, samples were treated with 0.1 mol/L HCl at 50°C for 20 h, leading to the appearance (2.071%) of a degradation product identified as 1,6-DCF and a slight decrease of the impurity identified as 'dichlorosucrose'. Alkaline degradation treatment, with 0.1 mol/L NaOH at 50°C for 20 min, resulted in a new degradation product (4.930%) (see below for more information on the identity of this degradation product under alkaline conditions). Oxidative degradation was performed using 25% hydrogen peroxide at 50°C for 19 h, leading to the formation (0.196%) of a new degradation product, of not identified structure and a slight increase of the impurity 'dichlorosucrose'.

In a further study, sucralose was exposed to 1% HCl or 1% NaOH at 40°C, both for 5.5 and 20.5 h. Under acidic conditions, 1,6-DCF was identified as the degradation product (near 8% after 20.5 h correlating with the decrease observed in

sucralose). Simultaneous 4-CG[27] was also detected. This indicated that sucralose undergoes glycosidic bond cleavage under acidic conditions.

Under alkaline conditions, two new peaks were observed, corresponding to sucralose molecules that had lost one and two HCl molecules by intramolecular dehydrochlorination. As the alkaline degradation time increases, sucralose almost disappears and the degradation product resulted from the loss of one HCl molecule (substance corresponding to the one identified as impurity G (Table 1), which is further transformed to the degradation product resulting from the loss of two HCl molecules (see Figure 2).

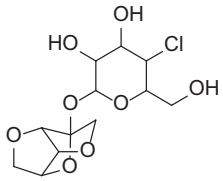


FIGURE 2 Structure of the sucralose (E 955) degradation product identified under basic conditions.

The study also included the investigation of sucralose stability under simulated gastric and intestinal conditions at acidic and alkaline pHs at a concentration of 10 g/L and 37°C. The effects of temperature (33°C–37°C) and time (5–72h) on the stability of sucralose (pH 2) were evaluated. At 45°C for 72 h, 1,6-DCF reached a concentration of 0.950%, while at 37°C for 24 h, it was only 0.362%. The effect of pH (0.9–4) was evaluated at different holding times (5–72 h) at 37°C. At pH 0.9 for 72 h, 1,6-DCF reached 12.8%, while at pH 3 it was 0.3%. Under alkaline conditions (pH 8–11, 37°C), minor amounts of the degradation product resulting from the loss of two HCl molecules was observed at pH 11 (after 5 h and does not increase with the time), as well as for the other dehydrochlorinated product (loss of one HCl molecule) at pH 8–9. Only at pH 11, the degradation product resulting from the loss of one HCl molecule appeared at 3% after 5 h, and above 6% after 48 and 72 h.

The study showed that sucralose undergoes glycosidic bond cleavage under acidic conditions degrading into 1,6-DCF and 4-CG. Under alkaline conditions, it undergoes intramolecular dehydrochlorination. Degradation is minimal under simulated gastric conditions, with 1,6-DCF formation being very low (0.172% at pH 2, 37°C, 24 h).

A summary of information available in the open literature on the stability of sucralose (E 955) as such, as well as in liquid systems or solid foods, was provided by the same IBO (Documentation provided to EFSA No 1, 4).

Studies on the melting point and thermal behaviour of sucralose have reported that, decomposition and melting can occur simultaneously, depending on the rate of heating. Jenner and Smithson (1989) determined the melting point for sucralose of 114.5°C at an average heating rate of 1°C/min, and 125.5°C at a rate of 5°C/min. Bannach et al. (2009) attempted to relate the melting/decomposition temperatures of sucralose to its behaviour in baked products using thermogravimetry and differential thermal analysis (TG-DTA). Their findings indicated that sucralose is stable up to 119°C, with the initial stage of thermal degradation involving the loss of water and subsequently HCl at around 130°C. Additional studies on sucralose conducted by de Oliveira et al. (2015), using differential scanning calorimetry/thermogravimetric analysis coupled with Fourier transform infrared spectroscopy (DSC/TGA-FTIR) and hot stage microscopy (HSM), found that sucralose melts and decomposes at the same temperature (125°C) as reported by Jenner and Smithson (1989).

Miller (1991) reported that sucralose can breakdown to its monosaccharide components 1,6-DCF and 4-CG in aqueous solution at pH around 3 and at a temperature of 30–40°C. Moreover, Grotz et al. (2012) reported that the stability of sucralose in aqueous solution depends on pH and time/temperature treatment. At a pH of 3 and 30°C, a loss of around 3% of sucralose was observed after 52 weeks, whereas within the pH range of 4–6, the loss of sucralose was approximately 1%.

Barndt and Jackson (1990) investigated the stability of sucralose (E 955) in food and beverage matrices. Common bakery goods (yellow cake, cookies and graham crackers) were tested under typical industry baking conditions (180–230°C). These experiments were conducted using ^{14}C-labelled sucralose. A recovery of radioactivity of 100% was obtained in the baked goods and the TLC distribution of radioactivity matched that of the sucralose standard solution, demonstrating that no detectable sucralose degradation took place during the baking process. The Panel noted that the sensitivity of the radioactivity TLC plate scanner analysis was not reported, and so minor degradation products (if any) may not have been detected.

Additionally, the stability of sucralose was explored in other processed food products: instant black coffee stored for 24 h at 80°C (Quinlan and Jenner, 1990); cola (pH 2.8) and carbonated lemon/lime (pH 3.8) drinks stored for 4 weeks at 20°C in the light (Quinlan and Jenner, 1990); cola drinks (pH 3) stored for up to 6 months at 20°C (Quinlan et al., 1999); lassi samples (pH 4.56) stored for up to 15 days under refrigerated conditions (6–8°C) (George et al., 2010). The Panel noted that minimal or no loss of sucralose was observed at the end of the storage period across all these studies.

In lemon-lime flavoured carbonated beverages (pH 3.14–3.21) stored for 60 days at 37°C, the loss of sucralose was around 2% at the end of the storage period (Malik et al., 2002). In burfi samples, stored at 5, 30 and 45°C for up to 28 days, and analysed by HPTLC plates, no products of hydrolysis (1,6-DCF and 4-CG) were detected, the limit of detection was 1 mg/L (Morlock & Prabha, 2007).

[27]The Panel noted that it was named as '1-chloroglucose' in the report.

Grotz et al. (2012) reported that in low acid/neutral pH heat-treated products, such as pasteurised beverages, sterilised or ultra-high temperature (UHT) products (beans in sauce, dairy desserts and vanilla milk), sucralose was 100% recovered after thermal treatment.

The reactivity of sucralose in model systems was reviewed by Miller (1991). Four model systems were developed: (i) bases (niacinamide, monosodium glutamate); (ii) oxidising and reducing agents (hydrogen peroxide, sodium bisulphite); (iii) aldehydes and ketones (acetaldehyde, ethyl acetoacetate); (iv) metal salts (ferric chloride solution). Each model system was 0.1% in a 1% aqueous sucralose solution. These solutions were stored at 40°C at pH 3, 4, 5 or 7, for 7 days. At the end of the storage time, the sucralose content was analysed by HPLC and found to be higher than 98% of the starting concentration in all cases, except for the ferric chloride solution (95.9%).

An additional study on the stability of sucralose was retrieved from the scientific literature by the Panel. In fibre-enriched, reduced-calorie biscuits baked at 165°C for 16 min and stored for 180 days at 34°C, sucralose showed a recovery rate of 98.6% (Aggarwal et al., 2017).

In 2019, the German Federal Institute for Risk Assessment (BfR) reviewed the potential occurrence of chlorinated compounds in foods containing sucralose during conditions simulating industrial heat processing and consumer cooking (BfR, 2019; Eisenreich et al., 2020). The BfR concluded that the results of the reviewed studies indicated that, when heated to temperatures of approx. 120°C–250°C, sucralose (E 955) is dechlorinated, possibly leading to the generation of chlorinated organic compounds exhibiting potential health risk (e.g. polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) or chloropropanols). In addition to the studies described above, the Panel considered the following studies included in the BfR's review which are described below.

Rahn and Yaylayan (2010) investigated the thermal degradation and dehydrohalogenation of sucralose under high dry heating conditions. For this purpose, a sucralose/glycerol model mixture was prepared and subjected to heating at 250°C for 20 s. The analysis, conducted by pyrolysis-gas chromatography–mass spectrometry (Py-GC/MS), revealed the formation of chloropropanols, including 3-monochloropropandiol (3-MCPD) and 1,2 and 1,3-dichloropropanols (1,2 and 1,3 DCP). The Panel noted that the yield of degradation products from sucralose was not reported.

Wu et al. (2011) investigated PCDD/PCDF formation during the heating of sucralose or 1,3-DCP in soybean oil or in a soybean oil/minced beef mixture. Sucralose (44.4 g) was heated in soybean oil (203 g) for 25 min on an electric hotplate and the oil temperature reached on around 250°C. During heating, the sample was swept with air. The trapped gas phase ('smoke') and the heated oil sample were then analysed by isotope dilution high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC-HRMS). PCDD/PCDF congeners (the sums of tetra-, penta-, hexa- and octa-) were found especially in the gas/smoke phase, albeit at very low yields in the order of 1–5 ng per gram of chlorine content of the sucralose (or DCP) heated. They then heated minced beef (ca. 600 g) in soybean oil (ca. 200 g) containing 0, 42 mg, 400 mg, 4.1 g or 45 g of sucralose under the same experimental conditions. Due to the water content of the minced beef and evaporative cooling effect, the temperature of the beef did not exceed 200°C during the heating tests. The temperature of the oil was not reported. They reported an increase in the concentrations (WHO-TEQ) of PCDD/PCDF by a factor of around 5 in the beef samples compared to the control (heated, no sucralose). There was no relationship with increased sucralose amount used, with toxic equivalent (TEQ) values of ca. 0.3, 0.2, 0.25 and 0.4 pg/g beef for heating with 42 mg, 400 mg, 4.1 g or 45 g sucralose, respectively. PCDD/PCDF were found in a dose-dependent manner in the trapped gas/smoke phase, rising to ca. 2.5 pg/g TEQ on a fresh beef weight basis when the heating tests used 45 g of sucralose.

In a separate publication, Dong et al. (2011) analysed the same samples for dioxin-like polychlorinated biphenyls (dl-PCB). The concentrations of the 12 dL-PCB congeners in the raw beef decreased by more than 50% on cooking, and the concentrations and TEQ values remained below the raw beef values even when up to 45 g of sucralose (5.3% w/w of the total heated mix) was used. In contrast, the presence of sucralose during the cooking process caused an increase in dl-PCB in the trapped gas/smoke phase, and the authors concluded that sucralose (as well as DCP) could act as a chlorine source and promote the formation of dl-PCBs in oil fumes.

Moreover, Dong, Liu, Hu, and Zheng (2013) investigated the generation of PCDD/PCDF congeners by heating 5 g of sucralose in contact with metal cooking utensils with or without the presence of metal oxides (Al_2O_3, Fe_2O_3 and CuO; 1 g each) to simulate rust of oxidised utensils, and between 200°C and 400°C. Analyses were conducted by isotope dilution HRGC-HRMS. Results showed that, in the presence of sucralose at temperatures of 350°C and 400°C, and when using metal cooking utensils, various PCDD/PCDF compounds were generated (31 ng/g at 400°C which is 25 times higher than the concentration produced at 350°C). The total levels of PCDD/PCDF congeners produced at 400°C were reported higher in the presence of metal oxides (CuO: 4.2×10^3 ng/g; Fe_2O_3: 1.2×10^3 ng/g; Al_2O_3: 97 ng/g). No significant amounts of PCDD/PCDF were detected when sucralose was heated in the presence of the metal oxides at 300°C.

Dong, Liu, Zhang, et al. (2013) also reported the formation of different polychlorinated naphthalene (PCN), besides dl-PCB and PCDD/PCDF congeners when heating sucralose (5 g) with cooking oil (either 50 g of peanut oil or olive oil) for 15 min up to 245°C in a stainless-steel pan. The concentrations of the PCNs present in the cooking oils and oil fumes generated during the heating process were determined by an HRGC-HRMS method. The results indicated that the heating of cooking oil in the presence of sucralose promoted the formation of PCNs. Although PCNs were detected in the oil fumes, no PCNs were found in the heated oils. The total concentrations of PCNs found in peanut and olive oil fumes were 490 and 240 pg/g, respectively. To quantify the produced PCNs, sucralose used in the experiments were about 2 or 3 orders of magnitude higher than in the normal cooking conditions for foods. PCNs were not detected under the normal cooking conditions. The total concentrations of dl-PCBs in the fumes of peanut oil and olive oil were 18 and 14 pg/g. While for 2,3,7,8-substituted

PCDDs the total concentration in the fumes of peanut oil and olive oil amounted to 54 and 28 pg/g, respectively, the total concentration of 2,3,7,8-substituted PCDFs were reported as 130 and 86 pg/g, respectively.

In a study published after the BfR report, Dong et al. (2019) looked into the effects of temperature (up to 400°C), container type (stainless steel, quartz, aluminium or copper containers and the presence of metal oxides (Al_2O_3, Fe_2O_3 and CuO) on the formation and distribution of PCN during the heating of sucralose (5 g). PCN congeners were generated during heating in stainless-steel containers at both 350°C and 400°C but were undetectable in the residues or smoke when sucralose was heated in quartz, aluminium or copper containers at 400°C. Similar to the findings for PCDD/PCDF (Dong, Liu, Hu, & Zheng, 2013), the presence of Al_2O_3, Fe_2O_3 and CuO significantly increased the concentrations of PCN congeners, with CuO exhibiting the most pronounced catalytic effect.

Upon a request from EFSA, an IBO provided data to assess the effects of typical baking heat processing treatments on the potential formation of 3-MCPD and 3-MCPD esters and PCDD/PCDF from sucralose (E 955) (Documentation provided to EFSA No 2, 4, 10, 11, 12).

3-MCPD and 3-MCPD esters

Four model food systems were formulated and processed under controlled conditions: i.e. wafers,[28] cupcakes,[29] biscuits (cookie)[29] and pizza sauce.[30] Each model food system was formulated to include a 'control' (containing sugar), 'sucralose' (containing sucralose and a maltodextrin bulking agent, when appropriate) and 'maltodextrin' (containing maltodextrin bulking agent only). According to the IBO, maltodextrin was included to replace sugar's bulk properties and aid in convenient sucralose measurement. Sucralose was used either in its pure form or as a mixture with maltodextrin. Detailed descriptions of recipes, food preparation and sampling procedures were provided. Specific sucralose concentrations were detailed for each food type and the respective baking conditions were described (Documentation provided to EFSA No 4).

3-MCPD and 3-MCPD esters analysis was conducted using gas chromatography–tandem mass spectrometry (GC–MS/MS) (American Oil Chemists' Society (AOCS) OM Cd29b-13 (2013), modified). The LOQs for free 3-MCPD and 3-MCPD esters (expressed as 3-MCPD) are given as 5 and 10 μg/kg, respectively. Spiking experiments were performed at the 5 and 10 μg/kg level and showed recoveries between 100% and 133%.

For 3-MCPD, the mean value in wafers prepared with sucralose amounted to 8.3 μg/kg compared to 5.0 and 5.1 μg/kg for the other recipes, respectively. For cupcakes, there were no differences in the analyses of the three different formulations, each reported as 5 μg/kg. In cookies prepared with the mixture of sucralose and maltodextrin, the mean value for 3-MCPD is given as 9.6 μg/kg compared to 6.7 and 7.1 μg/kg, respectively for the other two recipes. For the pizza sauce, the mean value for 3-MCPD is given as 4.8 μg/kg compared to 4 μg/kg (which is below the LOQ) for the control sauce.

In most samples, the levels for 3-MCPD esters (expressed as 3-MCPD) were below the LOQ (10 μg/kg). The highest amount of 3-MCPD esters (expressed as 3-MCPD) at 29 μg/kg was found in the pizza sauce prepared with 0.0017% sucralose. In the control sauce (formulated with sugar), the mean value for 3-MCPD esters (expressed as 3-MCPD) was reported as 23 μg/kg.

The Panel considered that the recipes and the cooking conditions used were appropriate to mimic household cooking of such products. The Panel also considered that the levels of sucralose used in these baking tests were appropriate.

Taking into consideration the scatter of results and the sensitivity of the analysis (LOQ values of 5 and 10 μg/kg for 3-MCPD and 3-MCPD esters, respectively), it can be concluded by inspection of the 3-MCPD data, that there is no evidence of any increase/decrease in levels due to the use of sucralose, no more than +/– 4 μg/kg (2 μg/kg for 3-MCPD plus 2 μg/kg for 3-MCPD esters).

The Panel conducted some calculations considering the data from the EFSA Comprehensive database[31] to determine whether the LOQ values of the analyses conducted were adequate to draw conclusions about the safe use of sucralose from these results. In toddlers, the maximum mean combined consumption of biscuits, cakes including muffins and wafers across the surveys, for consumers-only, is 2.6 g/kg bw per day.

Assuming that an increase of up to 4 μg/kg (3-MCPD and 3-MCPD esters) may have gone undetected, this level of food consumption would correspond to an exposure of $(0.0026 \times 4) = 0.0104$ μg/kg bw per day which is only 0.5% of the group tolerable daily intake (TDI) of 2 μg/kg bw per day for 3-MCPD and its fatty acid esters. So, it can be concluded that for the baking tests conducted, the inclusion of sucralose as a sweetening agent did not give rise to any significant increase in risk from 3-MCPD and 3-MCPD esters.

PCDD/PCDF

In response to a request from EFSA, an IBO submitted a published study on the potential formation of PCDD/PCDF from the use of sucralose in cooked foods (Gujral et al., 2021) and submitted also the underlying raw analytical data (Documentation provided to EFSA No 2, 4, 10, 11, 12, 13).

[28]Authorised at a MPL of 800 mg/kg in food category 7.2 with the following restrictions: 'only essoblaten-wafer paper'; 'only cornets and wafers, for ice-cream, with no added sugar' (Regulation (EC) 1333/2008).

[29]Not authorised in cupcakes according to Regulation (EC) 1333/2008.

[30]Authorised at a MPL of 450 mg/kg (food category 12.6) (Regulation (EC) 1333/2008).

[31]Available online: http://www.efsa.europa.eu/en/food-consumption/comprehensive-database.

The Panel noted that the sample weights were relatively low and there was substantial scatter in the analytical data due to different and insufficient LOQ values for the PCDD/PCDF congeners. The spiked amounts of native/unlabelled PCDD/PCDF were substantially higher than the analytes of interest and results for the laboratory blank samples were missing. It was impossible to differentiate between 'within' and 'between' sample scatter. Based on all these shortcomings the Panel considered this study as inconclusive.

The IBO therefore conducted a further study (Documentation provided to EFSA No 12).

The baking study assessed PCDD/PCDF concentrations in two food model systems: biscuits and wafers. Raw ingredients were purchased for both 'standard' and 'organic' ingredients. The individual ingredients, the raw dough mixes and the biscuits and wafers after cooking were analysed. For each model food, samples were prepared using different recipes: (i) sucrose, (ii) sucralose and maltodextrin or (iii) maltodextrin. According to the IBO, the use of a bulking agent, such as maltodextrin, is common in the food industry for solid foods to replace the bulk properties of sugar when a much lower amount of an intense sweetener is used. Five samples of each recipe were prepared, and each sample was analysed in triplicate before and after cooking, giving 15 analytical results for each recipe, to allow for sufficient analysis to assess within-sample and between-sample scatter of results.

The wafers and biscuits, made using sucralose, contained 250 and 500 mg/kg wet weight of the recipe, respectively. Biscuit dough was baked in a pre-heated electric oven for 12 min at 180°C. Wafer dough was baked in a pre-heated stainless-steel non-stick electric waffle cone baker for 75 s at 160°C. The cooking temperature was monitored using a calibrated thermocouple thermometer. Colour photographs of each sample of the product were recorded to demonstrate comparable (and consumer-acceptable) product appearance for the different recipe variations.

Raw ingredients, raw dough and cooked samples were analysed for 17 PCDD/PCDF congeners with 2,3,7,8-chlorine substitution by an accredited external laboratory using isotope dilution high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The LOQ was 0.02 pg. TEQ/g wet weight upper bound (UB) calculated according to Commission Regulation (EU) No 2017/644. The results of PCDD/PCDF were expressed as TEQ UB reported on a dry weight basis according to European Regulation (EC) No. 1881/2006.[32] The TEQ was calculated using 2005 WHO-TEFs. Upper bound (UB) concentrations were calculated by the laboratory assuming that all congeners with reported values below the LOQ are equal to the LOQ. A summary of the results is reported in Tables 3 and 4.

TABLE 3 PCDD/PCDF TEQ in biscuits (UB, pg/kg dry weight).[a]

	'Standard' ingredients		'Organic' ingredients	
Recipe	**Raw**	**Cooked**	**Raw**	**Cooked**
Sucrose	38.5	36.5	26.3	24.2
Sucralose and maltodextrin	37.0	33.8	26.1	25.8
Maltodextrin	38.7	35.1	27.2	27.2

[a]Geometric means ($n = 15$; five batches each analysed in triplicate).

TABLE 4 PCDD/PCDF TEQ in wafers (UB, pg/kg dry weight).[a]

	'Standard' ingredients		'Organic' ingredients	
Recipe	**Raw**	**Cooked**	**Raw**	**Cooked**
Sucrose	33.9	31.2	30.0	23.2
Sucralose and maltodextrin	38.3	33.7	29.3	22.9
Maltodextrin	37.4	32.1	31.1	23.3

[a]Geometric means ($n = 15$; five batches each analysed in triplicate).

Statistical analysis was conducted by the IBO to test if there were any differences in the results between the raw and cooked products, with and without (i) sucrose, (ii) sucralose and maltodextrin and (iii) maltodextrin alone. The statistical analysis showed that for both biscuits and wafers, and for recipes using both 'standard' and 'organic ingredients', there was no difference between raw and cooked products, and there were no differences between using sucrose, or sucralose and maltodextrin, or maltodextrin alone. Thus, the use of sucralose did not give rise to any detectable increase in the concentration of PCDD/PCDF.

The Panel noted that after adjustment for moisture content, the recipe level of 500 mg/kg sucralose would give an average of 559 mg/kg in the cooked biscuits. Similarly, the recipe level of 250 mg/kg sucralose would give an average of 347 mg/kg in the baked wafers. The Panel noted that these concentrations are lower than the maximum permitted level (MPL) for sucralose in food category 7.2 (Regulation (EC) No. 1333/2008).

[32]European Regulation (EC) 1881/2006 has been replaced by Commission Regulation (EU) 2023/915.

The Panel considered that the baking conditions used were appropriate. Biscuits were baked for 12 min at 180°C and wafers were baked for 75 s at 160°C. The colour photographs of each batch of product indicated an acceptable product appearance. The moisture content of the baked biscuits and the wafers also seemed to be appropriate.

The method of analysis used for the determination of the 17 PCDD/PCDF congeners with 2,3,7,8-chlorine substitution was sensitive, accurate and precise and was fit for the purpose. The between-batch standard deviation (SD) was in the range of 0.3–2.5 UB pg. TEQ/kg dry weight. If a person eats around 2.6[33] g/kg bw per day of this type of baked products, then this SD corresponds to 0.005–0.046 UB pg. TEQ/kg bw per week. This represents only 0.25%–2.3% of the tolerable weekly intake (TWI) PCDD/PCDF of 2 pg/kg bw per week (EFSA CONTAM Panel, 2018). The Panel considered that in the experiments conducted, the formation of PCDD/PCDF due to the use of sucralose was neither statistically significant nor toxicological relevant.

The calculations by the IBO described above used the 2005 WHO-TEFs that are given in the relevant EU legislation. The Panel is aware that revised toxic equivalency factor (TEF) values have been proposed by the WHO (De Vito et al., 2024). The Panel recalculated the results from these baking trials using the proposed 2022 WHO-TEF values and the conclusions were unchanged, with no statistically significant nor toxicological relevant formation of PCDD/PCDF in these baking trials using sucralose.

The Panel also noted some limitations in the 3-MCPD and PCDD/PCDF studies. Only four- and two recipes/baking conditions were used for the 3-MCPD and PCDD/PCDF studies, respectively. It may be questioned if the same results would have been found if different recipes and especially if higher baking temperatures/times had been used – even to the extent that the foods had been allowed to 'burn' or 'char'. The Panel noted that overcooking would not be allowed to happen for food products cooked commercially and that general advice to consumers is to avoid overcooking, excessive crisping or burning in home cooking due to general concerns for heat-generated toxicants, including furan, acrylamide and polycyclic aromatic hydrocarbons (PAH). Overall, based on the information available, the Panel considered that the potential formation of 3-MCPD and PCDD/PCDF from sucralose used in baking conditions used in these studies seems to be low and does not give rise to concern.

A study published in 2024, after the IBO investigations described above, reported the formation of new degradation products from sucralose under thermal conditions (Hellwig, 2024). Sucralose was subjected to different kinds of heat treatment, either on its own, in the presence of protein or as an ingredient in food. Sucralose in the dried state was heated in sealed tubes at temperatures between 80°C and 120°C in the absence of proteins. In comparison to sucrose, that was also tested in the experiment, sucralose was less stable and intense browning after heating at 90°C for 1 h was reported. The author reports that this browning was accompanied by a pungent smell from the residual material, which they attributed to the formation of HCl. After heating at temperatures between 90°C and 120°C, the sucralose samples showed a pH of about 2 following the addition of water. According to the author, at least 0.1 mmol of HCl was formed from 100 mg of sucralose (0.25 mmol).

At 80°C and 85°C, no formation of the carbohydrate degradation product 5-hydroxymethylfurfural (HMF) from sucralose was found. The Panel noted that HMF is also authorised as flavouring substance [FL-no: 13.139] previously evaluated by the Panel (EFSA FAF Panel, 2021). At higher temperatures, however, the HMF concentration rose in parallel to the absorbance of the whole reaction mixture at 280 nm. Up to approximately 0.2% sucralose was converted to HMF. Applying HPLC-TOF-MS a further UV-active peak eluted after HMF when sucralose was heated. This compound was identified as a chlorinated furane-3-one. No quantitative information on the formation of this degradation product was reported. In addition, different 'chlorinated dicarbonyl' compounds were reported by using HPLC–TOF–MS analysis after derivatisation with o-phenylenediamine. The formation of 3-chlorotyrosine was identified when sucralose was heated in the presence of casein.

Hellwig (2024) also tested the influence of the addition of sucralose (0.03%–0.1%) to dough on pH value, browning and HMF formation in baking experiments (muffins, coconut macaroons and cookies). It was shown that the addition of sucralose led to a small increase in the HMF concentration compared to the control with no added sucralose; from 24.8 to 33.3 mg/kg, this increase being around 2.5% of the added sucralose in the cookies experiment. HMF formation was not observed in baked muffins and macaroons samples formulated with sucralose, the author considered that this was probably due to the more alkaline pH in the dough and the lower baking temperature as compared to the cookies. Furthermore, a chlorinated 1,2-dicarbonyl compound was reported in cookies formulated with sucralose baked at 220°C.

One IBO submitted a review of the Hellwig (2024) publication on the use of sucralose in baked goods (Documentation provided to EFSA No 14). The IBO considered as a limitation that sucralose was used in its pure form rather than mixed with a carrier, such as maltodextrin, as would typically occur in products sold for home use. The IBO also noted that the heating experiments, which investigated the degradation of sucralose in a dry state, were not representative of real cooking conditions. In addition, the IBO highlighted that no authentic standards were used to confirm the identity of some of the degradation products proposed by the author.

The Panel acknowledges that there are indeed limitations in the study by Hellwig (2024), insofar as the tests were of an exploratory nature. The paper itself concludes with the statement that further studies are necessary.

Overall, the Panel considered that the study by Hellwig (2024) indicates that sucralose is not stable at raised temperatures, such as some baking temperatures.

[33]Combined mean consumption of biscuits, cakes and wafers.

3.2 | Authorised uses and use levels

Maximum levels of sucralose (E 955) have been defined in Annex II to Regulation (EC) No 1333/2008 on food additives, as amended. In this document, these levels are called MPLs.

Currently, sucralose (E 955) is an authorised food additive in the EU in 34 food categories (FCs) (corresponding to 45 authorised uses) with MPLs ranging from 10 to 3000 mg/kg and at quantum satis (QS) in 3 food categories. Table 5 lists the food categories with their restrictions/exceptions that are permitted to contain added sucralose (E 955) and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

TABLE 5 MPLs of sucralose (E 955) in foods according to Annex II to Regulation (EC) No 1333/2008.

Food category number	Food category name	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
01.4	Flavoured fermented milk products including heat-treated products	Only energy-reduced products or with no added sugar	400
03	Edible ices	Only energy-reduced or with no added sugar	320
04.2.2	Fruit and vegetables in vinegar, oil or brine	Only sweet–sour preserves of fruit and vegetables	180
04.2.3	Canned or bottled fruit and vegetables	Only fruit energy-reduced or with no added sugar	400
04.2.4.1	Fruit and vegetable preparations excluding compote	Only energy-reduced	400
04.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EC	Only energy-reduced jams jellies and marmalades	400
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EC	Only energy-reduced jams, jellies and marmalades	400
04.2.5.3	Other similar fruit or vegetable spreads	Only energy-reduced fruit or vegetable spreads and dried fruit-based sandwich spreads, energy-reduced or with no added sugar	400
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	Only energy-reduced or with no added sugar	800
05.2	Other confectionery including breath freshening microsweets	Only cocoa or dried fruit-based, energy-reduced or with no added sugar	800
05.2	Other confectionery including breath freshening microsweets	Only energy-reduced tablet form confectionery	200
05.2	Other confectionery including breath freshening microsweets	Only cocoa, milk, dried fruit or fat-based sandwich spreads, energy-reduced or with no added sugar	400
05.2	Other confectionery including breath freshening microsweets	Only starch-based confectionery energy-reduced or with no added sugar	1000
05.2	Other confectionery including breath freshening microsweets	Only confectionery with no added sugar	1000
05.2	Other confectionery including breath freshening microsweets	Only breath-freshening microsweets, with no added sugar	2400
05.2	Other confectionery including breath freshening microsweets	Only strongly flavoured freshening throat pastilles with no added sugar	1000
05.3	Chewing gum	Only with added sugars or polyols, as flavour enhancer[a]	1200
05.3	Chewing gum	Only with no added sugar	3000
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Only starch-based confectionery energy-reduced or with no added sugar	1000
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Only confectionery with no added sugar	1000
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Only cocoa or dried fruit-based, energy-reduced or with no added sugar	800

TABLE 5 (Continued)

Food category number	Food category name	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Only sauces	450
06.3	Breakfast cereals	Only breakfast cereals with a fibre content of more than 15%, and containing at least 20% bran, energy-reduced or with no added sugar	400
07.2	Fine bakery wares	Only cornets and wafers, for ice-cream, with no added sugar	800
07.2	Fine bakery wares	Only essoblaten – wafer paper	800
09.2	Processed fish and fishery products including molluscs and crustaceans	Only sweet–sour preserves and semi-preserves of fish and marinades of fish, crustaceans and molluscs	120
11.4.1	Table-top sweeteners in liquid form		*Quantum satis*
11.4.2	Table-top sweeteners in powder form		*Quantum satis*
11.4.3	Table-top sweeteners in tablets		*Quantum satis*
12.4	Mustard		140
12.5	Soups and broths	Only energy-reduced soups	45
12.6	Sauces		450
12.7	Salads and savoury-based sandwich spreads	Only *Feinkostsalat*	140
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		400
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)		320
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	Only energy-reduced or with no added sugar	300
14.1.4	Flavoured drinks	Only energy-reduced or with no added sugar	300
14.2.1	Beer and malt beverages	Only alcohol-free beer or with an alcohol content not exceeding 1,2% vol; 'Bière de table/Tafelbier/Table beer' (original wort content less than 6%) except for 'Obergäriges Einfachbier'; Beers with a minimum acidity of 30 milli-equivalents expressed as naoh; Brown beers of the 'oud bruin' type	250
14.2.1	Beer and malt beverages	Only energy-reduced beer	10
14.2.3	Cider and perry	Excluding cydr jakościowy, perry jakościowe, cydr lodowy, perry lodowe	50
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol		250
15.1	Potato-, cereal-, flour- or starch-based snacks		200
15.2	Processed nut		200
16	Desserts excluding products covered in categories 1, 3 and 4	Only energy-reduced or with no added sugar	400
17.1	Food supplements supplied in a solid form, excluding food supplements for infants and young children		800

(Continues)

TABLE 5 (Continued)

Food category number	Food category name	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
17.1	Food supplements supplied in a solid form, excluding food supplements for infants and young children	Only food supplements in chewable form	2400
17.2	Food supplements supplied in a liquid form, excluding food supplements for infants and young children		240
17.2	Food supplements supplied in a liquid form, excluding food supplements for infants and young children	Only food supplements in syrup form	2400

Abbreviation: MPL, maximum permitted level.

[a]If E 950, E 951, E 955, E 957, E 959 and E 961 are used in combination in chewing gum, the maximum level for each is reduced proportionally.

Sucralose (E 955) is not authorised according to Annex III of Regulation (EC) No. 1333/2008.

3.3 | Exposure data

3.3.1 | Concentration data

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, actual concentration data are required to perform a more realistic exposure assessment, especially for those food additives with an MPL at QS in at least one food category.

To obtain actual concentration data, EFSA issued a public call[34] for data (use levels and/or analytical data) on sucralose (E 955) in the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives. In addition, analytical data on sucralose (E 955) can be submitted yearly to EFSA through the open calls for food additive occurrence data in food and beverages intended for human consumption.[35]

In response to this public call, information on use levels of sucralose (E 955) in foods and beverages was made available to EFSA by 14 industry stakeholders by 1 October 2018 through batch 7 call for data (Documentation provided to EFSA No 19–32).

Analytical data on sucralose (E 955) in food and beverages were submitted to EFSA by 13 Member States and extracted in September 2024.

3.3.2 | Reported use levels of sucralose (E 955)

Industry provided EFSA with 336 use levels of sucralose (E 955) in foods for 25 out of 31 authorised food categories[36] according to Annex II to Regulation (EC) No. 1333/2008 (Documentation provided to EFSA No 19–32), see Table 5. Out of these, nine use levels were excluded: four due to unclear concentration in the final product (food supplements in solid form with levels above MPL to be dissolved in the water, but no further clarification on dilution was provided) , two because the data provider reported that the products are no longer on the market and one because the data provider reported that the product formulation has been updated and sucralose is no longer used. Additionally, two records were removed as they are no longer authorised (biscuits). The resulting dataset comprised 326 use levels on sucralose (E 955). The number of food categories available for exposure assessment remained the same after exclusion of these data and represented 31 uses of the 25 food categories. Annex A, Table A2 provides the use levels of sucralose (E 955) in foods as reported by industry.

The Panel noted that industry indicated that 95 use levels for 12 food categories referred to a niche product. Out of them three use levels were considered in the exposure assessment. The final dataset available for exposure assessment comprised 234 use levels.

[34]Available online: https://www.efsa.europa.eu/en/data/call/170621.

[35]5 March 2024: Open call for food additive occurrence data in food and beverages intended for human consumption: https://www.efsa.europa.eu/en/call/open-call-food-additive-occurrence-data-food-and-beverages-intended-human-consumption-2.

[36]Term 'authorised uses' refers to each single use considering each restriction/exception. Term 'authorised food categories' refers to food categories authorised regardless the restriction/exception.

3.3.3 | Summarised data on analytical results of sucralose (E 955) provided by member states

In total, 7636 analytical results of sucralose (E 955) were reported to EFSA by 13 Member States: Austria ($n = 1892$), Belgium ($n = 620$), Cyprus ($n = 30$), Czechia ($n = 36$), Germany ($n = 3026$), France ($n = 5$), Greece ($n = 57$), Croatia ($n = 1$), Hungary ($n = 522$), Ireland ($n = 668$), Italy ($n = 316$), Luxembourg ($n = 267$) and Slovakia ($n = 196$). These data were mainly for flavoured drinks (FC 14.1.4), sauces (FC 12.6) and other confectionery including breath-freshening microsweets (FC 05.2). In addition, 1450 analytical results referred to drinking water (well water and bottled water; FC 14.1.1) with almost all results being left-censored. Foods were sampled between 2010 and 2023. In the exposure assessment, samples from 2014 onwards were considered (516 samples were sampled between 2010 and 2014).

In total, 71% of the analytical results on sucralose (E 955) were left-censored. In addition, 6 records were excluded as they were indicated to be 'above the working limit'. It was noted that the analytical results reported for food categories not authorised to contain sucralose (E 955) were all or almost all left-censored (e.g. drinking water: 99% of data were left-censored). The assessment of exposure to sweeteners is based on the assumption that food and beverages containing the sweetener are identified from the data set and their levels are derived from the quantified analytical results only (EFSA, 2020a, 2020b). Therefore, the left-censored data were excluded from the present assessment ($n = 5455$). Analytical results with an unclear description of the food (e.g. defined as colours and flavourings, preparation aids) were also excluded ($n = 97$).

The Panel noted that 47 quantified analytical results were reported in five food categories in which the use of sucralose (E 955) is not authorised, including mostly cakes and biscuits (FC 7.2), and water (FC 14.1.1). In addition, 194 analytical results for authorised food categories were above the MPL, mostly for flavoured drinks (FC 14.1.4) and food supplements (FC 17). Exceedances were also observed for flavoured fermented milk products (FC 01.4), breath-freshening microsweets (FC 05.2), decorations, coatings and fillings (FC 05.4), breakfast cereals (FC 06.3), fine bakery wares (FC 07.2) and chewing gum (FC 5.3). Data on non-authorised food categories and data exceeding the MPL were excluded from the exposure assessment (EFSA FAF Panel, 2024a).

Overall, after the data cleaning, 1321 analytical results for sucralose (E 955) in foods were available for the exposure assessment corresponding to 27 food categories out of the 34 in which sucralose (E 955) is authorised as a food additive (Annex II to Regulation No 1333/2008).

Details on the available analytical results are provided in Annex A, Table A3.

3.3.4 | Summarised data extracted from Mintel's Global New Products Database

For the purpose of this Scientific Opinion, Mintel's GNPD was used for checking the labelling of food and beverages products and food supplements for sucralose (E 955) within the EU's food market as the database contains the compulsory ingredient information on the label. Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide currently having 24 out of its 27 member countries and Norway included.

According to Mintel's GNPD, sucralose (E 955) was labelled on 9596 products, mainly belonging to 'Nutritional & Meal Replacement Drinks' ($n = 1862$) 'Vitamins & Dietary Supplements' ($n = 1059$), 'Carbonated Soft Drinks' ($n = 853$) and 'Energy Drinks' ($n = 679$), between January 2021 and April 2025.

Annex A Table A5 lists the percentages of the food products labelled with sucralose (E 955) out of the total number of food products per food subcategory according to Mintel's GNPD food classification. The percentages ranged from 0.01% to 64%. The highest percentage was in Mintel's GNPD food subcategory 'Nutritional & Meal Replacement Drinks' followed by three subcategories in which the percentage was above 40% (Energy Drinks, Sports Drinks, Artificial Sweeteners). The average percentage of foods labelled to contain sucralose (E 955) was 3%. Note that these percentages may not consider the market share of the products listed per food category.

Table A5 also contains the list of corresponding food categories according to Annex II to Regulation (EC) No 1333/2008. However, as a one-to-one linkage between Mintel's GNPD food subcategories and these food categories was not possible, this list should be considered as an indicative approximation. The information from the Mintel's GNPD indicated use of sucralose (E 955) in certain authorised food categories (e.g. beer, breakfast cereals, mustard, nuts and soups) for which no use levels/analytical data were reported to EFSA. The Panel noted also that for a few food categories in which the use of sucralose (E 955) is not authorised, foods were labelled to contain sucralose (E 955) (e.g. cakes, meat products and liqueur). However, the number of non-authorised food products labelled with sucralose (E 955) was low (< 0.004% with approximately 36 out of a total of 9596 products).

3.3.5 | Food consumption data used for exposure assessment

3.3.5.1 | *EFSA Comprehensive European Food Consumption Database*

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

in Exposure Assessment'; EFSA, 2011). The version of the Comprehensive database taken into account in this assessment was published in July 2021.[37] Data from EU member states were considered for the estimations, and only countries with food consumption surveys covering more than 1 day were included.

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

Food consumption data from different population groups, infants, toddlers, children, adolescents, adults and the elderly, were used in the exposure assessment. For the present assessment, food consumption data were available from 44 different dietary surveys carried out in 22 EU Member States (Table 6). Not all Member States provided consumption information for all population groups, and in some cases food consumption data from more than one consumption survey of one country was available. In most cases, when, for one country and age class, different dietary surveys were available, only the most recent was used. However, when two national surveys from the same country gave a better coverage of the age range than using only the most recent one, both surveys were kept. For details on each survey, see Table A1 of the Annex A.

TABLE 6 Population groups considered for the exposure estimates of sucralose (E 955).

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

[a]The term 'toddlers' in the Comprehensive Database (EFSA, 2011) corresponds to 'young children' in Regulations (EC) No 1333/2008 and (EU) No 609/2013.
[b]The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in Comprehensive Database (EFSA, 2011).

Since 2018, all consumption records in the Comprehensive Database are codified according to the FoodEx2 classification system (EFSA, 2015). Nomenclature from the FoodEx2 classification system has been linked to the food categorisation system of Annex II of Regulation (EC) No. 1333/2008, part D, to perform the exposure assessments. In practice, the FoodEx2 food codes were matched to the food categories. For a detailed description of the methodology used to link FoodEx2 codes to the food categories, see section 5.2.1 of EFSA FAF Panel (2024b). In FoodEx2, facets are used to provide further information about different properties and aspects of foods recorded in the Comprehensive Database. Facets were used in the exposure assessment of sucralose (E 955) to further identify foods to be included in the assessment (e.g. sweetener-related facets for foods in relevant food categories, see details in Annex A, Table A4).

Specific considerations on table-top sweeteners

A first exposure assessment of sucralose (E 955) showed that the exposure via table-top sweeteners in tablet form was unrealistically high. An examination of the consumption records of these tablets in the Comprehensive Database showed peaks in the consumption distributions at integer numbers of 1 and above in certain dietary surveys. These amounts very likely reflect the number of tablets consumed instead of the consumption in the requested unit of grams. EFSA has asked the data providers of the 16 relevant surveys if they could confirm this and, to date, has received an affirmative response from the data providers of seven dietary surveys. No response has yet been received for the other nine surveys.

For the current assessment, the consumed amounts of table-top sweetener in tablet form in integer numbers of 1 and above in all 16 dietary surveys were converted to grams by multiplying the reported consumption with the highest unit weight of tablets reported to contain sucralose (E 955) in Mintel's GNPD, i.e. 0.085 g per tablet. The same correction was applied to the consumption records of unspecified table-top sweeteners (i.e. those for which the form, tablet or otherwise, was not reported) in the same 16 dietary surveys, as the same consumption pattern was found for these table-top sweeteners.

[37]Available online: https://www.efsa.europa.eu/en/data-report/food-consumption-data.

For this opinion, this ad-hoc correction was applied to the dataset extracted from the Comprehensive database in May 2025.

3.3.5.2 | *Food categories considered for the exposure assessment of sucralose (E 955)*

The food categories for which MPLs have been set, including FC 11.4 Table-top sweeteners for which use and analytical levels of sucralose (E 955) were provided, were selected from the nomenclature of the Comprehensive Database (FoodEx2 classification system), at the most detailed level possible (up to FoodEx2 Level 7) (EFSA, 2015).

Facets were used to identify eating events of foods reported to contain sweeteners and of foods that are related to specific restrictions/exceptions defined in the legislation (see details in Annex A, Table A4).

Facets were not used to identify relevant eating events for FCs 11.4 Table-top sweeteners and 05.3 Chewing gum, and for gum drops in FC 05.2 Other confectionery including breath refreshening microsweets, energy drinks in FC 14.1.4 Flavoured drinks, and vitamin and mineral supplements in FC 17 Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children. These food categories and foods are expected to be major contributors to the exposure according to the literature and represent a relatively high percentage of products labelled to contain at least one sweetener. Thus, all eating events referring to these categories and foods were included in the dietary exposure assessment of sucralose (E 955) as defined in the protocol (EFSA FAF Panel, 2024a).

As FC 17 Food supplements does not consider food supplements for infants and toddlers as defined in the legislation, the exposure to sucralose (E 955) for these two population groups does not include the exposure via food supplements.

Eating occasions belonging to FCs 13.2 Dietary foods for special medical purposes and 13.3 Dietary foods for weight control diets intended to replace total daily food intake or an individual meal were reclassified under food categories in accordance with their main component (e.g. gluten-free pasta reclassified as pasta). Therefore, concentration data for these two food categories could not be considered in the exposure assessment.

Some restrictions/exceptions of certain food categories are not referenced in the Comprehensive Database and therefore the whole food category was considered in the exposure assessment. This was the case for the restriction 'only strongly flavoured throat pastilles with no added sugar' and 'only energy-reduced tablet form confectionary' for FC 05.2 Other confectionery including breath refreshening microsweets, and 'only breakfast cereals with a fibre content of more than 15%, and containing at least 20% bran, energy reduced or with no added sugar' for FC 06.3 Breakfast cereals (Annex A, Table A4). By not considering these restrictions the exposure via these food categories may have been overestimated. In addition, FC 04.2.5.1 Extra jam and extra jelly as defined by Directive 2001/113/EC cannot be distinguished from FC 04.2.5.2 Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EC in the Comprehensive Database and therefore, it was considered in the exposure assessment via the consumption of jam (FC 04.2.5.2).

Sucralose (E 955) can also be added to FC 05.3 Chewing gum as a flavour enhancer, only with added sugars or polyols (see Table 5). To reflect this use, relevant concentration data were linked to consumption events of chewing gum with sugar.

When, within the same food category, specific restrictions/exceptions defined in the legislation could not be identified in the Comprehensive Database, the highest MPL/use level/the highest reliable percentile of analytical level among the closest authorised uses for the same food category was used for all as indicated in Annex A Table A4.

Overall, out of the 34 food categories in which sucralose (E 955) is authorised, 32 were included in the *regulatory maximum level exposure scenario* (29 food categories with an MPL and three with the maximum reported use/analytical level for FCs 11.4.1, 11.4.2 and 11.4.3). For the refined scenarios (i.e. *refined regulatory maximum level exposure assessment scenario* and *refined brand-loyal exposure assessment scenario*), 27 food categories were included. Compared to the *regulatory maximum level scenario*, the following five food categories were not included in the two refined scenarios: FCs 06.3 Breakfast cereals, 12.4 Mustard, 12.5 Soups and broths, 14.2.1 Beer and malt beverages and 15.2 Processed nuts.

The assigned concentrations to each food category in each scenario are detailed in Table A4 of Annex A.

3.4 | Exposure estimates

3.4.1 | Exposure to sucralose (E 955) from its use as a food additive

The Panel considered appropriate, in the remit of the re-evaluation of sweeteners, to estimate a chronic exposure to sucralose (E 955) (EFSA FAF Panel, 2024a). As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011), dietary surveys with only 1 day per subject were not considered as they are not adequate to assess repeated exposure. Similarly, subjects who participated only 1 day in the dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic exposure assessment.

Exposure assessments of sweeteners under the re-evaluation programme are carried out by the Panel based on two different sets of concentration data: (a) MPLs set down in the EU legislation (in the regulatory maximum level exposure assessment scenario) and (b) use levels and/or analytical data provided through the calls for data (in the refined brand-loyal exposure assessment scenario).

To calculate the chronic dietary exposure to sucralose (E 955), food consumption and body weight data at the individual level were extracted from the Comprehensive Database and linked to the concentration data, as described in section 5.2.1 of the Protocol (EFSA FAF Panel, 2024a).

Chronic dietary exposure was calculated by combining MPLs/concentration levels of sucralose (E 955) in each food with the average daily consumption for each food at individual level in each dietary survey and population group. Exposure estimates per individual were divided by the individual's body weight resulting in a distribution of daily individual average exposures per kilogram body weight. Based on these distributions, the mean and 95th percentile (P95) exposures were calculated per survey and per population group. Mean estimates based on dietary surveys/population groups with less than six consumers and P95 estimates with less than 60 observations are not presented (EFSA, 2011).

In this evaluation, as stated in section 5.2.3 in the Protocol (EFSA, 2020a, 2020b), the dietary exposure was assessed for only consumers of at least one food category that could contain sucralose (E 955)[38] for all scenarios. Exposure estimates for these population groups are assumed to be the best approximation reflecting the exposure levels in diabetics, i.e. the population with the highest expected exposure to sweeteners (EFSA, 2020a, 2020b). Depending on the food categories considered in the exposure assessment, the exposure was estimated based on different numbers of consumers. Exposure estimates based on fewer food categories could be higher than those based on a larger number of food categories due to the higher number of non-consumers within certain food categories.

In order to evaluate whether consumers of a single food category would have a higher exposure than consumers of at least one food category, the exposure to sucralose (E 955) of consumers of each single food category (while still considering the whole diet) was also calculated in the *refined brand-loyal exposure assessment scenario*. These exposure estimates are discussed if they are higher than the exposure estimates for consumers of at least one food category.

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 (EC) No 1333/2008 and in case of QS, on maximum reported use level/ the highest reliable percentile of the analytical level when available. For sucralose (E 955), the MPLs used in the assessment are listed in Table A4 of Annex A. For the three table-top sweetener food categories in which sucralose (E 955) is authorised according to QS, the maximum of the reported use levels per food category was used.

Refined brand-loyal exposure assessment scenario

The *refined brand-loyal exposure assessment scenario* for sucralose (E 955) was based on use levels reported by food industry or analytical results reported by Member States. This exposure scenario considers only those food categories for which these data were provided. In this scenario, it is assumed that a consumer is exposed long-term to sucralose (E 955) present at the maximum reported use level/the highest reliable percentile of the analytical data for one food category, and at the mean of typical use levels/mean of analytical data for the other authorised food categories as explained in the protocol (EFSA, 2024).

Annex A, Table A4 summarises the concentration levels of sucralose (E 955) used in the *refined brand-loyal exposure assessment scenario*.

Refined regulatory maximum level exposure assessment scenario

Results of the *regulatory maximum level exposure assessment scenario* cannot be compared directly to the exposure estimates of the refined scenarios since the food categories considered are different ($n = 32$ for the regulatory scenario and $n = 27$ for the refined scenario), and therefore the underlying populations of consumers only are not the same. Therefore, the Panel performed a *refined regulatory maximum level exposure assessment scenario* based on the same food categories as included in the *refined brand-loyal exposure assessment scenario*.

The *refined regulatory maximum level exposure assessment scenario* considers only those food categories for which use levels or analytical data were provided to the Panel (Annex A, Table A4). In this scenario, it is assumed that a consumer is exposed long-term to sucralose (E 955) present at the MPL for these food categories, instead of at use level/analytical level as in the refined brand-loyal exposure assessment scenario.

3.4.1.1 | *Results of the dietary exposure assessment to sucralose (E 955)*

Table 7 summarises the estimated exposure to sucralose (E 955) from its use as food additive in six population groups (Table 6) according to the three exposure scenarios among consumers only of at least one food category containing sucralose (E 955).

[38]Part of the survey population may have no exposure to the additive because they did not report eating a food from one of the food categories that could contain the additive (these are 'non-consumers'). The sub-group that reports eating these foods are called consumers.

TABLE 7 Summary of dietary exposure to sucralose (E 955) from its use as food additive in the regulatory maximum level exposure assessment scenario and in the two refined exposure assessment scenarios, in six population groups among consumers only of at least one food category containing sucralose (E 955) (minimum–maximum across the dietary surveys in mg/kg bw per day and number of corresponding dietary surveys in brackets).[a]

	Infants (12 weeks-11 months)	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[a]						
Mean[b]	0.1–1.6 (10)	0.2–3.9 (15)	0.2–2.8 (19)	0.2–1.6 (21)	0.2–1.2 (22)	0.03–0.9 (23)
95th percentile[c]	0.4–2.4 (2)	0.5–13.5 (14)	0.6–8.7 (19)	0.6–5.2 (20)	0.5–3.9 (22)	0.2–3.7 (22)
Refined regulatory maximum level exposure assessment scenario						
Mean[b]	0.1–4.1 (5)	0.4–5.0 (13)	0.1–2.9 (19)	0.1–1.6 (21)	0.2–2.8 (22)	0.1–2.0 (21)
95th percentile[c]	0.8 (1)	1.2–15.2 (4)	0.5–8.8 (15)	0.7–5.1 (14)	0.8–4.5 (19)	0.7–11.6 (13)
Refined brand-loyal exposure assessment scenario						
Mean[b]	0.1–4.1 (5)	0.2–4.7 (13)	0.1–2.6 (19)	0.1–1.5 (21)	0.2–2.8 (22)	0.1–1.9 (21)
95th percentile[c]	0.4 (1)	0.7–14.3 (4)	0.5–8.1 (15)	0.6–4.8 (14)	0.7–4.5 (19)	0.7–11.6 (13)

[a]Results of the *regulatory maximum level exposure assessment scenario* and the *refined exposure assessment scenarios* can not be compared directly as the underlying populations of consumers differ. This is due to a difference in the number of food categories considered ($n = 32$ and 27, respectively) and because facets are only considered in the regulatory maximum level exposure assessment scenario when the restriction 'only energy-reduced products or with no added sugar' applies. (EFSA FAF Panel, 2024a).

[b]Mean estimates based on dietary surveys/population classes with less than six consumers may not represent the population group and are thus not included in this table.

[c]95th percentile estimates based on dietary surveys/population classes with less than 60 observations may not be statistically robust (EFSA, 2011) and are thus not included in this table.

For the *regulatory maximum level exposure assessment scenario*, the highest mean and P95 exposure was found in toddlers (3.9 and 13.5 mg/kg bw per day, respectively).

In the *refined regulatory maximum level exposure assessment scenario*, mean exposure to sucralose (E 955) ranged from 0.1 mg/kg bw per day in infants, children, adolescents and the elderly to 5 mg/kg bw per day in toddlers. The 95th percentile of exposure ranged from 0.5 mg/kg bw per day in children to 15.2 mg/kg bw per day in toddlers.

In the *refined brand-loyal exposure assessment scenario*, mean exposure to sucralose (E 955) ranged from 0.1 mg/kg bw per day in infants, children, adolescents and the elderly to 4.7 mg/kg bw per day in toddlers. The 95th percentile of exposure ranged from 0.4 mg/kg bw per day in infants to 14.3 mg/kg bw per day in toddlers.

The Panel noted that the 95th percentile exposure levels in the refined scenarios were based only for one dietary survey for infants. (See Table 7).

Detailed results per population group and survey are presented in Annex A, Table A6.

3.4.1.2 | *Main food categories contributing to the exposure to sucralose (E 955)*

In the *regulatory maximum level exposure assessment scenario*, the main contributing food categories to the total mean exposure were FCs 14.1.4 Flavoured drinks and 12.6 Sauces for all population groups. In addition, FC 11.4.3 Table-top sweeteners in tablets contributed considerably for adults and the elderly.

The main contributor in the *refined regulatory maximum level exposure assessment scenario* was FC 14.1.4. Flavoured drinks for all population groups, except the elderly for which it was the second most important contributor to the total mean exposure. In addition, FCs 05.2 Other confectionery, 05.1 Cacao and chocolate products and 05.3 Chewing gum contributed considerably for younger population groups and FC 11.4.3 Table-top sweeteners in tablets for adults and the elderly. FC 01.4 Flavoured fermented milk products appeared also frequently as a main contributor in all population groups. A similar pattern was observed for the *refined brand-loyal exposure assessment scenario*.

For details on the contribution of each food category in the three scenarios, see Tables A7, A8 and A9 in Annex A.

3.4.1.3 | *Dietary exposure for consumers of a single food category containing sucralose (E 955)*

Among consumers only of a single food category (Annex A, Table A10), in the *refined brand-loyal exposure assessment scenario*, the mean exposure estimates for the following food categories exceeded the highest mean exposure estimates of consumers of one or more food categories: FCs 01.4 Flavoured fermented milk products in toddlers (5.7 mg/kg bw per day), 05.2 Other confectionery including breath freshening microsweets in toddlers (6.7 mg/kg bw per day), 11.4 Table top sweeteners in different forms in children, adolescents, adults and elderly (up to 18.8 mg/kg bw per day in adults) and 14.1.4. Flavoured drinks in infants and toddlers (up to 5.6 mg/kg bw per day in toddlers).

For the 95th percentile exposure of consumers only of a single food category FC 01.4 Flavoured fermented milk products in toddlers (14.4 mg/kg bw per day), 11.4 Table-top sweeteners (form not specified) in the elderly (15.1 mg/kg bw per day) and FC 14.1.4 Flavoured drinks in toddlers (15.2 mg/kg bw per day) exceeded the highest overall 95th percentile exposure estimates of consumers of one or more food categories. It was noted that the highest exposure (mean exposure of 18.8 mg/kg bw per day) was derived for 24 adult consumers of table-top sweeteners (form not specified).

3.4.2 | Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 8.

TABLE 8 Qualitative evaluation of influence of uncertainties on the dietary exposure estimate.

Sources of uncertainties	Direction[a]
Consumption data	
Different methodologies/representativeness/underreporting/misreporting/no portion size standard/only a few days	+/–
Underreporting of food descriptors (facets) concerning the presence or potential presence of sweeteners	–[b]
Not considering some of the restrictions specified in the legislation (e.g. specific requirements for breakfast cereals)	+/–
Use of the additive in table-top sweeteners, in particular in powder form, used as alternative for sugar added to home-made products might not be captured	–
Use level of sweetener in home-made products may differ from industrial counterpart	+/–
Table-top sweeteners in tablets and unspecified form: food consumption data assumed to be reported in units were adjusted with the highest tablet size in Mintel's GNPD	+
Unspecified table-top sweeteners in the consumption database matched with the occurrence data for tablet top sweeteners in tablets (highest available among the table-top sweeteners categories)	+
Concentration data	
Correspondence of reported use levels and analytical data to the food items in the Comprehensive Database: uncertainties to which types of food the levels refer	+/–
Uncertainty in possible national differences in use levels of food categories	+/–
Regulatory maximum level exposure assessment: 32 out of the 34 authorised food categories to contain sucralose (E 955) were considered in the exposure assessment	–
Refined regulatory maximum level exposure assessment and brand-loyal scenario: 27 out of the 34 authorised food categories to contain sucralose (E 955) were considered in the exposure assessment	–
Refined regulatory maximum level and brand-loyal exposure assessment scenario: 86 out of 92 Mintel food subcategories in which sucralose (E 955) was labelled were included in the current exposure assessment representing ~98% of the products labelled with sucralose (E 955)	–
Use levels/MPLs considered applicable to all foods within the entire food category, while the percentage of foods labelled with sucralose (E 955) in a corresponding food subcategory labelled with sucralose (E 955) in Mintel was maximally 64% (FC 13.2 and 13.3)	+
Methodology	
Regulatory and *refined maximum level exposure assessment scenario*: – exposure calculations based on the MPL according to Annex II to Regulation (EC) No 1333/2008	+
Refined brand-loyal exposure assessment scenario: – exposure calculations based on the maximum (highest reliable percentile) or mean/median levels	+/–
Use of data from food consumption survey covering only a few days to estimate high percentile(95th) of long-term (chronic) exposure	+

[a]+, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.
[b]Direction of the uncertainty is based on the assumption that the underlying population of consumers does not change.

The dietary exposure to sucralose (E 955) estimated for the *refined regulatory maximum level exposure assessment scenario* and the *refined brand-loyal exposure assessment scenario* covers the majority of food categories in which the sweetener is authorised (27 out of the 34 authorised food categories and 86 out of 92 food subcategories recorded in Mintel, and corresponding to authorised food categories).

An underestimation of the exposure could be expected for consumers who use sucralose (E 955) as an alternative for sugar for home-made food preparation, including baking.

Although there was a high percentage of foods for special medical purposes (FC 13.2. and 13.3) labelled to contain sucralose (E 955) (64%) in Mintel, it was not possible to assess the exposure to sucralose for consumers of these products due to insufficient consumption data.

The Panel acknowledged that the assumption that all foods meeting the facets' criteria (see Section 3.3.5.2) and for which concentrations were available were assumed to contain E 955 resulted in an overestimation of the exposure to sucralose (E 955). According to Mintel, the percentage of foods containing sucralose (E 955) compared to foods available on the market was maximally 64% (for the subcategory of Nutritional & Meal Replacement Drinks). The average percentage was 3% among all Mintel subcategories.

Overall, the Panel considered the exposure to sucralose (E 955) from its use as food additive (excluding population groups under medical supervision), to be overestimated in all three exposure scenarios. The Panel considered the *refined brand-loyal exposure assessment scenario* the most appropriate exposure scenario for the risk assessment of sucralose (E 955).

3.4.3 | Concentrations of and dietary exposure to sucralose (E 955) relevant for the EU population

A literature search (Appendix A) was carried out to gather data on levels of sucralose (E 955) in food and beverages, and on dietary exposure estimates of this sweetener in Europe published between 1999 and 2025.[39] This comparison included the results of studies that reported concentration or exposure over the last 10 years because of changes in the use of the food additives in the EU market and in the consumption behaviour.

3.4.3.1 | *Concentration data*

Several European studies have analysed sucralose (E 955) in food and beverages. The results of the studies are discussed below. The mean concentrations reported are based on quantified values.

Silva et al. (2021) reported concentrations of sucralose in soft drinks ($n = 68$) from the Portuguese market targeted to contain the sweetener according to the label. The soft drinks were grouped as colas ($n = 16$), juice drinks ($n = 28$), iced teas ($n = 13$) and lemon-flavoured drinks ($n = 11$). Sucralose was found in all samples at concentrations ranging from 17 to 99 mg/L.

Concentrations of sucralose in food and food supplements on the Italian market were reported by Janvier et al. (2015). Foods included were flavoured drinks ($n = 57$), fruit nectars ($n = 18$), syrups ($n = 3$), jams ($n = 14$), ketchups ($n = 1$), confectionary ($n = 84$), yogurts ($n = 42$), ice creams ($n = 3$), table-top sweeteners ($n = 14$) and food supplements ($n = 54$), with no quantification of sucralose in ice creams and ketchups. In 37 flavoured drinks (65%), sucralose was found at a mean concentration of 69 mg/L. Out of 14 samples of table-top sweeteners, sucralose was found in four (29%) at a mean concentration of 56 g/kg. Sucralose was also found in 26 food supplements (48%) at a mean concentration of 2079 mg/kg, indicating that there were food supplements exceeding the MPL.

Analysis of artificial sweeteners in 66 beverage products from local markets in Santiago de Compostela in Spain included energy drinks, soft drinks, juices, teas, soy beverages, dairy-based drinks, beers and spirit alcoholic drink (Lorenzo et al., 2015). In 24 (36%) of the beverages, sucralose was present at concentrations ranging from 21 to 224 mg/L. No quantified concentrations were found in soy beverages, beers and spirit alcoholic drink. The two highest concentrations were found in energy drinks (177 and 224 mg/L).

Buffini et al. (2018) analysed different sweeteners in 377 food samples from the Irish market, belonging to 17 food categories. Sucralose was the most frequently found sweetener, found in 66 samples, mainly energy-reduced dairy products ($n = 21$), flavoured drinks ($n = 15$) and carbonated flavoured drinks ($n = 8$). Concentrations above the MPL were reported for food supplements and flavoured drinks. The reported mean concentrations included also results reported as below the LOD and so they cannot be compared to the concentrations reported in this opinion.

Krmela, Kharoshka, Schulzova, Pulkrabova, and Hajslova (2020) reported results for 76 food samples collected at a Czech supermarket: 14 soft drinks, 19 energy drinks and 43 alcoholic beverages. In three samples of energy drinks, sucralose was quantified at concentrations ranging from 47 to 155 mg/L.

Kubica et al. (2015) analysed food products with a multi-method able to determine steviol glycosides and other sweeteners, including sucralose. They analysed 21 samples of soft and alcoholic drinks as well as three drink powders from the market in Poland. The samples were mainly selected for the presence of steviol glycosides according to the label, and this is likely the reason why in none of the samples sucralose was detected.

Three out of eight analysed samples of sugar-free beverages (energy drinks, iced teas and carbonated drinks), purchased from a supermarket located in Brno, Czech Republic, contained sucralose (Diviš et al., 2020). The measured concentrations ranged from 75 to 270 mg/L.

In a study that analysed beverage samples obtained from local supermarkets in Spain, 13 out of 25 samples contained sucralose in a range of 20 to 101 mg/L (Ordoñez et al., 2015). Detects were reported in soft drinks (6 out of 11 samples), energy drinks (3 out of 4), isotonic drinks (2 out of 2), a milk-multi-juice mix (1 out of 2) and a beer-mix (1 out of 2) whereas in the two analysed nectars, one soda and one ice-tea, none were detected for sucralose.

In summary, the quantified concentrations of sucralose in food and beverages reported in the above-mentioned studies in Europe are comparable to the analytical data reported to EFSA (see Section 3.3.3).

3.4.3.2 | *Dietary exposure*

Carvalho et al. (2022) estimated the dietary exposure to sucralose based on the Portuguese dietary survey IAN-AF 2015–2016. Using the MPLs as defined in Regulation No 1333/2008, dietary exposure estimates ranged from 0.54 to 3.69 mg/kg bw per day at the mean and from 1.50 to 7.38 mg/kg bw per day at the 95th percentile in the different age groups (children: 3–10 years; adolescents: 10–17 years; adults: 18–64 years; and elderly: 65–84 years). Considering MPLs only for food categories where sucralose was labelled, exposure estimates varied between 0.05 and 0.25 mg/kg bw per day at the mean, and 0.26 and 1.07 mg/kg bw per day at the 95th percentile.

Based on the analytical data and food consumption data from the National Adults Nutrition Survey described by Buffini et al. (2018; see above), the exposure to sucralose in the adult Irish population was estimated to be 0.06 mg/kg bw per

[39]To cover the period from the latest SCF evaluation with an overlap of 1 year (i.e. 1999) until 4 March, 2025.

day for the mean and 0.68 mg/kg bw per day at the P99 in a consumers only approach. In another Irish study in children (1–4 years), the exposure to sucralose was estimated using food survey data and analytical data in foods and beverages of the Irish market (Martyn et al., 2016). Sucralose exposure of consumers only was 0.65 mg/kg bw per day at the mean and 1.97 mg/kg bw per day at the 95th percentile.

Chazelas et al. (2021) reported a mean dietary exposure to sucralose of 0.02 mg/kg bw per day and a high (95th percentile) exposure of 0.10 mg/kg bw per day for French adults in the NutriNet-Santé cohort that are consumers of foods containing sweeteners. The approach used by the authors deviates from the EFSA approach. Especially, it is not clear whether the adults are consumers of sucralose only or of all sweeteners, and the concentration data used in the NutriNet-Santé cohort was a mix of analytical data, use levels and MPLs.

A Belgian study assessed sucralose exposure of children with type 1 diabetes based on analytical data and a specific food survey within three age groups: 4–6 years, 7–12 years and 13–18 years (DeWinter et al., 2015). The mean exposure to sucralose across the three age groups ranged from 1.05 to 2.57 mg/kg bw per day with children aged 4–6 years having the highest exposure levels. The 95th percentile estimates for consumers only was between 4.87 to 11.00 mg/kg bw per day, also with the highest for children aged 4–6 years.

Estimates of sucralose exposure in a 3 to > 65 years old Italian population ranged from 0.04 (mean of total population) to 0.15 mg/kg bw per day (95th percentile of total population) (Le Donne et al., 2017). The estimates were based on analytical data and a food label survey.

Tennant and Vlachou (2019) assessed sucralose exposure based on reported use levels with the FAIM template and by excluding so called 'minor' food categories. The exclusion criteria applied was that food categories with < 1% labelled with sucralose according to the Mintel GNPD database, were excluded. This approach resulted in an average sucralose exposure for brand-loyal European consumers of the different age-classes and countries that ranged between < 0.1 and 3.6 mg/kg and an exposure at the P95 between < 0.1 and 12.8 mg/kg bw per day with fermented milk products as the main contributor.

Tran et al. (2021) assessed the sucralose exposure for UK based on the National Diet and Nutrition Survey Rolling Programme and reported use levels. Average sucralose exposure ranged between 2.7 and 8.3 mg/kg bw per day for the different age groups (1.5 years and older).

The publications summarised above report dietary exposure assessments performed using methodologies different from the one described in the protocol for assessing exposure to sweeteners (EFSA FAF Panel, 2024a).

In summary, it is not possible to directly compare dietary exposure estimates reported in the cited literature to those estimated in this opinion, primarily because (i) different numbers of food categories are considered, (ii) approaches are different (consumers only approach vs. whole population), (iii) concentration data used in the opinion are from across all European countries vs. country specific data in the literature, (iv) and/or the population groups considered are different. Nonetheless, the Panel noted that estimates from the literature tend to be in the same order of magnitude as those in the current opinion.

3.4.4 | Dietary exposure to sucralose (E 955) considering a proposed extension of use

Jointly with the re-evaluation of the already permitted uses of sucralose (E 955), the Panel considered a request for a proposed extension of use for this food additive in FC 07.2 'Fine bakery wares' with no added sugar or energy reduced at a maximum use level of 700 mg/kg (Documentation provided to EFSA No 45). Table 9 summarises the estimated exposure to sucralose (E 955) from its use as food additive in six population groups (Table 6) according to the *regulatory maximum level exposure assessment scenario* and *refined regulatory maximum level exposure assessment scenario* among consumers only of at least one food category containing sucralose (E 955) considering the proposed extension of use. Detailed results per population group and survey are presented in Annex A, Table A11.

TABLE 9 Summary of dietary exposure to sucralose (E 955) from its use as food additive in the regulatory maximum level exposure assessment scenario and refined maximum level exposure assessment scenario in six population groups among consumers only of at least one food category containing sucralose (E 955) (minimum–maximum across the dietary surveys in mg/kg bw per day and number of corresponding dietary surveys in brackets) considering the proposed extension of use.

	Infants (12 weeks-11 months)	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 considering the proposed extension of use on FC 07.2						
Mean[a]	0.3–2.5 (12)	0.4–4.8 (15)	0.6–4.0 (19)	0.4–2.3 (21)	0.3–1.6 (22)	0.2–1.4 (23)
95th percentile[b]	1.0–5.9 (8)	1.9–14.3 (14)	2.4–10.3 (19)	1.4–6.1 (20)	0.9–4.6 (22)	1.0–4.0 (22)
Refined maximum level exposure assessment scenario considering the proposed extension of use on FC 07.2						
Mean[a]	0.1–2.3 (5)	0.5–5.2 (14)	0.1–2.9 (19)	0.1–1.6 (21)	0.2–2.2 (22)	0.1–2.0 (21)
95th percentile[b]	0.8 (1)	1.2–16.0 (4)	0.5–9.3 (15)	0.6–5.3 (14)	0.7–4.5 (19)	0.7–11.6 (13)

[a]Mean estimates based on dietary surveys/population classes with less than 6 consumers may not represent the population group and are thus not included in this table.
[b]95th percentile estimates based on dietary surveys/population classes with less than 60 observations may not be statistically robust (EFSA, 2011) and are thus not included in this table.

As part of the re-evaluation of sucralose (E 955), FC 07.2. Fine bakery wares was included by considering the restrictions 'only cornets and wafers, for ice-cream, with no added sugar' and 'only essoblaten – wafer paper' at MPL of 800 mg/kg (see Table 5). For the proposed extension of use in FC 07.2, all remaining foods covered by this food category with no added sugar or energy reduced were considered at 700 mg/kg in addition to the already considered authorised uses.

Reporting of a low number of facets for this food category explains the minor differences in the exposure estimates compared to the current authorisation. This was in line with the information retrieved from Mintel's GNPD database where 10% of the bakery products are labelled with other sweeteners in the EU.

3.5 | Biological and toxicological data

The biological and toxicological studies that were assessed as relevant and reliable according to the inclusion criteria established in the revised protocol on hazard identification and characterisation of sweeteners (EFSA FAF Panel, 2023), are listed in Annex B, C, F1 and F2. The identified studies were provided to EFSA following the public call for biological and toxicological data[8,10] and in response to related clarification requests and/or also identified by EFSA from the literature. The identified studies included unpublished study reports (Documentation provided to EFSA No 1), which are summarised in Annex C.

An evaluation of the RoB was performed (Annex E) and a WoE approach for the reliable studies was applied for each health outcome for both human and animal studies (Annex F1, Annex F2). A narrative synthesis of the WoE analysis is reported in Section 3.5.4.

Studies on ADME were not subject to RoB assessment but were evaluated independently by two experts. Information from mechanistic studies, and studies not directly relevant for the identification of a reference point were considered as supporting information and were summarised if relevant under Sections 3.5.4.1, 3.5.4.2 and Appendix C.

Genotoxicity studies were evaluated according to the approach outlined in the revised protocol (EFSA FAF Panel, 2023).

3.5.1 | Absorption, distribution, metabolism and excretion

The ADME of sucralose and its impurities and degradation products[40] (1,6-DCF and 4-CG) were previously evaluated by the SCF (SCF, 2000) and the studies considered in that evaluation were made available by one IBO (Documentation provided to EFSA No 1). The SCF evaluation did not include detailed descriptions and evaluations of individual studies. Therefore, the Panel deemed it appropriate to revisit these studies along with the studies retrieved from the literature search (Appendix A). These encompassed studies in mice, rats, rabbits, dogs and humans for sucralose and studies in rats for its impurities and degradation products (1,6-DCF and 4-CG; see below and Annex B). The Panel considered also the statement on the ADME of sucralose submitted by the IBO (Documentation provided to EFSA No 37).

3.5.1.1 | *Sucralose*

Sucralose kinetics was assessed in one study in **mice**, by administering ^{14}C-labelled sucralose at doses between 100, 1500 and 3000 mg/kg bw per day, given orally or intravenously (Documentation provided to EFSA No 1; reference ID (RefID)[41] 1728, also published in John et al. (2000a)). After oral administration of ^{14}C-labelled sucralose (100, 1500 or 3000 mg/kg bw), 15%–23% of the radioactivity was excreted in the urine and 70%–74% in the faeces. More than 90% of the excreted material was unchanged sucralose. Male and female mice exhibited the same pattern of excretion. In the same study, after a single intravenous dose (20 mg/kg bw), 80% was excreted in the urine and 22% in the faeces. Two components were found in urine, one of which was a glucuronide conjugate based on its co-elution, whereas the other component was not identified. Neither of the two components corresponded to 4-CG and 1,6-DCF.

Sucralose kinetics was assessed in nine studies in **rats** (Documentation provided to EFSA No 1; RefIDs 1730, 772, 1693 (also published by Mann et al. (2000)), 1694, 1697, 1692, 1751, 1752) and Bornemann et al. (2018) and, at doses between 50 and 1000 mg/kg bw, given orally or intravenously. In eight oral studies ^{14}C- or ^{36}Cl-labelled sucralose was used. Urinary and faecal excretion following oral administration accounted for 5.0%–14.9% and 74.4%–93.9% of the dose, respectively. In bile of male rats, 3.2% and 0.8% of the radioactivity was found, following oral doses of 50 mg and 1000 mg/kg bw, respectively. Biliary excretion in females was higher, with 8.9% and 2.2% following the same oral doses of 50 mg and 1000 mg/kg bw, respectively. The results may indicate a dose-dependent, sexually dimorphic biliary excretion process. In studies following intravenous administration, between 76 and 83% of the radioactivity was excreted in the urine and 9%–11% in the faeces. From the urinary radioactive excretion data, the Panel estimated an oral sucralose absorption of about 10%. In urine, most of the studies identified a single radioactive peak by TLC. One study reported that 1%–4% of the radioactivity was associated with more polar metabolites. From the percent of the radioactive dose excreted in the urine after 24 and 96 h, the Panel estimated a sucralose half-life of roughly 4 h.

[40]Referred to as 'hydrolysis products' in the documentation provided by the IBO (Documentation provided to EFSA No 1).

[41]Numerical identifier generated by the DistillerSR tool reported for easier reference to Annex B.

Accumulation of sucralose was not observed in a 28-day study in rats dosed by gavage as indicated by comparison of the AUCs on day 1 and day 28 (Documentation provided to EFSA No 1; RefID 1752), both confirming the estimated half-life of sucralose of roughly 4 h. Chemical analysis was done to measure the sucralose concentration in urine sampled at 26 weeks in animals non-pretreated (Documentation provided to EFSA n.1; RefIDs 1693, 1694). Because of lack of further information (i.e. volume of the urine excreted) the given information on the measured concentrations does not contribute to the understanding of the kinetic of sucralose.

Following 40 days of oral dosing by gavage in rats (average dosage of 80.4 mg/kg bw per day; Bornemann et al., 2018) with unlabelled sucralose, two metabolites, which have not been reported until this study, were found in both the urine and faeces using UHPLC–MS/MS. These metabolites were both acetylated forms of sucralose, were more lipophilic than sucralose, were present in urine and faeces throughout the dosing period and were still present in urine 11 days after dosing ceased. Despite the authors claim that the impurities in the tested sucralose were below the LOD, no information on the identity of the impurities analysed is indicated. No quantification of the impurities in the test item was performed. However, the same group (Schiffman et al., 2023; Documentation provided to EFSA No 33 and 34[42]) referred to one of the metabolites as sucralose-6-acetate which is an impurity of sucralose. According to the EU specifications, sucralose-6-acetate (considered a chlorinated disaccharide) can be present at up to 0.5% as an impurity. Hence, the Panel considered that the occurrence of these substances in faeces and urine is not necessarily an indication of metabolism, but that they were likely impurities in the administered sucralose. In Bornemann et al. (2018), sucralose was found in adipose tissue 14 days after dosing was stopped in four rats (two female, two male) at concentrations between 0.5 and 7.2 μg/g fat (Bornemann et al., 2018). The authors claimed that these findings indicated accumulation of sucralose in fat tissue. The Panel considered the findings as unexpected, given the low partitioning of sucralose in fat tissue due to its physicochemical properties (i.e. log Pow of –1.5).

Secretion of 10% of radioactivity into breast milk was shown in RefID 1692 (Documentation provided to EFSA No 1) using ^{36}Cl-labelled sucralose. In the same study, in 16-day pregnant rats, low levels of radioactivity were found in tissues, including uterus, placenta, foetus and amniotic fluid. In pregnant rats, the influence of multiple dosing and coprophagy on the internal exposure was studied in RefID 1751 (Documentation provided to EFSA No 1). Sucralose was dosed (2000 mg/kg bw by gavage) either repeatedly every day from GD 6 to GD 15 (group 1) or at only a single dose on GD 15 (groups 2 and 3). Group 3 rats were hindered by an 'Elizabethean' collar to prevent coprophagy. The concentrations of sucralose were measured in plasma by a validated method using gas chromatography–electron capture detector (GC–ECD). The comparison of the AUC from the repeated dose group with the AUCs of the single dosed groups suggested that no accumulation occurred, consistent with a half-life of 4–6 h. Animals with hindered coprophagy had a 40% lower internal sucralose exposure than those free to perform coprophagy.

Sucralose kinetics was assessed in **rabbits** in two studies (Documentation provided to EFSA No 1, RefIDs 1751 and 1695 also published in John et al., 2000b). In John et al. (2000b), the urinary and faecal excretion of ^{14}C-labelled sucralose was followed after oral gavage of 10 mg/kg bw as a single dose. Based on urinary radioactivity excretion, an oral absorption of at least 20% was calculated by the authors. TLC of urine samples showed the presence of one major radioactive component which corresponded to unchanged sucralose. The remainder of the radioactivity appeared to be associated with more polar chromatographically-coalesced compounds.

In RefID 1751 (Documentation provided to EFSA No 1), three groups of pregnant New Zealand white rabbits were administered sucralose (dose 350 mg/kg bw per day) by gavage either repeatedly every day from GD 6 to GD 19 (group 1) or a single dose on GD 19 (groups 2 and 3). Plasma concentrations of sucralose were measured by a validated GC-ECD method. In group 3, the rabbits were prevented from performing coprophagy by an 'Elizabethean' collar. From the AUCs, the Panel estimated a sucralose half-life of about 24 h and an accumulation factor of about 2 in all three groups of rabbits. Animals with hindered coprophagy had a 30% lower internal sucralose exposure than those free to perform coprophagy.

The study reported in refID 1695 (Documentation provided to EFSA No 1) was performed to obtain information on the excretion of orally administered ^{14}C-labelled sucralose in pregnant ($n = 3$) and non-pregnant ($n = 3$) female rabbits. There were no notable differences in the absorption and excretion of single oral doses of ^{14}C-labelled sucralose between the two groups. Urinary excretion after 120 h accounted for 21.5% (non-pregnant) and 22.3% (pregnant). Faecal excretion at 120 h accounted for 65.2% and 54.7% of the radioactivity in non-pregnant and pregnant animals, respectively. There were no apparent differences in the profiles of urinary radioactivity between the two groups. Chromatographic analysis of the radioactivity in urine indicated that ^{14}C-labelled sucralose was mainly excreted unchanged and accounted for about 70%–80% of total radioactivity in most urine samples.

Sucralose kinetics was assessed in **dogs** in four studies (Documentation provided to EFSA n. 1, RefIDs 1692, 1700, 1683 (also published by Wood et al. (2000)). In RefID 1692, Beagle dogs (two males, two females) were dosed with 10 or 100 mg/kg bw ^{14}C-sucralose in a capsule. The dogs excreted an average of 17.2% of the radioactivity in the urine and 61.5% in the faeces in 96 h post-dose. The Panel noted that total recovery in this study was low. The authors reported that the elimination rate constants (Ke) were 0.239 and 0.277 h-1 following the doses of 10 and 100 mg/kg bw, respectively, corresponding to half-lives of 2.0 and 2.7 h. In this study, analysis of urine by TLC indicated the presence of sucralose as the only radioactive compound.

[42]Please refer to Section 2.1 Data.

In refID 1700 (Documentation provided to EFSA n. 1), ^{14}C-labelled sucralose was injected intravenously in two male Beagle dogs, on two occasions, 7 days apart at doses between 1.6 and 1.8 mg/kg bw. In all four experiments radioactivity in plasma was detectable up to 6 h after injection and the decline in radioactivity in plasma was described by a three-compartment model. The excretion of radioactivity in urine accounted for between 45.3% and 106.6% of the dose; in the faeces, collected only occasionally 27.6% and 66.3% of the dose were found, indicating most probably high biliary excretion. Most of the radioactivity was excreted within 48 h.

In refID 1683 (Documentation provided to EFSA n. 1), four beagle dogs (two females, two males) were dosed with 2 mg ^{14}C-labelled sucralose/kg bw intravenously and 14 days later orally with 10 mg/kg bw. Methanol extracts of urine and faeces as well as native urine and faeces were analysed by TLC. Within 12 h after intravenous dosing, 74.1% of the dose was excreted in the urine. Urinary excretion accounted for 80.9 ± 4.2% of the administered dose after 5 days. Excretion of radioactivity in the faeces accounted for 10.4% of the dose after 24 h, and 11.9 ± 1.4% of the dose after 5 days. In the same study, following oral dosing, radioactivity declined in a multi-exponential fashion after peak concentrations were reached; the concentrations at 96 h were near or below the background level. Urinary excretion was 26.5% of the radioactive dose following oral dosing within 24 h and 27.6 ± 9.6% within 120 h. Excretion of radioactivity in the faeces accounted for 65.9% of the dose after 24 h, increasing to 68.4% ± 9.4% of the dose after 5 days. Comparing the urinary excretion between intravenous and oral dosing, the Panel estimated an absorption of 40%. The major radioactive component (90%) excreted in the urine was sucralose as determined by TLC. Ten percent of the radioactivity was associated with the other component (Component A). During 3–6 and 6–12 h after dosing, the proportion of Component A constituted approximately 33% of total urinary radioactivity with a corresponding decrease in the proportion of unchanged sucralose. During 12–24 h after intravenous dosing, urinary radioactivity (about 75%) was associated with unchanged ^{14}C-sucralose. Samples of urine incubated with beta-glucuronidase/sulphatase in terms of concentrations of sucralose with untreated samples; this may indicate that component A is either not conjugated with glucuronic acid and/or sulphate or is a conjugated product resistant to hydrolysis. Comparing dog urine, given sucralose, with human urine by examination of the corresponding autoradiograph indicated that unchanged sucralose and component A were present in human urine samples. The identity of component A as a glucuronic acid conjugate was established in a separate study using electron impact and chemical ionisation mass spectra of the TMS-derivatised compound A (Ref ID 1696).

Sucralose kinetics was assessed in 12 **human** studies (Documentation provided to EFSA No 1, RefIDs 1664, 1756, 1684 (also published in Roberts et al. (2000)), 1705, 1711 (also published in Roberts et al. (2000)), 1729; Rother et al. (2018), Stampe et al. (2022), Sylvetsky et al. (2015, 2017, 2024), Leth-Moller et al. (2023)). In Documentation provided to EFSA n. 1, RefID 1664, in two healthy human volunteers following oral administration of 100 mg sucralose dissolved in mineral water, urinary excretion amounted to about 5.0% of the dose. The Panel analysed the data by graphical analysis and detected a biphasic elimination pattern with a half- life of approximately 4 h in the first phase (up to 8 h following administration) and a half-life of approximately 12 h in the second phase. Low absorption was also shown in further studies, varying between 8.8% and 21.7%.

In an oral study with ^{14}C- labelled sucralose (dissolved in aqueous solution; 1 mg/kg bw; RefID 1684, Documentation provided to EFSA n. 1), the plasma concentration-time curves declined in a biphasic manner with a half-life of approximately 4 h in the first phase and a mean terminal half-life of 24.6 h for the second phase. The biphasic decline was also seen in the urinary excretion. Absorption varied between 8.8% and 21.7% of the dose. The main component identified in urine samples was sucralose (about 80% of the radioactivity). Two metabolites accounted together for approximately 10% of the urinary radioactivity and were identified by TLC separation as glucuronide conjugates of sucralose.

RefID 1711 (Documentation provided to EFSA No 1) is a follow-up study to refID 1684 (Documentation provided to EFSA No 1) aimed at identifying one of the metabolites present in urine after oral administration of ^{14}C-labelled sucralose. Two male volunteers received a dose of 10 mg sucralose/kg bw (approximately 20 μCi). The 3–6 h urinary sample from Subject 1 was purified by HPLC fractionation and was analysed by combined HPLC–thermospray mass spectrometry (HPLC–TS–MS). The 0–3 h urine from Subject 1 was incubated for 24 h with 0.1M acetate buffer in the presence and absence of a beta-glucuronidase/sulphatase mixture, sulphatase alone or beta-glucuronidase alone). Aliquots were also incubated 60 h in the presence and absence of aryl-sulphatase. Radioactivity was excreted mainly in the faeces, accounting for about 85% of the dose after 120 h. 10%–13% of the dose was eliminated in urine within 120 h. Mean recovery in urine and faeces was 96% after 5 days. Radio chromatographic analysis of urine samples taken at 0–3, 3–6 and 6–12 h intervals, showed three distinct radioactive peaks where the major peak was unchanged sucralose. It accounted for about 75% of urinary radioactivity in the 0–12 h samples. Two minor components which were detected appeared to be the same as those observed in the previous study (Documentation provided to EFSA n. 1, refID 1684). Over the 12 h, M1 accounted for approximately 10–15% of urinary radioactivity, while the faster running component, M2, accounted for about 9%–11% of urinary radioactivity. Together, metabolites M1 and M2 account for about 2% of the total dose. HPLC analysis of urine revealed similar findings. HPLC–TS–MS analysis of metabolite M1 was inconclusive.

Incubation with either the beta-glucuronidase or the beta-glucuronidase/sulphatase mixture resulted in the complete hydrolysis of M1 which resulted in a proportional increase in radioactivity associated with the sucralose peak but did not significantly alter M2. The data suggest that M1, but not M2, is a glucuronide conjugate of sucralose susceptible to enzymatic hydrolysis with beta-glucuronidase.

In a 12 weeks study in human volunteers (Documentation provided to EFSA No 1, RefID 1705), plasma concentrations of sucralose did not suggest accumulation of sucralose which is in accordance with a half-life of 4–6 h.

In Sylvetsky et al. (2017), the concentration-time course of sucralose in plasma was followed in three groups: group A (11 adults) randomised to consume 355 mL water containing 0 mg, 68 mg, 170 mg or 250 mg sucralose; group B (11 adults) randomised to consume 355 mL seltzer water, 355 mL caffeine-free Diet Rite Cola™ sweetened with 68 mg sucralose and 41 mg acesulfame-K, or 68 mg sucralose and 41 mg acesulfame-K in 355 mL of seltzer water; group C (11 children) randomised to consume 0 mg or 68 mg sucralose in 240 mL water. In children the peak sucralose concentrations in plasma reached 145–400 ng/mL (mean 262.3 ± 24.6 ng/mL). Most adults showed similar peak concentrations after 250 mg (mean 365.6 ± 69.9 ng/mL). Concentrations were similar regardless of whether sucralose was administered in water, in combination with acesulfame-K or in diet soda.

In RefID 1756 (Documentation provided to EFSA No 1), the kinetics of ^{14}C-labelled sucralose was investigated in 3 healthy males (aged 36, 49, 65 years and weighing 69, 65, 80 kg, respectively) following oral administration of 1 mg/kg bw (20 µCi) sucralose. Biological samples were collected in intervals over a period of 12 h (blood) or 120 h (urine, faeces) post-dose. Expired CO_2 was collected in intervals up to 8 h. Blood sampling was performed at 0, 0.5, 1, 2, 3, 4, 6, 8 and 12 h; urine and faeces were collected in the following intervals 0–3, 3–6, 6–12, 12–24, 24–48, 48–72, 72–96 and 96–120 h. No $^{14}CO_2$ was detected in expired air. Chromatographic separation of the total radioactivity in urine (collected 0–3 h) demonstrated a radioactive peak with the same Rf-value as sucralose (constituting 80% of total plate radioactivity). A second unidentified peak contained 5% of the radioactivity which increased to between 10% and 19% in an artificial 3–24 h sample. The Panel considered that this study demonstrated that sucralose is absorbed in man to an extent of at least 13.5%. In urine, most of the radioactivity measured was attributed to sucralose. As no $^{14}CO_2$ was detected in the expired air, the Panel considered that sucralose does not undergo appreciable oxidation (e.g. via the glycolysis and citrate cycle) in the body.

The objective RefID 1729 (Documentation provided to EFSA No 1) study was to identify by mass spectrometry or by HPLC–TS–MS the radioactive components present in urine after oral administration of ^{14}C-labelled sucralose to human volunteers. Samples of human urine were obtained from those collected in study described in RefID 1711. Samples were taken 3–6 h after a single oral dose of ^{14}C-labelled sucralose. Unchanged sucralose accounted for about 65% of the urinary radioactivity in the 3–6 h samples analysed. Approximately 10% of the urinary radioactivity was attributed to a slightly more polar metabolite identified as sucralose-glucuronide based on HPLC–TS–MS. A second radiolabelled metabolite was detected but could not be identified under the HPLC conditions adopted for sucralose and sucralose-glucuronide conjugate No chlorine-containing fragment ions were identified and therefore no information could be obtained as to the site of glucuronide conjugation. The secretion of sucralose in human breast milk was demonstrated in different studies (Rother et al., 2018; Stampe et al., 2022; Sylvetsky et al., 2015, 2024), the mean milk/plasma ratio being 0.15 (SD 0.06) and the calculated mean dose in infants being 4% (SD 2%) of the external dose of the mother.

The study by Leth-Moller et al. (2023) was performed to investigate the transplacental transport of different sweeteners, among them sucralose. Pregnant women, who underwent a planned caesarean section were given 250 mL of blackcurrant-flavoured juice containing 85 mg acesulfame-K, 100 mg aspartame, 60 mg cyclamate, 20 mg saccharin and 75 mg sucralose 2 h before the planned C-section. Maternal blood samples were taken immediately before the caesarean section. During the caesarean section the surgeon obtained amniotic fluid samples and cord-blood samples after delivery. The concentration of the sweeteners was measured by LC–MS/MS. Cord plasma concentrations of sucralose had a significant, positive, linear dependency on the maternal plasma concentrations. The data show that sucralose crossed the placental membranes and was renally excreted by the foetus into the amniotic fluid.

3.5.1.2 | *Impurities and degradation products (1,6-DCF and 4-CG)*

The kinetics of 1,6-DCF and 4-CG was assessed in several studies in rats (Documentation provided to EFSA n. 1, RefID 1652, 1661, 1709, 1714, 1720; Jones et al., 2002). These studies demonstrated extensive metabolism and renal and biliary excretion of the two compounds. (see Annex B for further details).

Additional in vitro studies (Documentation provided to EFSA No 1, RefID 1714) were performed to investigate the distribution of several putative metabolites of 1,6-DCF such as 1,6-dichloro-1,6-dideoxymannitol, 6-chloro-deoxyfructose and 6-chloro-deoxyfructose-glutathione, these studies are summarised in Annex B.

Summary and conclusion on ADME

The kinetics of sucralose was investigated in mice, rats, rabbits, dogs and man. In all species the absorption following oral administration was low (not more than approximately 20%) and variable. Absorption following oral administration in humans varied between 8.8% and 21.7% of the dose. Elimination of systemically available sucralose is mainly by urinary excretion; below 10% of the urinary excreted radioactivity is associated with metabolites with characteristics of glucuronide conjugates. As faecal excretion was observed following intravenous administration in rats and dogs, this indicates that biliary excretion and/or secretion into the gut lumen also occurs. In rats, direct evidence by sampling of bile was obtained for biliary excretion which accounted for between 0.8% and 8.9% of the dose. In dogs, after intravenous administration, 27.6% and 66.3% of the dose were found in the faeces, independent of the dose.

In several experiments, including studies in humans, with ^{14}C-labelled sucralose, no $^{14}CO_2$ was detected in the expired air.

After administration, the decrease in both unlabelled and radioactively labelled plasma sucralose showed a fast first phase (within about the first 8 h) and thereafter a second slower phase in rats, dogs and man. The half-lives were 4–6 h in

the first phase and 12 h in the second phase, in all species (with the exception of rabbits in which the decay was characterised by a single phase with a half-life of 24 h). Urinary excretion data confirm the existence of two phases in rats, dogs and in humans. The mechanism underlying these two phases and the observed interspecies differences remains unclear. Apart from rabbits, where an accumulation factor of 2 was estimated, no accumulation was observed in rats or in humans.

Concerning 1,6-DCF and 4-CG, studies in rats demonstrate extensive metabolism and renal and biliary excretion.

3.5.2 | Animal toxicity

No new data on acute toxicity were received by IBOs; no new data on acute toxicity were identified in the literature. Repeated dose toxicity studies were assessed systematically and are discussed in Section 3.5.4.1.

3.5.3 | Genotoxicity

The genotoxicity of sucralose was evaluated by the SCF (1989, 2000). Based on the studies evaluated, the SCF concluded that there was adequate evidence, both for sucralose and its impurities and degradation products (1,6-DCF and 4-CG), that there was no concern about mutagenicity (SCF, 2000). However, no description and evaluation of the individual studies was provided. Moreover, the FAF Panel noted that, since the last SCF evaluation, refined study protocols and criteria for the evaluation of study results were adopted by the OECD, e.g. in TG 474 and TG 475 (OECD, 2016a, 2016b), and endorsed by EFSA Scientific Committee (2011, 2017, 2021b), together with criteria for the evaluation of the reliability and relevance of genotoxicity studies (EFSA, 2023). Therefore, the FAF Panel deemed it appropriate to consider in this re-evaluation (i) the studies already considered in the previous SCF opinions and provided by one IBO (Documentation provided to EFSA No 1), (ii) those published since the year 2000 and retrieved through a systematic literature search (Appendix A) (iii) the new studies provided by the IBO in reply to the call for data and subsequent communications (Documentation provided to EFSA No 36–43). A systematic literature search was also performed to retrieve studies on the impurities and degradation products of sucralose 1,6-DCF and 4-CG and the degradation product chlrorinated-furan-3-one (see Appendix A), however, additional studies on their genotoxicity were not retrieved.

A total of 41 studies related to in vitro and in vivo genotoxicity of sucralose and its impurities degradation products were available. The available studies were assessed for reliability and relevance (see Annex D). Of these, eight in vitro studies and six in vivo studies on sucralose were considered relevant (high or limited relevance) and are shown in Tables 10 and 11. For sucralose impurities and degradation products (1,6-DCF and 4-CG), the numbers of relevant in vitro and in vivo studies were 14 (8 with 1,6-DCF, 3 with 4-CG, 2 with sucralose-6-acetate and 1 with different mixtures of impurities and degradation products) and 6 (all with 1,6-DCF), respectively (Tables 12 and 13). The remaining studies evaluated as of low relevance due to important flaws or limitations are not considered in the WoE evaluation and excluded from Tables 12 and 13 but are reported in Annex D.

3.5.3.1 | *Genotoxicity assessment of sucralose (E 955)*

In vitro studies

In vitro, sucralose was tested with negative results in a bacterial reversion assay (Ames test) (Documentation provided to EFSA No 36) and an in vitro micronucleus test in human lymphocytes (Documentation provided to EFSA No 38) of high relevance. Negative results of limited relevance were also obtained in bacterial reversion assay (Documentation provided to EFSA No 1), chromosomal aberration test in human lymphocytes (Documentation provided to EFSA No 1), a forward mutation test in mouse lymphoma cells (Documentation provided to EFSA No 1) and an unscheduled DNA synthesis (UDS) in primary rat hepatocytes (Jeffrey & Williams, 2000). In ToxTracker, a stem cell based reporter assay of limited relevance, no activation of DNA damage response, oxidative stress or cell cycle perturbation was observed after sucralose exposure, with or without metabolic activation (Documentation provided to EFSA n. 36). Positive results of limited relevance were reported in a flow cytometry assay for DNA damage where an increase in γH2AX, a possible indication of DNA double strand breaks, was reported in TK 6 cells in the absence of metabolic activation with high sucralose concentrations (2.5 mM and above) (Schiffman et al., 2023; Documentation provided to EFSA n. 33 and 34).[43]

[43]Please refer to Section 2.1 Data.

TABLE 10 Summary table of the in vitro genotoxicity studies on sucralose (E 955). A comprehensive summary of the extracted data and detailed account of the evaluations performed is provided in Annex D.

Test system	Exposure conditions (concentration/testing conditions)	Information on the characteristics of the test substance	Result	Reliability/comments	Relevance of the test system/relevance of the result	Reference
Gene Mutation						
Mammalian Gene Mutation Assay (Forward mutation at the thymidine kinase locus) mouse lymphoma L5178Y cells	Exposure: 4 h Concentrations: 1335–10,000 µg/mL (8 doses, without S9) and 751–10,000 µg/mL (10 doses, with S9)	Sucralose, purity 100% ± 2%	Negative	Reliable with restrictions	High/Limited	Documentation provided to EFSA No 1[44]
Bacterial reverse mutation assay (Ames test) in *S.* Typhimurium TA1535, TA1537, TA98, TA100 and *E.coli* WP2 uvrA	Plate incorporation (1st exp) and pre-incubation (2nd exp) test with concentrations 0, 1.58, 5.0, 15.8, 50, 158, 500, 1580, 5000 µg/plate (with and without S9)	Sucralose purity > 98%	Negative	Reliable without restrictions	High/High	Documentation provided to EFSA No 36[45]
Bacterial reverse mutation assay (Ames test) in *S.* Typhimurium TA1535, TA1537, TA1538, TA98 and TA100	Concentrations: 8, 40, 200, 1000 and 5000 µg/plate (buffer hydrolysate of TGS), 250, 500, 1000, 2500 and 5000 µg/plate (1,6-DCF) or 16, 80, 400, 2000 and 10,000 µg/plate (all the remaining test materials)	Sucralose purity 100% ± 2%	Negative	Reliable with restrictions	High/Limited	Documentation provided to EFSA No 1
Bacterial reverse mutation assay (Ames test) in *S.* Typhimurium TA98, TA100, TA1535 and TA1537, and *E. coli* WP2 *uvr*A	Experiment 4 Concentrations: 1.5, 5, 15, 50, 150, 500, 1500, and 5000 µg/plate In the presence and absence of an exogenous metabolic activation system (S9)	Sucralose Obtained from Sigma-Aldrich It contained 0.5% sucralose-6-acetate.	Negative	Reliable with restrictions	High/limited	Documentation provided to EFSA No 33 and 34 Schiffman et al. (2023)[46]
Chromosomal aberration and Micronuclei						
Chromosomal aberration in cultured human lymphocytes	Exposure 24 h (with S9 added for the first 2 h in the activation assays) Concentrations: 8, 40 and 200 µg/mL (tested only without S9)	Sucralose– no further information reported (e.g. purity)	Negative	Reliable with restrictions	High/Limited	Documentation provided to EFSA No 1[47]
Micronucleus test in human lymphocytes	Concentrations: 500, 1000 and 2000 µg/mL Treatment: 4 h with S9 and 44 h without S9	Sucralose purity > 98%	Negative	Reliable without restrictions	High/High	Documentation provided to EFSA No 38

[44]This study was presented also in Brusick et al. 2010, see Annex D.

[45]This study was presented also in Brusick et al. 2010, see Annex D.

[46]Please refer to Section 2.1 Data.

[47]This study was presented also in Brusick et al. 2010, see Annex D.

TABLE 10 (Continued)

Test system	Exposure conditions (concentration/testing conditions)	Information on the characteristics of the test substance	Result	Reliability/comments	Relevance of the test system/relevance of the result	Reference
Other endpoints						
Reporter genes-based assay for Aneugen Clastogen Evaluation (ToxTracker) No OECD TG No GLP compliance	Concentrations: 0, 625, 1250, 2500, 5000, 10,000 µM With and without S9 Exposure: 24 h	Sucralose purity > 98%	Negative	Reliable with restrictions	Limited/limited	Documentation provided to EFSA No 36
In vitro MultiFlow® DNA damage (p53, γH2AX and Phospho-histone H3) assay in TK6 cells	Experiment 1 Concentration: 20 values of sucralose (maximum 10 mM or 3980 µg/mL) with a dose spacing of 1.4142 (square root of 2) in the presence (+S9) and absence (–S9) of metabolic activation	Sucralose Obtained from Sigma- Aldrich The authors reported that it contained 0.5% sucralose-6-acetate.	Positive A greater than 2-fold rise in γH2Ax by 4 successive increasing concentrations of sucralose beginning at 994 µg/mL (2.5 mM) in the non-activated treatment	Reliable with restrictions	Limited/limited	Documentation provided to EFSA No 33 and 34 Schiffman et al. (2023)[48]
Rat hepatocyte DNA repair analysis in vitro. Hepatocytes from male F344 and SD rats	Hepatocyte cultures prepared from male F334 and Sprague–Dawley rats were exposed for 20 h to $1 \times 10-2$M and $2 \times 10-1$ M sucralose in presence of 10 µCi/mL methyl[3 H]thymidine and then fixed and processed for the autoradiographic determination of methyl[3 H] thymidine incorporation in 50 nuclei from each of three slides per dose.	Sucralose Purchased from Toronto Research Chemicals, Inc. (North York, Ontario, Canada); purity not stated	Negative	Reliable with restrictions	Limited/Limited	Jeffrey and Williams (2000)

[48]Please refer to Section 2.1 Data.

In vivo studies

Negative results were obtained with sucralose in cytogenetic studies in erythropoietic cells of orally exposed rodents, namely a highly relevant micronucleus test in mice (Documentation provided to EFSA No 42), a limited relevance chromosomal aberration test in rats (Documentation provided to EFSA No 1) and a highly relevant micronucleus test in mice (Documentation provided to EFSA No 1). No signs of toxicity to bone marrow were observed in these studies. However, the bioanalysis of plasma (concurrent or in separate toxicokinetic studies) provides a line of evidence of systemic availability of orally administered sucralose and hence of bone marrow exposure.

Negative results of high relevance were obtained in a transgenic rodent mutation assay in the Muta™Mouse strain, where the oral administration of sucralose for 4 weeks up to the limit dose of 1000 mg/kg bw did not induce an increase of mutations in the *lacZ* transgene in any of the tissues analysed (liver, lung, stomach and colon) (Documentation provided to EFSA No 41).

In an in vivo comet assay in rats (Documentation provided to EFSA No 40), the oral administration by gavage of sucralose to the maximum recommended dose level (2000 mg/kg bw) and two lower doses (660 and 200 mg/kg bw) was associated with a statistically significant and dose-related increase in %Tail Intensity (TI) in stomach, colon, liver and, to a lesser extent, in lung cells. In blood cells, no treatment-related increase in %TI was observed. A parallel increase of hedgehogs was observed, especially in stomach and colon samples, that could be linked to a cytotoxic effect. However, there was no evidence of necrosis or apoptosis in the target tissues at the end of treatment. No adverse findings were reported either in an additional pathology analysis, including immunohistochemistry, gross macroscopic investigations and organ weights, performed as a follow-up study using a 24 h delayed sampling time. In addition, previous in vivo comet assay in mice reported a positive response of limited relevance in DNA damage in the same tissues (Sasaki et al., 2002).

TABLE 11 Summary table of the in vivo genotoxicity studies on sucralose (E 955). A comprehensive summary of the extracted data and detailed account of the evaluations performed is provided in Annex D.

Test system	Exposure conditions (dose/testing conditions) (administration)	Information on the characteristics of the test substance	Result	Reliability/comments	Relevance of the test system/relevance of the result	Reference
Gene mutation						
Transgenic rodent mutation assay Liver, lung, glandular stomach and colon from male Muta™Mice	Route: Oral gavage Doses: 250, 500 and 1000 mg/kg bw (limit dose). 5 mice/group Exposure: daily for 28 days Tissue sampling 3 days after last administration Positive control DNA from ENU treated mice.	Sucralose, purity 99.7%	**Negative**	**Reliable without restrictions**	High/High	Documentation provided to EFSA No 41
Chromosomal aberration and Micronuclei						
Micronucleus assay in mouse bone marrow erythrocytes	Fifteen animals per sex per treatment group. Route: Oral gavage Doses: 1000 and 5000 mg/kg Exposure: 24, 48 and 72 h	Sucralose, purity 99.4%	Negative	Reliable without restrictions	High/High	Documentation provided to EFSA No 1[49]
Chromosomal aberrations in bone marrow of rats	Five animals per sex per treatment group. Route: Oral gavage Doses: 500. 1000 and 2000 mg/kg Exposure: 5 successive days	Sucralose, purity 100%	Negative	Reliable with restrictions	Limited/Limited	Documentation provided to EFSA No 149[50]
Micronucleus test in bone marrow erythrocytes Sprague–Dawley rats (Crl:CD(SD)	Forty-two male rats, 6 animals per group Route: Oral gavage Doses: 0, 500, 1000, 2000 mg/kg per day Exposure: 48 h At least 4000 PCE/animal were examined for the presence of MN.	Sucralose, purity 100.7%	Negative	Reliable without restrictions	High/High	Documentation provided to EFSA No 42

(Continues)

[49]This study was presented also in Brusick et al. 2010, see Annex D.

[50]This study was presented also in Brusick et al. 2010, see Annex D.

TABLE 11 (Continued)

Test system	Exposure conditions (dose/testing conditions) (administration)	Information on the characteristics of the test substance	Result	Reliability/comments	Relevance of the test system/relevance of the result	Reference
DNA damage						
Comet assay in mice Stomach, colon, liver, kidney, bladder, lung, brain and bone marrow	Route: Oral gavage Single Dose 24 h Exposure: 100, 1000 and 2000 mg/kg 3 h exposure: 2000 mg/kg dose	Sucralose from San-Ei Gen F.F.I. Inc., Osaka, Japan; Purity not specified	Positive Increase in glandular stomach, colon and lung at 24 h treatment and the top dose of 2000 mg/kg (limit dose). No evidence of necrosis or apoptosis in tested organs.	Reliable with restrictions	High/Limited	Sasaki et al. (2002)
Comet assay (OECD TG 489) Male Crl: CD(SD) rats (5 per group) Stomach, colon, liver, lung and peripheral blood	Route: Gavage Doses: 200, 666 and 2000 (limit dose) mg sucralose/kg bw twice 23 apart; EMS (200 mg/kg bw) as single oral dose; sampling 1 h (T_{max}) after second admin; 3 h after EMS gavage Samplings: stomach, colon, liver, lung, peripheral blood Analysis: %TI determined in 150 cells (from 3 slides) per animal	Sucralose, purity 100.3%	Positive in stomach, colon and liver, and statistically significant trend. Positive results mainly associated with high hedgehogs %, but no other clear toxicity findings Equivocal in lung Negative in blood cells	Reliable without restrictions	High/High	Documentation provided to EFSA No 40

3.5.3.2 | *Genotoxicity assessment of sucralose degradation products and impurities*

In vitro studies

The sucralose impurity and degradation product 1,6-DCF produced dose-related increases in revertant colonies in an Ames test in the *S*. Typhimurium strain TA1535 (up to 6.7-fold) and *E. coli* WP2 *uvrA* (up to 2.6-fold) with and without S9 (Documentation provided to EFSA No 36). A concentration-related increase in revertant colonies (less than 2-fold) was also observed in strain TA100. A positive response in strain TA1535 was also reported in several other limited Ames studies (Documentation provided to EFSA n No 1). A weak mutagenic activity was also observed for 1,6-DCF in a gene mutation tests in mammalian cells (mouse lymphoma) of limited relevance, only in the absence of metabolic activation (Documentation provided to EFSA No 1). In the ToxTracker system, exposure of mouse embryonic reporter cell lines to 1,6-DCF resulted in the activation of p53 and oxidative stress genes, but not of reporter genes responsive to bulky adduct and DNA double strand breaks (Documentation provided to EFSA No 36). Negative results were obtained in an in vitro micronucleus test in human lymphocytes (Documentation provided to EFSA No 39) of high relevance, and in an in vitro chromosomal aberration test (Documentation provided to EFSA n No 1) of limited relevance.

The same activity profile of 1,6-DCF in the Ames test was elicited in one study (Documentation provided to EFSA n. 1) by a buffer hydrolysate of sucralose-containing 1,6-DCF (44.6%) and 4-CG (52.7%). However, the Panel noted that in the same study a mixture of sucralose impurities and degradation products containing similar amounts of 1,6-DCF (49.4%) and 4-CG (47.7%) gave negative results. The latter component (4-CG) gave a negative response in all in vitro assays performed, i.e. Ames test (Documentation provided to EFSA No 1), chromosomal aberrations (Documentation provided to EFSA No 1) and mouse lymphoma assay (Documentation provided to EFSA No 1).

Other degradation products and impurities of sucralose, namely 6-chloroglucose, 6-chlorofructose, 6,1,6′-trichlorosucrose, 6′-chlorosucrose, 1′,6′-diclorosucrose, 4,1′-dichlorogalactosucrose, 4,6′-dichlorogalactosucrose and 4,6,6′-trichlorogalactosucrose, and the mixture TGSHP (49.4% 1,6-DCF +47.7% 4-CG) were tested with negative results in a limited Ames test (Documentation provided to EFSA No 1).

Sucralose 6-acetate was found to give a prototypical clastogenic signature in a MultiFlow® DNA damage assay in TK6 cells (both the assay system and the results were of limited relevance) but a negative response in bacterial mutation assays (a test with high relevance) with results of limited relevance (Documentation provided to EFSA No 33, 34[51]; Schiffman et al., 2023).

Concerning sucralose thermal degradation products, no genotoxicity data were retrieved for chlorinated furan-3-one; a literature search was not performed for 5-hydroxymethylfurfural (HMF), as this compound was considered not to raise concern for genotoxicity based on the conclusions of the previous evaluation as flavouring substance [FL-no 13.139] performed by the EFSA CEF Panel (2011), EFSA FAF Panel (2021).

TABLE 12 Summary table of the in vitro genotoxicity studies on sucralose (E 955) degradation products and impurities. A comprehensive summary of the extracted data and detailed account of the evaluations performed is provided in Annex D.

Test system	Exposure conditions (concentration/testing conditions)	Information on the characteristics of the test substance	Result	Reliability/ comments	Relevance of the test system/ relevance of the results	Reference
Gene mutation						
Bacterial reverse mutation assay (Ames test) in Salmonella Typhimurium TA1535, TA1537, TA98, TA100 and *E. coli* WP2 uvrA	Plate incorporation (1st exp) and pre-incubation (2nd exp) test with concentrations 0, 1.58, 5.0, 15.8, 50, 158, 500, 1580, 5000 µg/plate (with and without S9)	1,6-DCF, purity 99.8%	Positive in TA1535 and *E. coli* WP2 *uvrA* with and without S9. Equivocal positive in TA100.	Reliable without restrictions	High/High	Documentation provided to EFSA No 36
Bacterial reverse mutation assay (Ames test) in Salmonella Typhimurium TA1535, TA1537, TA1538, TA98 and TA100 (Salmonella/ mammalian-microsome plate incorporation mutagenesis assay)	Concentrations: 60, 300, 1500, 3000 and 6000 µg/plate (with and without S9)	1,6-DCF, purity not reported	Weakly positive (in presence of S9)	Reliable with restrictions	High/Limited	Documentation provided to EFSA No 1

(Continues)

[51]Please refer to Section 2.1 Data.

TABLE 12 (Continued)

Test system	Exposure conditions (concentration/testing conditions)	Information on the characteristics of the test substance	Result	Reliability/ comments	Relevance of the test system/ relevance of the results	Reference
Bacterial reverse mutation assay (Ames test) in Salmonella Typhimurium TA1535, TA1537, TA1538, TA98 and TA100	Concentrations: 16, 80, 400, 2000, 10,000 µg/plate (first test with 5 strains); 1000, 2000, 3000, 4000, 5000 µg/plate (repeat test with TA1535 only) With and without S9	1,6-DCF, purity not reported	Positive (TA1535) Negative (other strains)	Reliable with restriction	High/Limited	Documentation provided to EFSA No 1
Bacterial reverse mutation assay (Ames test) in Salmonella Typhimurium TA98, TA100, TA1535 and TA1537, and *Escherichia coli* WP2 *uvr*A	Experiment 43 Concentrations: 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate In the presence and absence of an exogenous metabolic activation system (S9)	Sucralose-6-acetate, purity 99.7%	Negative	Reliable with restrictions	High/limited	Documentation provided to EFSA No 33 and 34 Schiffman et al. (2023)[52]
Bacterial reverse mutation assay (Ames test) in Salmonella Typhimurium TA1535, TA1537, TA1538, TA98 and TA100	Concentrations: 8, 40, 200, 1000 and 5000 µg/plate (buffer hydrolysate of TGS), 250, 500, 1000, 2500 and 5000 µg/plate (1,6-DCF) or 16, 80, 400, 2000 and 10,000 µg/plate (all the remaining test materials)	Mixture Impurities and degradation products of TGS (TGSHP) consisting of 4-chlorogalactose (47.7%) and 1,6-dichlorofructose (49.4%); buffer hydrolysate of TGS, consisting of 4-chlorogalactose (52.7%) and 1,6-diclorofructose (44.6%); 6-chloroglucose; 6-chlorofructose; 6,1,6′-trichlorosucrose; 1,6-diclorofructose; 6′-chlorosucrose; 1′,6′-diclorosucrose; 4,1′-dichlorogala ctosucrose; 4,6′-dichlorogala ctosucrose; 4,6,6′-trichlorogal actosucrose	Positive Buffer hydrolysate of TGS and 1,6-DCF in strain TA1535. All the remaining chemicals were negative.	Reliable with restrictions	High/Limited	Documentation provided to EFSA No 1
Bacterial reverse mutation assay (Ames test) in Salmonella Typhimurium TA1535, TA1537, TA1538, TA98 and TA100	Concentrations: 16, 80, 400, 2000 and 10,000 µg/plate (with and without S9)	4-CG, purity > 99%	Negative	Reliable with restriction	High/Limited	Documentation provided to EFSA No 1
Forward mutation at the thymidine kinase locus in mouse lymphoma L5178Y cells	Concentrations: 13, 18, 24, 32, 42, 56, 75, 100 and 133 µg/mL (with and without S9)	1,6-DCF, purity not reported	Positive in the absence of metabolic activation and negative with activation	Reliable with restrictions	High/Limited	Documentation provided to EFSA No 1
Forward mutation at the thymidine kinase locus in mouse lymphoma L5178Y cells	Concentrations: 10, 13, 17, 23, 30, 40, 53, 71, 95, 127 (with and without S9) and 169 µg/mL (only with S9)	1,6-DCF, purity > 98%	Positive in the absence of metabolic activation and negative with activation	Reliable with restrictions	High/Limited	Documentation provided to EFSA No 1

[52]Please refer to Section 2.1 Data.

TABLE 12 (Continued)

Test system	Exposure conditions (concentration/testing conditions)	Information on the characteristics of the test substance	Result	Reliability/ comments	Relevance of the test system/ relevance of the results	Reference
Forward mutation at the thymidine kinase locus in mouse lymphoma L5178Y cells	Concentrations: 1335, 1780, 2373, 3164, 4219, 5625, 7500 and 10,000 µg/mL (with and without S9)	4-CG, purity > 98%	Negative	Reliable with restrictions	High/Limited	Documentation provided to EFSA No 1
Chromosomal aberration and Micronuclei						
In vitro micronucleus test in human lymphocytes	Concentrations: 25, 50, 100 µg/mL (I exp. without S9); 10, 50, 100 µg/mL (I exp. with S9); 25, 50, 75 µg/mL (II exp. without S9) Treatment: 4 h with S9 and 44 h without S9	1,6-DCF, purity 99.4%	Negative	Reliable without restrictions	High/High	Documentation provided to EFSA No 39
In vitro cytogenetic test (metaphase analysis) in cultured human lymphocytes, with and without metabolic activation	Concentrations: 1.5, 8 and 40 µg/mL (with S9) Treatment: 24 h (S9 added for the first 2 h in the activation assays)	1,6-DCF, purity not reported	Negative	Reliable with restrictions	High/Limited	Documentation provided to EFSA No 1
In vitro cytogenetic test (metaphase analysis) in cultured human lymphocytes, with and without metabolic activation	Concentrations: 40, 200 and 1000 µg/mL (with S9) Treatment: 24 h (S9 added for the first 2 h in the activation assays)	4-CG, purity not reported	Negative	Reliable with restrictions	High/Limited	
Other endpoints						
ToxTracker. Reporter genes-based assay for Aneugen Clastogen Evaluation	Concentrations: 0, 39.1, 78.1, 156.3, 312.5, 625 µM With and without S9 Duration: 24 h	1,6-DCF, purity 99.8%	Induction of p53 and oxidative stress response genes	Reliable with restrictions	Limited/limited	Documentation provided to EFSA No 36
In vitro MultiFlow® DNA damage assay in TK6 cells	Experiment 1 Concentration: 20 values of sucralose-6-acetate (maximum 4.5489 mM or 2000 µg/mL) with a dose spacing of 1.4142 (square root of 2) in the presence (+S9) and absence (−S9) of metabolic activation	Sucralose-6-acetate, purity 99.7%	Sucralose-6-acetate exhibited a prototypical clastogenic signature for both +S9 and -S9 conditions. Sucralose-6-acetate did not display an aneugenic signature	Reliable with restrictions	Limited/limited	Documentation provided to EFSA No 33, 34 Schiffman et al. (2023)[53]

In vivo studies

In cytogenetic tests in rodent erythropoietic cells, negative results of high relevance were obtained in a micronucleus test in rats (Documentation provided to EFSA No 42), in which systemic exposure to 1,6-DCF was indicated by plasma bioanalysis. Other studies with limited relevance include a chromosomal aberration assay in rat bone marrow with equivocal results (Documentation provided to EFSA No 1), and a negative micronucleus assay in mice (Documentation provided to EFSA No 1). Negative results of limited relevance were obtained with 1,6-DCF in a sister chromatid exchange assay in mouse bone marrow (Documentation provided to EFSA No 1), only considered as supporting information.

[53]See Section 2.1 Data.

Negative results of high relevance were obtained in a transgenic rodent mutation assay in the Muta™Mouse strain, where the oral administration by gavage of 1,6-DCF for 4 weeks up to the maximum tolerated dose of 500 mg/kg bw did not induce an increase of mutations in the lacZ transgene in any of the tissues analysed (liver, lung, stomach and colon) (Documentation provided to EFSA No 42, 43).

In an in vivo comet assay in rats (Documentation provided to EFSA No 40), the oral administration by gavage of 1,6-DCF to the maximum tolerated dose (1750 mg/kg bw) and two lower dose levels (175 and 577 mg/kg bw) was associated with a statistically significant and dose-related increase in %TI in stomach, colon, liver and, to a lesser extent, in lung cells. No treatment-related increase in %TI was observed in blood cells. A parallel increase of hedgehogs was observed in stomach and colon. No clear evidence of organ or cellular toxicity was observed at the end of treatment, nor in an additional pathology investigation performed 24 h after the last treatment. However, the Panel noted that in the study report it is stated that in the additional pathology experiment all animals in the high dose group were found dead 24 h after treatment. This finding is in line with the high delayed acute toxicity of orally administered 1,6-DCF.

TABLE 13 Summary table of the in vivo genotoxicity studies on sucralose (E 955) degradation products and impurities. A comprehensive summary of the extracted data and detailed account of the evaluations performed is provided in Annex D.

Test system	Exposure conditions (dose/testing conditions) (administration)	Information on the characteristics of the test substance	Result	Reliability/ comments	Relevance of the test system/ relevance of the results	Reference
Gene mutation						
Gene Mutation In vivo transgenic rodent mutation assay (OECD TG 488) Liver, lungs, glandular stomach and colon from Muta™Mice	Route: Oral gavage Forty-four male mice, 9 weeks old, 7 mice/group Doses: 0, 125, 250, 500 mg/kg per day via oral gavage Exposure: daily for 28 consecutive days	1,6-DCF, purity 99.8%	Negative	Reliable without restrictions	High/High	Documentation provided to EFSA No 42, 43
Chromosomal aberration and Micronuclei						
Chromosomal aberrations in bone marrow of rats	Five animals per sex per treatment group. Oral (gastric intubation): Single dose study 1000 mg/kg; Sub-acute study dosed 5 successive doses. Doses: 50, 150 and 500 mg/kg per day	1,6-DCF, purity not stated	Equivocal. Positive linear trend, not statistically significant at any dose.	Reliable with restrictions	High/Limited	Documentation provided to EFSA No 1[54]
Micronucleus assay in mouse bone marrow	Single dose of 1660 mg/kg Exposure: 24, 48 and 72 h Positive control: cyclophosphamide	1,6-DCF	Negative	Reliable with restrictions	High/Limited	Documentation provided to EFSA No 1
Micronucleus test in the PCE of the bone marrow in rats Sprague–Dawley rats (Crl:CD(SD)	Fifty-one male and nine female, 7–8 weeks old, out-bred rats Route: Oral gavage Doses: 0, 375, 750, 1500 mg/kg per day via oral gavage Exposure: 48 h At least 4000 PCE/animal were examined for the presence of MN.	1,6-DCF, purity 99.8%	Negative	Reliable without restrictions	High/High	Documentation provided to EFSA No 42

(Continues)

[54]This study was presented also in Brusick et al. 2010, see Annex D.

TABLE 13 (Continued)

Test system	Exposure conditions (dose/testing conditions) (administration)	Information on the characteristics of the test substance	Result	Reliability/ comments	Relevance of the test system/ relevance of the results	Reference
DNA damage						
Comet assay (OECD TG489) Male CD rats (5 per group)	Route: Gavage Doses: 175, 577.5 and 1750 (MTD) mg 1,6-DCF/kg bw twice 23 apart; EMS (200 mg/kg bw) as single oral dose; sampling 3 h after last admin Samplings: stomach, colon, liver, lung, peripheral blood Analysis: %TI determined in 150 cells (from 3 slides) per animal	1,6-DCF, purity 99.8%	Positive in stomach, colon, liver and lung, with statistically significant increase of %TI (above 95% upper confidence limit of HC) and statistically significant trend; positive results mainly associated with high hedgehogs %, but no other clear toxicity findings. Negative in blood cells.	Reliable without restrictions	High/High	Documentation provided to EFSA No 40
Other endpoints						
Sister chromatid exchange assay in mouse bone marrow	Five animals per sex per treatment group Route: Oral gavage Doses: 200, 1000 and 2000 mg/kg Positive control: cyclophosphamide	1,6-DCF, purity 99%	Negative	Reliable with restrictions	Limited/Limited	Documentation provided to EFSA No 1

In silico studies

An in silico assessment of the potential genotoxicity of sucralose (E 955) impurities was performed using a suite of VEGA statistical models and the knowledge-based OECD QSAR Toolbox resource (version 4.4.1) (see Appendix B). The VEGA statistical models aimed to predict the activity in in vitro (Ames, chromosomal aberrations and micronucleus) and in vivo (micronucleus) mutagenicity assays based on the presence of common molecular substructures in the compounds analysed and in a training set of compounds for which experimental data are available. The OECD QSAR Toolbox, which is basically intended for grouping and read-across, identifies alerting structures in the compounds potentially associated with toxicity endpoints based on previous knowledge, with no specific consideration of the structure of the compounds investigated. Both general mechanistic profilers for DNA binding capacity and endpoint specific profilers for in vitro (reverse mutation in the Ames test, in vitro chromosomal aberrations and micronuclei) and in vivo (micronucleus) mutagenicity were applied.

The main conclusions from the in silico assessment are summarised below. For further details see Appendix B.

A consistent prediction of mutagenicity was provided by VEGA models for the sucralose thermal degradation product chlorinated furan-3-one (referred to as alpha-beta unsaturated carbonylic compound # 15 in Appendix B). This compound was predicted to be mutagenic in the Ames test, and active in the in vitro and in vivo cytogenetic assays.

For the other compounds, most of the molecules were out of the applicability domain of the VEGA models because the accuracy and concordance of predictions for similar substances in the training set were inadequate: the predictions have low reliability and were not further considered. The only reliable prediction provided was for a positive response of 1,6-DCF in the Ames test, which is in line with the experimental evidence provided by several experimental studies in vitro but overruled by the available negative experimental studies. No consistent prediction of mutagenicity in mammalian cells was obtained for any of the compounds investigated.

In the QSAR Toolbox, the presence of the alpha-beta unsaturated carbonylic structure was identified by all profilers as an alert for DNA binding and in vitro and in vivo genotoxicity and genotoxic carcinogenicity for chlorinated furan-3-one (see Appendix B).

For the remaining compounds, a similar output was obtained with all impurities structurally related to sucralose (i.e. all compounds with the exception of TPPO, for which no alerting structure was identified). The presence of the same potentially alerting substructures in sucralose and its impurities supports the read-across among this set of compounds for genotoxicity evaluation. In these compounds the presence of the aliphatic halogen structure was identified as an alert for DNA binding capacity and mutagenicity in the Ames test. However, the Panel noted that these predictions for sucralose are overruled by the available negative experimental data in vitro and in vivo and considered that a similar conclusion can be reached by read-across for all impurities structurally related to sucralose.

Overall, the Panel concluded that the in silico assessment of sucralose impurities and degradation indicates a concern for genotoxicity of the thermal degradation product chlorinated furan-3-one, while no genotoxicity is anticipated for the other analysed impurities and degradation products.

3.5.3.3 | *Conclusion on the genotoxicity of sucralose and its degradation products and impurities*

In tests in vitro with sucralose, highly relevant, negative results were obtained in the bacterial reverse mutation (Ames) and micronucleus assays, the basic test battery recommended by the EFSA Scientific Committee for genotoxicity testing (EFSA Scientific Committee, 2011) also applicable to food additives (EFSA ANS Panel, 2012). Negative results of less relevance were also obtained in other mutagenicity assays (gene mutations in bacteria and mammalian cells, and chromosomal aberrations) and in an indicator test (the ToxTracker assay). Positive findings, of limited relevance, were only reported in a flow cytometry assay for DNA damage where an increase in γH2AX, a possible indication of DNA double strand breaks, was reported in TK 6 cells in the absence of metabolic activation with high sucralose concentrations (2.5 mM and above) (Documentation provided to EFSA No 33 and 34; Schiffman et al., 2023)[55].

In vivo sucralose was negative in cytogenetic tests in rodent erythropoietic cells, which include a highly relevant micronucleus test in mice. In a comet assay in rats, sucralose induced a positive response both in the gastrointestinal tract (stomach and colon) and at distant sites (liver and partially in lung), but not in blood cells. Negative results of high relevance were obtained in a mutation test in transgenic mice and, in which no increase of mutations was observed in liver, lung, colon and stomach. These are the same tissues presenting positive data in comet assay in the other study.

Among the impurities and degradation products of sucralose, only 1,6-DCF showed a consistent mutagenic activity in vitro. 1,6-DCF directly induced base substitution mutations in bacteria in several studies. It was also weakly positive in a gene mutation study in mammalian cells, only in the absence of exogenous metabolic activation. A positive result, indicative of oxidative stress induction and p53 activation, was obtained in the ToxTracker assay. Negative results of high and limited relevance were obtained in the in vitro micronucleus and chromosomal aberration assays.

In tests in vivo with 1,6-DCF, highly relevant negative results were obtained in a micronucleus test in rat erythropoietic cells, and in a gene mutation study in transgenic mice in liver, lungs, glandular stomach and colon. On the other hand, similarly to sucralose, highly relevant positive results were reported in the same tissues in an oral comet assay in rats.

Overall, for both sucralose and 1,6-DCF highly relevant negative results in vivo covering different apical genetic endpoints (gene mutation, clastogenicity and aneugenicity) are available. However, with both compounds a positive response, suggestive of the induction of primary DNA damage, was observed in several tissues in comet assay in rats.

[55]Please refer to Section 2.1 Data.

As recommended in the EFSA Scientific Committee statement 'Clarification of some aspects related to genotoxicity assessment' (2017), other data may assist in reducing the uncertainty in case it is not possible to conclude on genotoxicity with confidence based on the available genotoxicity experimental data. In this respect the Panel noted that:

- In silico data indicate oxidative stress secondary to GSH depletion as an indirect mechanism for DNA damage for both sucralose and 1,6-DCF as haloalcohols (see Appendix B);
- Several studies have provided evidence that sucralose can produce an oxidative stress in a range of mammalian and bacterial systems under a variety of conditions in vitro (Kundu et al., 2020; Wu et al., 2022; Yu et al., 2021, 2023 and Yu & Guo, 2022). The TOXTracker data for 1,6-DCF also gave an indication of oxidative stress. Sucralose-induced oxidative stress has also been reported in human subjects (Zafrilla et al., 2021);
- In additional mechanistic studies in non-mammalian systems, the generation of reactive oxygen species associated with oxidative stress was observed along with DNA damage detected by the Comet assay and micronucleus formation in blood cells of Carp fish (*C. carpio*) exposed to sucralose (Heredia-García et al., 2019);
- Oxidative DNA damage (8-hydroxydeoxyguanosine) in association with lipid peroxidation and a reduction of glutathione concentrations has been reported in human microglial clone 3 (HMC3) cells exposed to sucralose (1 mM) albeit in long-term culture (7, 14 and 21 days) (Hacioglu, 2024);
- The available in silico and experimental data indicate the plausibility of reactive oxygen playing an indirect role in the DNA damage observed in comet assays with sucralose and 1,6-DCF;
- The available toxicological data on sucralose, which also include a 2-year chronic toxicity/carcinogenicity study in rats, indicate no concern for the possible long-term consequences of DNA damage observed in comet assays.

Overall, considering the negative results obtained in mutagenicity studies in vivo on apical genotoxicity endpoints (gene mutation and chromosomal damage), also including the same tissues that revealed positive effects in comet assay, and the plausible indirect genotoxic mode of action secondary to induced oxidative stress, the Panel concluded that there is no concern for the genotoxicity of sucralose when used as food additive (E 955). The absence of adverse effects related to genotoxicity such as carcinogenicity further supports this conclusion (see Section 3.5.4.3).

The Panel noted that based on read-across the same conclusions apply to impurities and degradation products structurally related to sucralose, including 1,6-DCF for which adequate in vivo mutagenicity data are available, including in tissues tested positive in the comet assay.

For chlorinated furan-3-one, in silico data indicates a potential genotoxicity. However, based on general knowledge of food processing, the formation of chlorinated furan-3-one is not expected under the temperature and time conditions used in currently authorised uses (see Table 5).

3.5.4 | Synthesis of systematically appraised evidence and weight of evidence assessment

For sucralose (E 955), a total of 3228 references were screened based on title and abstract. These references included studies retrieved from the literature (timeframe: 1.1.1999–4.3.2025) as well as the key studies on which the derivation of the ADI set by SCF (2000) and JECFA (1991) was based.

The toxicological studies provided by one IBO (Documentation provided to EFSA No 1) were already considered and evaluated by the SCF or were among the publications retrieved in the systematic literature search (see Appendix A). After title and abstract screening, 1381 studies were screened at the full text level; 392 studies were further subjected to categorisation and confirmation of relevance screening, resulting in 110 studies (62 animal studies, 48 human studies) for inclusion in RoB assessment (Appendix A, Figure A1).

The studies previously considered by the SCF (SCF, 2000) for deriving the current ADI (hereafter reported as 'key studies') encompassed areproductive toxicity study in rats by dietary exposure, a 104-week combined toxicity and carcinogenicity study in rats by dietary exposure, a 26-week study in rats with exposure by gavage and a pair fed dietary restriction study in rats (Documentation provided to EFSA No 1; see alsoAnnex C). These studies were evaluated for RoB, along with relevant literature published since the previous evaluation by the SCF, allowing 1 year of overlap (cut-off date: 1999). All the key studies were of low to moderate risk of bias (tier 1 and tier 2). Therefore, following the approach described in the protocol (EFSA, 2020a; EFSA FAF Panel, 2023), the systematic approach for the appraisal of the evidence was applied to: (i) the new evidence available since the cut-off date and (ii) the studies identified as key in the previous evaluation (Documentation provided to EFSA No 1).

3.5.4.1 | *Animal studies*

The studies included in the assessment encompassed 27 animal studies. Following a RoB evaluation, among these studies, 6 studies were allocated to tier 1, 16 studies tier 2 and 5 studies were allocated tier 1 or tier 2 depending on the endpoint, see Table 14.

TABLE 14 Animal studies included in the assessment. The data extraction for the unpublished study reports (Documentation provided to EFSA No 1) is provided in Annex C.

Authors	Study type[a]	Exposure duration	Species/strain	No of animals	Dose level	Dose level in mg/kg bw per day	RoB tier
Martínez et al. (2010)	Sub-chronic	73 days	Rats, Wistar	9 males/group	0.19% sucralose in drinking water, ad libitum	Equivalent to 169.1 mg/kg bw per day sucralose	1
Pałkowska-Goździk et al. (2018)	Sub-acute	21 days	Rats, Sprague–Dawley	35 animals/sex/group	0.0167 g of sucralose/100g diet	Equal to 14.2 mg/kg bw per day sucralose	1
Documentation provided to EFSA No 1	Sub-chronic	182 days (26 weeks)	Rats, CD Strain	20 animals/sex/group	1% sucralose ad libitum 1% sucralose 90% diet 3% sucralose ad libitum 3% sucralose 90% diet	1% sucralose ad libitum: equal to 636 (M) and 787 mg/kg bw per day (F) 1% sucralose 90% diet: equal to 628 (M) and 787 mg/kg bw per day (F) 3% sucralose ad libitum: equal to 1978 (M) and 2435 mg/kg bw per day (F) 3% sucralose 90% diet: equal to 1973 (M) and 2455 mg/kg bw per day (F)	1
Documentation provided to EFSA No 1[e]	Sub-chronic	182 days (26 weeks)	Rats, CD Strain	21 animals/sex/group	Gavage with sucralose in purified water – all formulations were prepared daily to provide the daily dosage at a constant volume of 10/mL/kg body weight per day)	Equal to 0, 750, 1500 and 3000 mg/kg bw per day sucralose	1 and 2
Shi et al. (2021a)	Sub-chronic	77 days (11 weeks)	Mice, C57BL/6	10 females/group	0, 0.1 mg/mL sucralose solution	Equivalent to 0, 16.4 mg/kg bw per day sucralose	1
Shi et al. (2021b)	Sub-chronic	77 days (11 weeks)	Mice, C57BL/6	10 females/group	0, 0.1 mg/mL sucralose solution	Equivalent to 0, 16.4 mg/kg bw per day sucralose	1
Rathaus et al. (2024)	Sub-chronic	140 days (20 weeks)	Mice, C57BL/6J	10 males/group	0, 179.5 mg/L sucralose in drinking water	Equivalent to 0, 26 mg/kg bw per day sucralose	1
Documentation provided to EFSA No 1[f]	Chronic toxicity and carcinogenicity	728 days (104 weeks)	Rats, Sprague–Dawley, CD strain	50 animals/sex/group	3000 (0.3%), 10,000 (1.0%) and 30,000 (3%) ppm continuously via diet. The concentration of 30,000 ppm was reduced to 10,000 ppm during lactation	Males: equal to 94, 323 and 1005 mg/kw bw per day (weeks 1–104) Females: equal to 135, 458 and 1395 mg/kg bw per day (weeks 1–104)	1 and 2
Documentation provided to EFSA No 1[g]	Reproductive toxicity	28 weeks (F0 and F1 parental; (two generation study)	Rats, Sprague–Dawley, CD strain	30 animals/sex/group Litters reduced to 8 pups (4 males and 4 females when possible) on post-partum day 4	Sucralose in diet – concentrations not reported	Males F0 (week 10): equal to 0, 155, 514, 1580 mg/kg bw per day Females F0 (week 10): equal to 0, 194, 656, 2090 mg/kg bw per day Males F1 (week 10): equal to 0, 154, 524, 1680 mg/kg bw per day Females F1 (week 10): equal to 0, 192, 642, 2100 mg/kg bw per day	1 and 2
Glendinning et al. (2020)	Sub-acute	28 days	Mice, C57BL/6 (B6)	5 animals/sex/group	0, 30 mM (1.2%) sucralose in deionised water	Equivalent to 0, 2160 mg/kg bw per day sucralose	1 and 2

(Continues)

TABLE 14 (Continued)

Authors	Study type[a]	Exposure duration	Species/strain	No of animals	Dose level	Dose level in mg/kg bw per day	RoB tier
Guo et al. (2021)	Short-term (sub-acute)	42 days (6 weeks)	Mice, C57BL/6	6 males/group	0, 1.5 mg/mL sucralose in drinking water, and 1.5 mg/mL sucralose in drinking water, ad libitum	Equivalent to 0, 261 mg/kg bw per day sucralose	1 and 2
Haq et al. (2019)	Sub-acute	56 days (8 weeks)	Rats, Wistar albino	10 animals/group (number per sex per group not stated)	–	Equal to 0,5 or 1000 mg/kg bw per day, 6 days per week	2
Ren et al. (2021)	Sub-acute	32 days	Mice, Kunming	10 males/group	10 mM sucralose solution	Equivalent to 692 mg/kg bw per day	2
Barakat et al. (2023)	Sub-acute	6 weeks	Rats, albino Wistar. Normal rats (NR) and STZ-induced diabetes rats (DR)	6 males/group		0 (NR & DR), 92.5 mg/kg bw per day sucralose solution (NR & DR), 92.5 mg/kg bw per day sucralose solution + insulin (2 units per day) (DR only), 92.5 mg/kg bw per day sucralose solution + stevia (25 mg/kg bw per day) (NR & DR), 92.5 mg/kg bw per day sucralose solution + stevia (25 mg/kg bw per day) +insulin (2 units per day) (DR only)	2
Zhang et al. (2024)	Sub-acute	70 days (10 weeks)	Rats, Sprague–Dawley (SPF)	6 males/ group	0, 0.02% sucralose in drinking water ad libitum	Equivalent to 0, 17.8 mg/kg bw per day sucralose	2
Zhu et al. (2024)	Sub-acute	28 days	Guinea pigs, Harley-white	10 females/group	0, 167 mg/kg sucralose in the diet	Equivalent to 0, 6.8 mg/kg bw per day[b]	2
Ragi et al. (2021)	Short-term (sub-acute)	49 days (7 weeks)	Rats, Sprague–Dawley	6–7 males/group	0, 0.016% sucralose in the diet, in drinking water for 12 h (dark phase) and in both the diet and in drinking water for 12 h (dark phase) combined, all ad libitum.	Equivalent to 19 mg/kg bw per day sucralose in the diet, 10 sucralose in the water and 39 mg/kg bw per day for sucralose in both water and diet	2
Farid et al. (2020)	Sub-chronic	56 and 112 days (8 and 16 weeks)	Mice, BALB/c albino	5 animals/sex/group	5.2 mg/mL for 5 h per day in drinking water	Equivalent to 162.5 mg/mg bw per day	2
Santos et al. (2021)	Sub-chronic	42 days (6 weeks)	Mice, Swiss (SPF)	4/5 males per group	0, 0.03% sucralose solution in drinking water, ad libitum	Equivalent to 43.2 mg/kg bw per day sucralose	2
Morales-Ríos et al. (2022)	Sub-chronic	126 days (18 weeks)	Rats, Sprague–Dawley	10 males/group	0, 10% sucralose in drinking water ad libitum	Equivalent to 2 mg/kg bw per day	2

TABLE 14 (Continued)

Authors	Study type[a]	Exposure duration	Species/strain	No of animals	Dose level	Dose level in mg/kg bw per day	RoB tier
Shi et al. (2023)	Sub-chronic	84 days (12 weeks)	Mice, ICR wild-type, ICR germ-free & C57BL/6 Tas1r3 knockout mice	10 males/group ICR mice (wild-type and germ-free groups); 5 males/group C57BL/6 Tas1r3 knockout mice	0, 0.27, 0.33, 0.4 and 0.47 g/L sucralose solution ad libitum.	Equivalent to 0, 39, 48, 58 and 68 mg/kg bw per day sucralose	2
Zheng et al. (2022)	Sub-chronic	112 days (16 weeks)	Mice, C57BL/6J (M)	8 males/group	0, 0.0003, 0.003, 0.03 and 0.3 mg/mL sucralose solution	Equivalent to 0, 0.043, 0.43, 4.3 and 43 mg/kg bw per day sucralose	2
Concha Celume et al. (2024)	Sub-chronic	112 days for F0 only (16 weeks)	Mice, C57BL/6J	Animal no. per sex/ per group not stated	0, 0.1mg/mL sucrose solution (in water) ad libitum	Equivalent to 0, 15 mg/kg bw per day sucralose	2
Azad et al. (2020)	Reproductive toxicity	42 days (6 weeks)	Mice, C57BL/6	6 females/litters per group	–	Equal to 6.3 mg/kg per bw peray sucralose	2
Mendoza-Pérez, García-Gómez, et al. (2021), Mendoza-Pérez, Guzmán-Gómez, et al. (2021)	Chronic	480 days	Rats, Wistar HsdHan	10 animals/sex/group for childhood, adolescence and young adult groups; 5 animals/sex/group for adulthood and reproductive senescence groups	0, 0.019% sucralose in drinking water ad libitum	22.8 mg/kg bw per day for sub-acute exposures, 17.1 mg/kg bw per day for sub-chronic exposures and 9.5 mg/kg bw per day for chronic exposures[c]	2
Mendoza-Pérez et al. (2022)	Chronic	480 days	Rats, Wistar HsdHan	10 animals/sex/group for childhood, adolescence and young adult groups; 5 animals/sex/group for adulthood and reproductive senescence groups	0, 0.019% sucralose in drinking water ad libitum	22.8 mg/kg bw per day for sub-acute exposures, 17.1 mg/kg bw per day for sub-chronic exposures and 9.5 mg/kg bw per day for chronic exposures[d]	2
Soffritti et al. (2016)	Chronic toxicity and carcinogenicity	From 12 days of gestation until death	Mice, Swiss	5 groups of male (total $n = 457$) and 5 groups female (total $n = 396$) M: 117, 114, 90, 66, 70 F: 102, 105, 60, 65, 64	Concentrations of 0, 500, 2000, 8000 and 16,000 ppm of sucralose in feed	Equivalent to 0, 62.5, 250, 1000 and 2000 mg/kg bw per day sucralose[a]	2

[a]Studies were classified according to duration into (i) sub-acute (less than 8 weeks), (ii) sub-chronic (superior or equal to 8 weeks to less than a year) and (iii) chronic (equal to 1 year or longer).

[b]Default values for Guinea pigs: World Health Organization & Food and Agriculture Organization of the United Nations. (2009). Environmental health criteria 240: Principles and methods for the risk assessment of chemicals in food (Annex 2). WHO Press. WHO_EHC_240_14_eng_Annex2.pdf.

[c]In this chronic toxicity study euthanasia was performed for half of the animals on day 160 and for the other animals on day 480. Statistical analyses of body mass, feed, drink and energy intake were carried out on days 21, 35, 63, 210 and 504, which correspond to the beginning of the different life stages of the rats: childhood, adolescence, young adult, adulthood and old age, respectively. For this reason, the doses were calculated for different exposure durations.

[d]In this chronic toxicity study euthanasia was performed for half of the animals on day 160 and for the other animals on day 480, therefore, the dose calculation was done both for sub-chronic and chronic exposure.

[e]The data from this study were also published in Goldsmith et al. (2000).

[f]The data from this study were published in Mann et al. (2000).

[g]Data from this study were published in Kille et al. (2000 a,b).

The WoE assessment of the 27 animal studies (Table 16) is presented in detail in Annex (F1). Annex F1 shows the WoE rating according to predefined downgrading and upgrading elements for each health outcome category (HOC). Each HOC consists of groups of endpoints (see Table 15), each endpoint being addressed in one or more of the included animal studies.

TABLE 15 Health outcome categories and related endpoints of the appraised animal studies subjected to WoE evaluation (see Annex F1).

Health outcome categories (HOCs)	Endpoints
Body weight[a]	Body weight, body weight gain, feed intake, water intake
Additional clinical chemistry[b]	Serum triglyceride levels, cholesterol level (total, LDL, HDL, VLDL)
Haematotoxicity	Haematology parameters (number of erythrocytes, haemoglobin, haematocrit/packed cell volume, platelet count, white blood cells)
Glucose/insulin homeostasis	OGTT and ITT, glucose and insulin level, GLP-1, c-peptide
Inflammation/immunotoxicity	White blood cells, cytokines (IL-6, IL-8, IL-10), immunoglobulin (IgG, IgE and IgA), spleen and thymus weight, spleen and thymus histopathology
Liver toxicity	Bilirubin (total and direct), ALP, ALT, AST, serum albumin, plasma GGT and OCT, total bile acid, liver triglyceride content, liver cholesterol content, liver fat, liver weight, liver histopathology
Nephrotoxicity	Creatinine, urea/BUN, kidney weight, kidney histopathology
Carcinogenicity	Histopathology (neoplastic changes)
Reproductive toxicity	'Conception rates'[57], mating performance and fertility, fertility index[58], gestation length, pre-coital interval and gestation length, mammary gland histopathology, ovary histopathology, ovary weight (absolute), uterus histopathology, oestrus cycle, prostate histopathology, prostate weight (absolute), seminal vesicle weight (absolute), testis histopathology, testis weight (absolute), ididymis weight (absolute), epididymis histopathology
Maternal and developmental toxicity	Body weight gain and litter body weight, gestation length, lactation index, litter size, sex ratio, live birth index, PND1, live birth/PND1, litter viability/ PND4, viability index/ PND4, maternal body weight gain and maternal body weight, pups health status development landmarks (F1), abnormalities[59]
Neurotoxicity	Auditory and visual responses, brain histopathology, brain weight (absolute), neurotransmitters, elevated plus-maze, novel object recognition task (memory task), open field test
Thyroid toxicity	Thyroid histopathology, thyroid weight, T3, T4, TSH, fT3/T3, fT4/T4m TPO and deiodinase type 1 (DIO1) expression

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferases; AST, aspartate aminotransferases; BUN, blood urea nitrogen; DIO1, deiodinase type 1; fT3, free T3; fT4, free T4; GLP-1, glucagon-like peptide-1; GTT, gamma-glutamyl transferase; HDL, high density lipoprotein; Ig, immunoglobulin; IL, interleukin; ITT, insulin tolerance test; LDL, low-density lipoprotein; OGT, ornithine carbamyl transferase; OGTT, oral glucose tolerance test; PND, post-natal day; T3, triiodothyronine; T4, thyroxine; TPO, Thyroid peroxidase; TSH, thyroid-stimulating hormone; VLDL, very low-density lipoprotein.

[a]Feed and water intake were considered as supportive endpoints to interpret the changes in body weight.

[b]'Additional clinical chemistry' denotes the clinical chemistry not covered under other HOCs.

In addition to the apical and related endpoints reported in Table 15, other endpoints were investigated in the included studies but they were not included in the WoE evaluation as they were not considered relevant for the derivation of a possible HBGV. However, in some instances non-apical endpoints were evaluated as supporting evidence for the apical endpoints in the WoE assessment.

Based on the included animal data, the Panel evaluated the confidence in the body of evidence for the identified HOCs; see Table 16.

[57]Calculated in the study report as the ratio between the animals that achieve pregnancy and the animals mating.

[58]Calculated in the study report as the ratio between the animals that achieve pregnancy and the animals paired.

[59]Mean onset and completion times for pinna unfolding, hair growth, eye-opening and tooth eruption.

TABLE 16 Summary table of rating confidence in the body of evidence for each health outcome category: Animal studies. For a detailed assessment, see Annex F1).

		Elements for downgrading[b]				Elements for upgrading[b]				
Health outcome categories (HOCs) investigated	**Initial rating (no. of studies)[a]**	**Concern for risk of bias across studies**	**Concern for unexplained inconsistency across studies**	**Concern related to relevance of studies**	**Concern for imprecision**	**Magnitude of effect**	**Dose–response**	**Consistency across study population/ study design**	**Final rating of confidence**	**Effect/no effect**
Body weight	High (22)	No downgrading				No upgrading			High	Effect
Additional clinical chemistry	High (10)	Downgrading: concern for unexplained inconsistencies				No upgrading			Moderate	No effect
Haematotoxicity	High (3)	No downgrading				No upgrading			High	No effect
Glucose/insulin homeostasis	High (12)	No downgrading				No upgrading			High	No effect
Inflammation/immunotoxicity	High (6)	Downgrading: concern for risk of bias across studies				No upgrading			Moderate	No effect
Liver toxicity	High (14)	Downgrading: concern for risk of bias across studies/unexplained inconsistencies				No upgrading			Moderate to high	No effect
Nephrotoxicity	High (7)	Downgrading: concern for unexplained inconsistencies				No upgrading			Moderate	No effect
Carcinogenicity	High (2)	No downgrading				No upgrading			High	No effect
Reproductive toxicity	High (4)	No downgrading				No upgrading			High	No effect
Maternal and developmental toxicity	High (4)	No downgrading				No upgrading			High	No effect
Neurotoxicity	High (6)	No downgrading				No upgrading			High	No effect
Thyroid toxicity	High (3)	No downgrading				No upgrading			High	No effect

[a]The total number of studies assessed was 27. The number in parentheses refers to studies considered under the specific HOC.

[b]Please refer to Appendix A and Annex F1 and to the protocol (EFSA FAF Panel, 2023) for further explanations on what is assessed under each element.

Body weight

Effects on body weight (defined as percentage of change with respect to the control of the mean final body weight) or body weight gain were evaluated in 22 studies of different duration and route/mode of administration, and in three different species (rat, mice and guinea pig) (see Table 14).

Body weight gain data were reported in different studies (Pałkowska-Goździk et al., 2018; Ragi et al., 2021; Guo et al., 2021; Morales-Ríos et al., 2022; Zhu et al., 2024; Martínez et al., 2010), however, absolute weights were not reported and from the available data it was not possible to calculate the difference in the final body weight with respect to the control. Increases or decreases relative to the control of less than 10% were observed in four studies (Guo et al., 2021[60]; Morales-Ríos et al., 2022; Zhu et al., 2024; Martínez et al., 2010) of different duration and testing doses up to 225 mg/kg bw per day. In Ragi et al. (2021)[61] only small not consistent changes on body weight gain were reported: increase in body weight gain (10%) when sucralose was administered both via the diet and via drinking water, increase in body weight gain (1%) when sucralose was administered only via drinking water and decrease in body weight gain (–10%) when sucralose was administered exclusively via the diet. In Pałkowska-Goździk et al. (2018) an increase in body weight gain (63%) was observed in rats exposed for 3 weeks to sucralose at a dose of 14.2 mg/kg bw per day via the diet. The Panel noted that this increase in body weight gain was mirrored by a corresponding increase in feed consumption. Overall, the Panel considered the observed changes in body weight gain alone from the studies as above as not toxicologically relevant, and they were not considered further in the assessment.

The Panel focused on the endpoint **'body weight'** (defined as percentage of change with respect to the control of the mean final body weight) to assess the potential effects of sucralose (E 955) on body weight. When this the magnitude of effect for this endpoint was not reported by the study authors, a calculation was done by the Panel. In some instances, data on body weight changes were extracted from figures reported in the publications (Azad et al., 2020; Concha Celume et al., 2024; Glendinning et al., 2020; Mendoza-Pérez, García-Gómez, et al., 2021; Mendoza-Pérez, Guzmán-Gómez, et al., 2021; Rathaus et al., 2024; Shi et al., 2023; Shi, Lei, et al., 2021; Soffritti et al., 2016; Zhang et al., 2024). It is noted that, for the reproductive toxicity study (Documentation provided to EFSA No 1), only data from the F0 generation were considered, because it was not possible to quantify offspring exposure during gestation and lactation. In the case of the combined chronic and carcinogenicity study with in utero exposure (Documentation provided to EFSA No 1) only the data from the F1 generations were considered. The percentage of change with respect to the control of the mean final body weight in the respective study were plotted against the administered doses of sucralose in Figure 3.

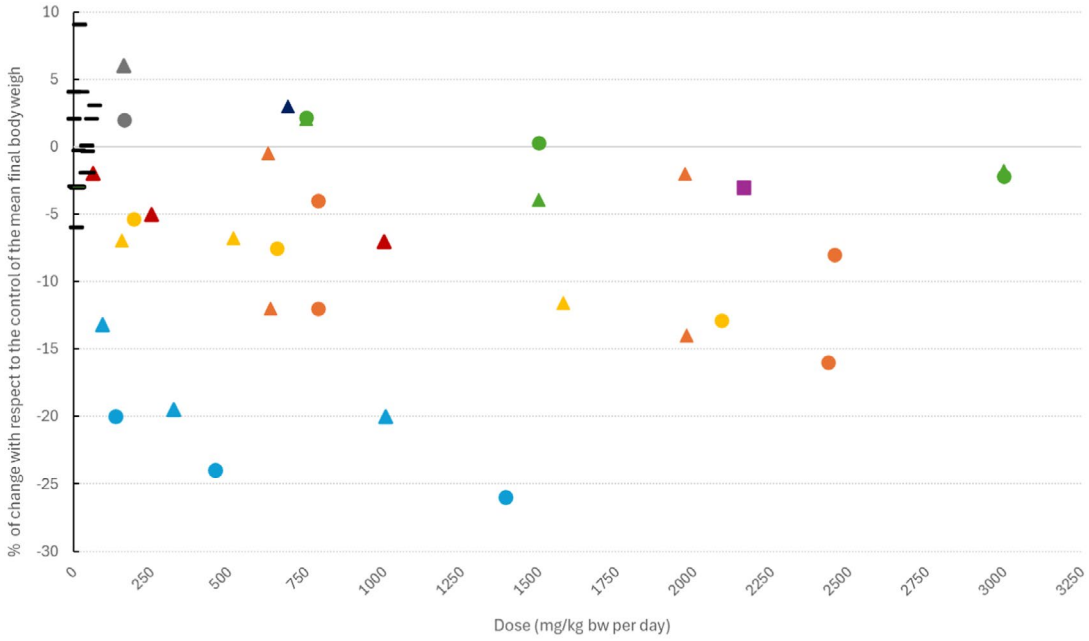


FIGURE 3 Percentage of change with respect to the control of the mean final body weight in rats or mice after exposure to sucralose doses in the range 62.5–3000 mg/kg bw per day (from 8 references: Soffritti et al., 2016; Glendinning et al., 2020; Ren et al., 2021; Farid et al., 2020; two generation reproductive toxicity study in rats, 104-week combined toxicity and carcinogenicity study in rats, a 26-week study in rats with exposure by gavage and pair fed dietary restriction study in rats; Documentation provided to EFSA No 1). In the studies with more than one time point measurement, only the values at the end of the study were included. Each colour represents data from one reference. Symbols: Triangles: M, Dots: F, Squares: M/F combined. Studies: Red: Soffritti et al., 2016 (chronic), Yellow: Reproductive toxicity study (Documentation provided to EFSA No 1), Blue: 104-week combined chronic and carcinogenicity study (Documentation provided to EFSA No 1), Orange: pair fed dietary restriction study-sub-chronic (Documentation provided to EFSA No 1), Green: 26 weeks gavage study-sub-chronic (Documentation provided to EFSA No 1), Purple: Glendinning et al., 2020 (sub-acute), Dark blue: Ren et al., 2021 (sub-acute), Grey: Farid et al., 2020 (sub-chronic). Black dashes indicate values after exposure to doses in the range 0.043–43.2 mg/kg bw per day.

[60]Data reported in a figure and it was not possible to estimate the magnitude of change.

[61]Estimated dose equivalent to 19 mg/kg bw per day when sucralose was administered in the diet, 20 mg/kg bw per day when sucralose was administered in the water and 39 mg/kg bw per day when sucralose was administered in both water and diet. Data on body weight gain extracted from the figure provided in the publication.

Reduced body weight relative to controls was observed in the rat studies following dietary administration of sucralose: reproductive toxicity study in rats (Documentation provided to EFSA No 1), 104-week combined toxicity and carcinogenicity study in rats (Documentation provided No 1), pair fed dietary restriction study in rats (Documentation provided to EFSA No 1), see Figure 3. Similar decreases in body weight were not observed in the available studies on mice with dietary or drinking water administration and in the studies in rats, in which sucralose was administered via drinking water only (Farid et al., 2020; Glendinning et al., 2020; Ren et al., 2021; Soffritti et al., 2016) or via gavage (26 weeks gavage study; Documentation provided to EFSA No 1). These differences could be either due to differences in the tested species, route of administration, doses or duration of studies.

In all dietary studies in rats, in which a decrease in final body weight was also feed consumption was reduced see Tables 18–20 and Annex F1. The magnitude of the reduction in feed consumption/feed conversion efficiency was not always proportionate to the reduction in body weight. The Panel considered that the feed consumption reduction might be due to the poor palatability of sucralose in the diet since no reduced feed consumption was observed after administration of sucralose by gavage for 26 weeks (Documentation provided to EFSA No 1). The Panel noted that the magnitude of the reduction in body weight in the high-dose group of the gavage study was small (up to 4%), the feed intake was similar to or slightly increased with respect to the controls, see Table 17.

TABLE 17 Body weight changes, feed intake and feed conversion efficiency in the 26-week study in rats with exposure by gavage (Documentation provided to EFSA No 1).

	Males				Females			
	C	750 mg/kg bw per day	1500 mg/kg bw per day	3000 mg/kg bw per day	C	750 mg/kg bw per day	1500 mg/kg bw per day	3000 mg/kg bw per day
BW week 26	685	699	658	673	369	377	370	361
% diff to C	–	+2	−4	−2	–	+2	+0.3	−2
Feed intake, total 0–26 week	5964	5984	5830	6255	4586	4686	4690	4651
% diff to C	–	+0.3	−2	+5	–	+2	+2	+1
Feed conversion efficiency week 1–26	9.35	9.63	9.18	8.82	5.74	5.75	5.70	5.35
% diff to C	–	+3	−2	−6	–	+0.2	−0.7	−7

Abbreviations: BW, body weight; C, control.

TABLE 18 Body weight changes, feed intake and feed conversion efficiency in the 104-week combined toxicity and carcinogenicity study in rats (Documentation provided to EFSA No 1).

	Males					Females				
	C1	C2	3000 ppm sucralose[a]	10,000 ppm sucralose[b]	30,000 ppm sucralose[c]	C1	C2	3000 ppm sucralose[a]	10,000 ppm sucralose[b]	30,000 ppm sucralose[c]
BW week 104	976	983	861	803	791	602	652	514	490	476
% diff to C	–		−13	−19	−20	–		−20	−24	−26
Feed intake as % of controls, total 1–104 week	100	10	95	92	92	100	100	93	90	89
% diff to C	–		−5	−8	−8	–		−7	−10	−11
Feed conversion efficiency week 1–104	2.4	2.5	2.4	1.9	1.9	3.2	2.9	2.5	2.8	2.3
% diff to C	–		−2	−22	−22	–		−18	−8	−25

Abbreviations: BW, body weight; C, control.

[a]Dose equal to 94 (males) and 135 (females) mg/kg bw per day.

[b]Dose equal to 323 (males) and 458 (females) mg/kg bw per day.

[c]Dose equal to 1005 (males) and 1395 (females) mg/kg bw per day.

TABLE 19 Body weight changes, feed intake and feed conversion efficiency in the pair fed dietary restriction study in rats (Documentation provided to EFSA No 1).

	Males							
	Controls				Sucralose			
	Basal diet ad libitum	95% of basal diet ad libitum	90% of basal diet ad libitum	85% of basal diet ad libitum t	1% sucralose[a] with Basal diet ad libitum	3% sucralose[b] with Basal diet ad libitum	1% sucralose[c] with 90% of basal diet ad libitum	3% sucralose[d] with 90% of basal diet ad libitum
BW week 26	796.6	720.4	663.7	657.5	703	681.7	660.6	648.6
% diff to C	–	–	–	–	−12	−14	−0.5	−2
Feed intake, total 0–26 week	5781.2	5263.8	4976.4	4792.4	5251.9	5276.6	4883.1	4963.5
% diff to C	–	–	–	–	−9	−9	−2	−0.25
Feed conversion efficiency week 1–26	12.0	11.8	11.3	11.6	11.4	11.0	11.5	11.0
% diff to C	–	–	–	–	−5	−8	+2	−3
	Females							
	Controls				Sucralose			
	Basal diet ad libitum	95% of basal diet ad libitum	90% of basal diet ad libitum	85% of basal diet ad libitum	1% sucralose[a] with basal diet ad libitum	3% sucralose[b] with basal diet ad libitum	1% sucralose[c] with 90% of basal diet ad libitum	3% sucralose[d] with 90% of basal diet ad libitum
BW week 26	407.2	353.4	343.4	328.5	360.1	340.9	328.7	316.1
% diff to C	–	–	–	–	−12	−16	−4	−8
Feed intake, total 0–26 week	4075.7	3623.0	3597.9	3452.3	3778.8	3707.6	3545.9	3505.3
% diff to C	–	–	–	–	−7	−9	−1	−3
Feed conversion efficiency week 1–26	7.8	7.4	7.2	7.0	7.2	6.8	6.8	6.6
% diff to C	–	–	–	–	−8	−13	−6	−8

Abbreviations: BW, body weight; C, control.

[a]Dose equal to 636 (males) and 787 (females) mg/kg bw per day.

[b]Dose equal to 1978 (males) and 2435 (females) mg/kg bw per day.

[c]Dose equal to 628 (males) and 787 (females) mg/kg bw per day.

[d]Dose equal to 1973 (males) and 2455 (females) mg/kg bw per day.

TABLE 20 Body weight changes, feed intake and food conversion efficiency in the two generation reproduction study in rats (Documentation provided to EFSA No 1).

	F0 males				F0 females			
	C	3000 ppm sucralose[a]	10,000 ppm sucralose[b]	30,000 ppm sucralose[c]	C	3000 ppm sucralose[a]	10,000 ppm sucralose[b]	30,000 ppm sucralose[c]
BW week 10	518	482	483	458	507	475	491	466
% diff to C	–	−7	−7	−11	–	−6	−3	−8
Feed intake (as % of controls), total 0–10 week	100	94	93	91	100	96	98	96
% diff to C	–	−6	−7	−9	–	−4	−2	−4
Feed conversion efficiency week 1–10	9	8	9	9	4	3	3	4
% diff to C	–	−11	0	0	–	−25%	−25%	0

Abbreviations: BW, body weight; C, control.

[a]Dose equal to 155 (males) and 194 (females) mg/kg bw per day.

[b]Dose equal to 514 (males) and 656 (females) mg/kg bw per day.

[c]Dose equal to 1580 (males) and 2090 (females) mg/kg bw per day.

In the pair fed dietary restriction study (Documentation provided to EFSA No 1) the relationship between feed intake and body weight changes in rats eating similar amounts of diet with and without sucralose was assessed (see Appendix G and Annex C). The SCF assessed this study in its opinion (SCF, 2000) and concluded that at doses up to 1% in the diet (628–787mg/kg bw per day), effects on feed intake and body weight were due to palatability of sucralose; only at dietary doses of 3% (1978 and 2435 mg/kg bw per day in males and females, in rats fed ad libitum respectively), a proportion of the effects on body weight was attributed to toxicity of sucralose.

The Panel agreed that the decrease in body weight following sucralose administration could not be attributed only to poor palatability of sucralose and noted that in particular in the available combined chronic and carcinogenicity study (Documentation provided to EFSA No 1), reductions in final body weight exceeded 10% (up to 26%) at all doses. The Panel further noted that an increase in caecum weight (absolute, empty and full) was observed in all animal studies in which it was measured (the 26 weeks gavage study, combined chronic and carcinogenicity study and reproductive toxicity study in rats; Documentation provided to EFSA No 1, see Appendix D). Using the scheme by Flamm et al. (2003), see Figure 4, and considering the evidence for reduced feed consumption, for palatability effect and the reduced body weight accompanied by increased weight of the caecum (full and empty) and decreased feed conversion efficiency, the Panel considered the decrease in body weight observed in the combined chronic and carcinogenicity study as adverse.

Considering the final rating of confidence in the body of evidence for the HOC 'body weight' as 'high' and identification of adverse effects (see Tables 16 and Annex F1), the Panel considered that there is high confidence in the body of evidence that the exposure to sucralose (E 955) is associated with decrease in body weight in rats.

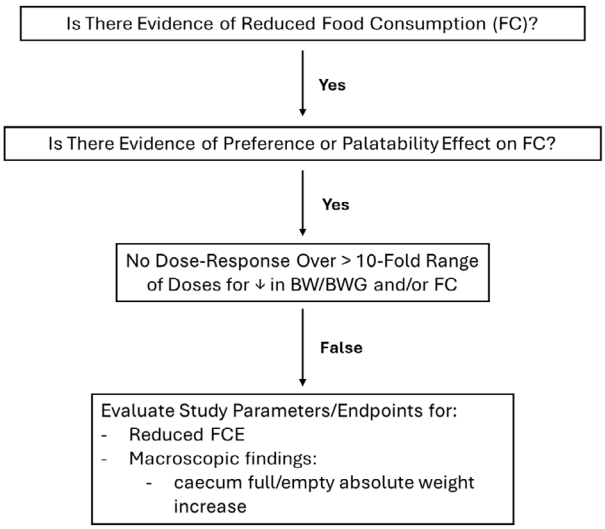


FIGURE 4 Scheme to consider the adversity of body weight changes, adapted from Flamm et al. (2003).

Additional clinical chemistry

Changes in clinical chemistry parameters (levels of HDL, LDL, VLDL, cholesterol and triglycerides) were evaluated in 10 studies of different duration and design in **mice** (four sub-chronic toxicity studies, highest dose tested 162.5 mg/kg bw per day; Rathaus et al., 2024; Shi, Lei, et al., 2021; Farid et al., 2020; Eisenreich et al., 2020), in **rats** (two sub-acute toxicity studies, highest dose tested 92.5 mg/kg bw per day; two sub-chronic toxicity studies, highest dose tested 17.8 mg/kg bw per day; one chronic toxicity study, highest dose tested 9.5 mg/kg bw per day; Ragi et al., 2021; Zhang et al., 2024; Morales-Ríos et al., 2022; Barakat et al., 2023; Mendoza-Pérez et al., 2022) and in **guinea pigs** (sub-acute toxicity study, only one dose tested 6.9 mg/kg bw per day; Zhu et al., 2024), see Table 14 and Annex F1. There were no consistent effects in the measured parameters in the available studies.

Considering the final rating of confidence in the body of evidence for the HOC 'additional clinical chemistry' as 'moderate' (Downgraded for unexplained inconsistencies across studies) and the absence of any toxicologically significant changes (see Tables 16 and Annex F1), the Panel considered that there is moderate confidence in the body of evidence that the exposure to sucralose (E 955) is not associated with adverse changes in clinical chemistry parameters.

Haematotoxicity

Haematological effects of sucralose (E 955) were evaluated in three studies of different duration and design in **mice** (sub-chronic toxicity study, single dose tested 162.5 mg/kg bw per day; Farid et al., 2020) and in **rats** (sub-chronic toxicity study, highest dose 3000 mg/kg bw per day and chronic toxicity study, highest dose 1005 mg/kg bw per day; Documentation provided to EFSA No 1), see Table 14 and Annex F1. No effects on number of erythrocytes, haemoglobin, haematocrit, platelet count and number of white blood cells were observed.

Considering the final rating of confidence in the body of evidence for the HOC 'haematotoxicity' as 'high' and the absence of any toxicologically significant changes (see Tables 16 and Annex F1), the Panel considered that there is high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse haematological effects.

Glucose and insulin homeostasis

Twelve studies of different duration and design addressed endpoints relevant for the assessment of effects on glucose and insulin homeostasis (OGTT and ITT, glucose and insulin level, GLP-1, c-peptide; see Table 14 and Annex F1). The studies were performed in **rats** (two sub-acute studies, highest dose 92.5 mg/kg bw per day; one sub-chronic study highest dose tested 3000 mg/kg bw per day; two chronic studies highest dose tested 1005 mg/kg bw per day; Barakat et al., 2023; Mendoza-Pérez, García-Gómez, et al., 2021; Mendoza-Pérez, Guzmán-Gómez, et al., 2021; Ragi et al., 2021; Documentation provided to EFSA No 1), in **mice** (two sub-acute toxicity studies, highest tested dose 692 mg/kg bw per day, three sub-chronic toxicity studies, highest dose tested 43.2 mg/kg bw per day; a reproductive toxicity study, single dose tested 6.3 mg/kg bw per day; Rathaus et al., 2024; Santos et al., 2021; Shi, Lei, et al., 2021; Ren et al., 2021; Azad et al., 2020; Glendinning et al. (2020) and in **guinea pigs** (sub-acute toxicity study, dose tested 6.9 mg/kg bw per day; Zhu et al., 2024). In Glendinning et al. (2020), OGTTs in four time points were performed and no effects were observed at 2160 mg/kg bw per day in both males and females. No effects on glucose and insulin levels, ITT, GLP-1 levels and c-peptide levels were observed in any of the available studies.

Considering the final rating of confidence in the body of evidence for the HOC 'glucose and insulin homeostasis' as 'high' and the absence of any toxicologically significant changes (see Tables 16 and Annex F1), the Panel considered that there is high confidence in the body of evidence that the exposure to sucralose (E 955) is not associated with an impairment of glucose/insulin homeostasis.

Inflammation/immunotoxicity

Six studies of different duration and design measured endpoints relevant for the assessment of immunotoxicological effects (white blood cells, cytokines (IL-6, IL-8, IL-10), immunoglobulin (IgG, IgE and IgA), spleen and thymus weight, spleen and thymus histopathology). The studies were performed in **rats** (one sub-chronic toxicity study, highest dose tested 3000 mg/kg bw per day; one chronic toxicity study, highest dose tested 1005 mg/kg bw per day and reproductive toxicity study, highest doses tested 1580 mg/kg bw per day (F0) and 1680 mg/kg bw per day (F1); Documentation provided to EFSA No 1) and in **mice** (one sub-acute toxicity study, dose tested 261 mg/kg bw per day; a sub-chronic toxicity study, dose tested 162.5 mg/kg bw per day and a reproductive toxicity study, dose tested 6.3 mg/kg bw per day; Farid et al., 2020; Guo et al., 2021; Azad et al., 2020), see Table 14 and Annex F1.

No adverse effects were observed in any study. In Farid et al. (2020), only small changes in immunoglobulins and cytokines were reported: increases in lipopolysaccharides (LPS), IgG, IgE, IgA, IL-8, IL-6 for males and females, mainly without time dependence. The Panel considered these changes as not toxicologically relevant.

Considering the final rating of confidence in the body of evidence for the HOC 'inflammation/immunotoxicity' as 'moderate' (Downgraded for risk of bias across studies) and the absence of any toxicologically significant changes on apical endpoints (see Tables 16 and Annex F1), the Panel considered that there is moderate confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse effects on the immune system.

Liver toxicity

Fourteen studies of different duration and design measured endpoints relevant for the assessment of effects of sucralose on liver (liver weight, liver histopathology, bilirubin (total and direct), ALP, ALT, AST, serum albumin, plasma GGT and OCT, total bile acid, liver triglyceride content, liver cholesterol content, liver fat). Studies were performed in **rats** (two sub-acute toxicity studies, highest dose tested 92.5 mg/kg bw per day; three sub-chronic toxicity studies, highest dose tested 3000 mg/kg bw per day; one chronic toxicity study, highest dose tested 1005 mg/kg bw per day and reproductive toxicity study, highest doses tested 1580 mg/kg bw per day (F0) and 2090 mg/kg bw per day (F1); Barakat et al., 2023; Haq et al., 2019; Martínez et al., 2010; Ragi et al., 2021; Documentation provided to EFSA No 1) and **mice** (six sub-chronic toxicity studies, highest dose tested 162.5 mg/kg bw per day; a reproductive toxicity study only dose tested 6.3 mg/kg bw per day; Azad et al., 2020; Farid et al., 2020; Rathaus et al., 2024; Santos et al., 2021; Shi, Lei, et al., 2021), see Table 14 and Annex F1. No toxicologically relevant effects were observed in all studies in any of the measured endpoints.

Considering the final rating of confidence in the body of evidence for the HOC 'liver toxicity' as 'moderate to high' and the absence of any toxicologically significant changes (see Tables 16 and Annex F1), the Panel considered that there is moderate to high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse effects on liver.

Nephrotoxicity

Seven studies of different duration and design measured endpoints relevant for the assessment of effects on kidneys (creatinine, urea/BUN, kidney weight and histopathology). Studies were performed in **rats** (one sub-acute toxicity study, highest dose tested 39 mg/kg bw per day; one sub-chronic toxicity study, highest dose tested 3000 mg/kg bw per day; one chronic toxicity and carcinogenicity study, highest dose tested 1005 mg/kg bw per day and reproductive toxicity study,

highest doses tested 1580 mg/kg bw per day (F0) and 2090 mg/kg bw per day (F1); Ragi et al. (2021, Documentation provided to EFSA No 1) and **mice** (two sub-chronic toxicity studies, highest dose tested 162.5 mg/kg bw per day; a reproductive toxicity study dose tested 6.3 mg/kg bw per day; Azad et al., 2020; Farid et al., 2020; Shi, Lei, et al., 2021)), see Table 14 and Annex F1. No toxicologically relevant effects were observed. Considering the final rating of confidence in the body of evidence for the HOC 'nephrotoxicity' as 'moderate' (Downgraded for unexplained inconsistencies across studies) and the absence of any toxicologically relevant changes (see Tables 16 and Annex F1), the Panel considered that there is moderate confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse effects on kidneys.

Carcinogenicity

A combined chronic and carcinogenicity study in **rats** (highest dietary concentration of 3% equal to the highest dose tested of 1005 mg/kg bw per day in males; Documentation provided to EFSA No 1) and a carcinogenicity study in **mice** (highest dietary concentration of 1.6% equivalent to the highest dose tested of 2000 mg/kg bw per day in males; Soffritti et al., 2016) were available (see Table 14 and Annex F1). No treatment-related neoplastic lesions were observed in the rat study. In Soffritti et al. (2016), the authors reported that sucralose-induced haematopoietic neoplasias in male Swiss mice. The results of this study were evaluated by the EFSA ANS Panel (2017), which concluded that the available data did not support the conclusions of the authors. The Panel agreed with the conclusion of EFSA ANS Panel (2017).

In the same statement, the ANS Panel also noted that negative results had been obtained in clastogenicity tests in haemopoietic cells of rats (chromosomal aberration test) and mice (micronucleus test) and in a comet assay in mice (EFSA ANS Panel, 2017). The recent negative in vivo comet assay in rats (Documentation provided to EFSA No 40) strengthen the evidence of the lack of genotoxic effects of orally administered sucralose in rodent erythropoietic cells, further supporting the lack of carcinogenic activity of sucralose in mice.

Considering the final rating of confidence in the body of evidence for the HOC 'carcinogenicity' as 'high' and the absence of neoplastic changes attributable to sucralose (see Tables 16 and Annex F1), the Panel considered that there is high confidence in the body of evidence that the exposure to sucralose (E 955) is not associated with carcinogenicity.

Reproductive toxicity

Effects on reproduction (mating/fertility/gestation, female reproductive organs, male reproductive organs, hormone levels) were assessed in three studies in **rats** (one sub-chronic toxicity study in rat, highest dose tested 3000 mg/kg bw per day; one reproductive toxicity study, highest doses tested 1580 mg/kg bw per day (F0) and 2090 mg/kg bw per day (F1); one combined chronic and carcinogenicity study with in utero exposure, highest dose tested 1005 mg/kg bw per day; Documentation provided to EFSA No 1)) and one study in **mice** (carcinogenicity study, highest dose tested 2000 mg/kg bw per day; Soffritti et al., 2016), see Table 14 and Annex F1). No toxicologically relevant effects were observed in any of the measured endpoints.

Considering the final rating of confidence in the body of evidence for the HOC 'reproductive toxicity' as 'high' and the absence of any toxicologically significant changes (see Tables 16 and Annex F1), the Panel considered that there is high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with reproductive toxicity.

Maternal and developmental toxicity

Effects on maternal and developmental toxicity (body weight gain and litter body weight, gestation length, lactation index, litter size, sex ratio, live birth index, PND1, live birth/PND1, litter viability/ PND4, viability index/ PND4, maternal body weight gain and maternal body weight, pups health status, developmental landmarks (F1), abnormalities) were assessed in two studies performed in **rats** (one reproductive toxicity study, highest doses tested 2090 mg/kg bw per day (F0) and 2100 mg/kg bw per day (F1) and one combined chronic and carcinogenicity study with in utero exposure, highest dose tested 2234 mg/kg bw per day mg/kg bw per day; Documentation provided to EFSA No 1) and two studies in **mice** (carcinogenicity study, highest dose tested 2000 mg/kg bw per day and reproductive toxicity study, dose tested 6.3 mg/kg bw per day; Soffritti et al., 2016; Azad et al., 2020), see Table 14 and Annex F1. No toxicologically relevant effects were observed in any of the measured endpoints; the observed slight decrease in body weight gain in offspring was not considered relevant as the final body weight was unchanged.

Considering the final rating of confidence in the body of evidence for the HOC 'maternal and developmental toxicity' as 'high' and the absence of any toxicologically significant changes (see Table 16 and Annex F1), the Panel considered that there is high confidence in the body of evidence that the exposure to sucralose (E 955) is not associated with maternal and developmental toxicity.

Neurotoxicity

Endpoints relevant for the assessment of neurotoxicity (auditory and visual responses, brain histopathology and weight, neurotransmitters, elevated plus-maze, novel object recognition task (memory task), open field test) were measured in six studies, including five studies in **rats** (three sub-chronic toxicity studies in rats, highest dose tested 3000 mg/kg bw per day; one reproductive toxicity study, highest doses tested 1580 mg/kg bw per day (F0) and 2090 mg/kg bw per day (F1); one

combined chronic and carcinogenicity study, highest dose tested 1005 mg/kg bw per day; Zhang et al., 2024; Morales-Ríos et al., 2022; Documentation provided to EFSA No 1) and a study in mice (sub-acute toxicity study, dose 692 mg/kg bw per day; Ren et al., 2021), see Table 14 and Annex F1.

No effects were observed on brain histopathology, weight, auditory and visual responses, novel object recognition task (memory task) and on neurotransmitters levels. Some effects were observed in in Zhang et al. (2024) (anxiety-like behaviours in open field and elevated plus-maze). However, this single sex, single dose level study had shortcomings[62] and therefore, also considering the lack of any other reports of effects on related endpoints, the Panel considered that the observed effect cannot be considered as a clear indication for neurotoxicity and that, accordingly, sucralose has no toxicologically relevant effects on neurotoxicological endpoints.

Considering the final rating of confidence in the body of evidence for the HOC 'neurotoxicity' as 'high' and the absence of any toxicologically significant changes (see Table 16 and Annex F1), the Panel considered that there is high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with neurotoxicity.

Thyroid toxicity

Three studies in rats (one sub-acute toxicity study, highest dose tested 14.2 mg/kg bw per day; one sub-chronic toxicity study, highest dose tested 3000 mg/kg bw per day; one combined chronic and carcinogenicity study, highest dose tested 1395 mg/kg bw per day; Pałkowska-Goździk et al., 2018; Documentation provided to EFSA No 1)) investigated potential effects on thyroid, see Table 14 and Annex F1.

In the study from Pałkowska-Goździk et al. (2018), there was no evidence of adverse perturbation of the HPT axis. An increase in the level of TSH (30%, 180 min after administration of the diet) was observed and was considered below the biological threshold of concern (50%) and was not associated to a corresponding decrease in thyroid hormones and thyroid weight relative to body weight. Existing differences are likely consequent to the metabolic-energetic status of the animal fed with sucralose. Despite few limitations (timing of TH and TSH sampling, lack of positive control, lack of thyroid histopathology) the Panel considered the study to be of sufficient quality to conclude on the status of the HPT axis.

In the 26 weeks gavage study in CD rats (Documentation provide to EFSA No 1), the HPT axis was assessed throughout the histological evaluation of the thyroid of animals belonging to the control and high dose group. There were no histological changes indicative of thyroid histopathology compatible with TSH stimulation (thyroid follicular cells hypertrophy or hyperplasia) nor toxicologically relevant changes in thyroid weight.

In the 104 weeks combined chronic and carcinogenicity study in CD rats (Documentation provide to EFSA No 1), the HPT axis was assessed throughout the histological evaluation of the thyroid of all animals and through the assessment of total T3 and T4. The hormonal assessment was only conducted in week 11 of the study. Although relevant limitations were noted (e.g. time of sampling, method for euthanasia and analytical method) the coefficient of variation (cv) was considered acceptable. There were no changes in T3 while a decrease of about 34% was reported for T4. This decrease is above the threshold of 25% that is considered as a potential concern of developmental neurotoxicity (Hassan et al., 2017). The Panel noted that several biases can have affected the inter-animal variability in T4 (see above) and that the TSH (which is less/not affected by the circadian rhythm) was not measured in this study. Moreover, there were no histological changes in the thyroid and thyroid follicular morphology (in the form of thyroid follicular cell hypertrophy/hyperplasia) which are recognised as very sensitive evidence of disruption of the HPT axis and in particular of persistent TSH stimulation (Capen, 1997). Therefore, the Panel considered that sucralose has no toxicologically relevant effects on the HPT axis.

Considering the final rating of confidence in the body of evidence for the HOC 'thyroid toxicity' as 'high' and the absence of any toxicologically relevant effects (see Table 16 and Annex F1), the Panel considered that there is high confidence in the body of evidence that the exposure to sucralose (E 955) is not associated with thyroid toxicity (Table 21).

TABLE 21 Translation of confidence ratings into level of evidence for conclusions of adverse effects or no adverse effects for the included animal studies for each of the HOC considered in the assessment.

HOC	Final rating of confidence	Level of evidence
Body weight	High	**High** There is high confidence in the body of evidence that exposure to sucralose (E 955) is associated with decreased body weight
Additional clinical chemistry	Moderate	**Moderate** There is moderate confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse changes in clinical chemistry parameters.
Haematotoxicity	High	**High** There is high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse haematological effects.

[62]The Panel noted that the number of entries in open arms in Zhang et al. (2024) was comparable to other literature data for Sprague–Dawley (Liu et al., 2017) and that the time (40s) spent in the centre by the control in this study was low compared to other studies, where control animals typically spend more than twice as long (≥ 100s) (Wang et al., 2017). This suggests that control values were unusual in this study.

TABLE 21 (Continued)

HOC	Final rating of confidence	Level of evidence
Glucose and insulin homeostasis	High	**High** There is high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with an impairment of glucose/insulin homeostasis.
Inflammation/immunotoxicity	Moderate	**Moderate** There is moderate confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse effects on the immune system.
Liver toxicity	Moderate to high	**Moderate to high** There is moderate to high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse effects on liver.
Nephrotoxicity	Moderate	**Moderate** There is moderate confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse effects on kidney.
Carcinogenicity	High	**High** There is moderate confidence in the body of evidence that exposure to sucralose (E 955) is not associated with carcinogenicity.
Reproductive toxicity	High	**High** There is high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with reproductive toxicity.
Maternal and developmental toxicity	High	**High** There is high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with maternal and developmental toxicity
Neurotoxicity	High	**High** There is high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with neurotoxicity.
Thyroid toxicity	High	**High** There is high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with thyroid toxicity.

Other endpoints

The Panel noted that additional endpoints were assessed in the available studies which were considered as supportive information for the re-evaluation of sucralose as food additive. These findings are summarised narratively in this section (please refer to Appendix C for further details).

Clinical condition and survival

Clinical conditions were monitored in four studies in rats including two sub-chronic toxicity studies (highest dose tested 3000 mg/kg bw per day), a combined chronic and carcinogenicity study (highest dose tested 1005 mg/kg bw per day) and a reproductive toxicity study (highest doses tested 1580 mg/kg bw per day (F0) and 1680 mg/kg bw per day (F1) (Documentation provided to EFSA No 1). No adverse changes in clinical conditions by oral exposure to sucralose were reported in these studies at the highest dietary concentrations/doses tested.

Three studies (a combined chronic and carcinogenicity study highest dose tested 1005 mg/kg bw per day, a carcinogenicity study highest dose tested 2000 mg/kg bw per day and a reproductive toxicity study highest doses tested 1580 mg/kg bw per day (F0) and 1680 mg/kg bw per day (F1)) assessed survival of animals following oral exposure to sucralose (Soffritti et al., 2016; Documentation provided to EFSA No 1). No effect on survival in laboratory rodents was observed.

Body composition

Body composition was addressed in a sub-acute study in male rats (highest dose tested 39 mg/kg bw per day (Ragi et al., 2021) and in two sub-chronic studies in male mice (highest dose tested 43.2 mg/kg bw per day (Rathaus et al., 2024; Santos et al., 2021).

In Ragi et al. (2021) exposure of rats to sucralose in the drinking water for 3 weeks (20 mg/kg bw per day) had no effect on fat mass gain, whereas a decrease (ca. -10%) in the group exposed to sucralose in the diet (19 mg/kg bw per day) and an increase (ca. +34%) in the group exposed to sucralose in both the diet and the drinking water (39 mg/kg bw per day) relative to the control group were reported. The observed effects on fat mass mirror the changes in body weight gain observed in this study (see section 'body weight') which were not considered toxicologically relevant.

No effect on lean body mass or fat mass was found in male mice exposed to 179.5 mg sucralose/L in drinking water (equivalent to 26.9 mg sucralose/kg bw per day) for 20 weeks (Rathaus et al., 2024).

There was no effect on the mass of brown and subcutaneous adipose tissue in male mice kept on a standard diet and receiving 0.03% sucralose in drinking water (equivalent to 45 mg/kg bw per day) for 16 weeks (Santos et al., 2021).

One study in mice investigated effects in progeny from dams exposed to sucralose during gestation and lactation (Azad et al., 2020); no effects were observed on lean body mass of offsprings from mothers exposed to 6.3 mg/kg bw per day of sucralose, but body fat mass was increased. The Panel noted that in this study the offspring were not exposed directly to sucralose, and this raises uncertainties in the interpretation of its findings.

Organ weight and histopathology

Organ weight and histopathology were assessed for several organs not considered in the weight of evidence under the HOCs assessed above (liver toxicity, nephrotoxicity, reproductive toxicity, neurotoxicity, inflammation/immunotoxicity and thyroid toxicity), please refer to Appendix C for further details. A combined chronic and carcinogenicity study (highest dose tested 1005 mg/kg bw per day (Documentation provided to EFSA No 1)) and a sub-chronic toxicity study (highest dose tested 3000 mg/kg bw per day (Documentation provided to EFSA No 1)) addressed organ toxicity by means of record of weights and macro-and microscopic changes in several organs, while a reproductive toxicity study (highest dose tested 1580 mg/kg bw per day (F0) and 1680 mg/kg bw per day (F1) (Documentation provided to EFSA No 1) by means of record of organ weights. In Azad et al. (2020) measurement of heart weight was included. Overall, no toxicologically relevant changes were observed.

3.5.4.2 | *Human studies*

The studies included in the assessment comprised 38 human studies following a RoB evaluation (Table 20). Among these studies, 19 studies were allocated to tier 1 and 19 to tier 2 following risk of bias evaluation. Annex F2 reports all the human studies evaluated, clustered by outcome within the different HOCs, for which a WoE analysis was performed. The outcomes considered and evaluated in the WoE for the included data are shown in Table 22.

TABLE 22 Human studies included in the assessment.

Authors, year	Type of study design	Dose (g/person or g/kg bw)[a]	Intervention/exposure	Number of subjects	Population (mean age in years)	RoB tier
Steinert et al. (2011)	HCT (Randomised controlled trial)	0.062 g sucralose in 250 mL tap water	Six treatments separated by at least 3–5 d, intragastric administration	12 (6 M and 6 F)	Mean: 23.3 (19–29)	1
Wu et al. (2012)	HCT (Randomised controlled trial)	0.060 g sucralose (in 400 mL water)	The evening before each study day subjects consumed a standardised evening meal and then fasted overnight. Sucralose consumed 15 min before a 13C-octanoic acid–labelled mashed potato meal.	10 (7 M & 3 F)	28.8 ± 4.0 years	1
Dhillon et al. (2017)	HCT (Randomised controlled trial)	Low calorie solid (LCS): sucralose-sweetened (0.066% w/w) gelatine cubes (16.4 cm^3) or low calorie beverage (LCB): sucralose-sweetened (0.013% w/w) beverage (59.2 mL) sham-fed for 15 s at 2-min intervals for 14 min; Phase 1: test load changed per week, Phase 2: one of the test loads consumed daily, Phase 3: test visit following Phase 2–50% group given same test load as phase 2 and 50% given save food form as phase 2 but with different sweetening agent.	'Phase 1 - single exposure, test load changed weekly for 4 weeks Phase 2 - daily exposure to one of the test loads for 14 consecutive days Phase 3 - test visit following Phase 2 'training' period'	64 (23 M & 41 F)	27.2 ± 8.6 years	1
Ginieis et al. (2018)	HCT (Randomised controlled trial)	Test drink (250 mL) sweetened with either sucralose (0.025 g), glucose (26.0 g), sucrose (14.5 g) or fructose (13.0 g) and flavoured by a non-caloric lemon flavouring agent.	The fasting group undertook a 10 h fasting period prior to each testing session. In the non-fasting group, participants were required to report the content of their most recent meal. Duration of exposure: 4 months	26 in the fasting group (11 M & 15 F) 23 in the non-fasting group (10 M & 13 F)	22.6 ± 4.2 years in fasting group24.3 ± 4.9 years in non-fasting group	1
Keesing et al. (2019)	HCT (Randomised controlled trial)	0.035g of sucralose	2 test days, each separated by 1 week: fasting from 10:00 to 12:00. At 12:00, a lunch comprising water and eight pieces of commercially purchased maki sushi. After eating, the participants received instructions not to eat anything else. At 14:00, the participants consumed the coded test beverage within 10 min.	77	18–60 years	1
Lertrit et al. (2018)	HCT (Randomised controlled trial)	0.2 g/day sucralose (capsules)	Duration of exposure: 4 weeks. Subjects were asked to maintain similar patterns of diet throughout the study.	15 participants exposed (4 M & 11 F)	31.9 ± 10 years	1

(Continues)

TABLE 22 (Continued)

Authors, year	Type of study design	Dose (g/person or g/kg bw)[a]	Intervention/exposure	Number of subjects	Population (mean age in years)	RoB tier
Nichol et al. (2019)	HCT (Randomised controlled trial)	0.048 g sucralose in 60 mL distilled water ingested or same amount of sucralose sham-fed and then expectorated after 5 secs during an OGTT	Single exposure per test condition (12 h overnight fast prior to each testing session)	21 (3 M & 7 F in normal weight group; 1 M & 10 F in the obese group)	27 ± 4.2 years in normal weight group; 29.5 ± 4.0 years in obese group	1
Yunker et al. (2021)	HCT (Randomised controlled trial)	1.5 mM, 2 mM or 3 mM sucralose in 300 mL drink (participants selected conc. to match sweetness of 75 g sucrose drink) mixed with 0.45g of non-sweetened zero calorie cherry flavouring (Kraft Foods Kool-Aid® Unsweetened Cherry Drink Mix)	Single exposure per test beverage (24 h dietary recall (avg. 5 per participant)	74 (31 M & 43 F); 72 received sucralose drink	23.4 ± 3.96 years [18.00–35.00] *Data for 74 participants	1
Ahmad et al. (2019)	HCT (Randomised controlled trial)	0.136 g/day sucralose (20% of ADI)	'Two 2-week periods of daily consumption of beverages containing pure aspartame or sucralose. Each participant consumed both sweeteners in separate phases separated by a minimum of a 4-week washout period'	17 healthy adults (10 F & 7 M)	18–45 years (mean age: 24 ± 6.8 years)	1
Eckstein et al. (2021)	HCT (Randomised controlled trial)	0.2 g sucralose in water (300 mL)	Single exposure per test beverage	15 (10 M & 5 F)	25.4 ± 2.5 years	1
Eckstein et al. (2022)	HCT (Randomised controlled trial)	0.2 g sucralose in water (300 mL)	Single exposure per test beverage	Healthy; 15 (10 M & 5 F)	25.4 ± 2.5 years	1
Kochem et al. (2024)	HCT (Randomised controlled trial)	0.6 g sucralose +75 g glucose in 300 mL water; participants gargled with the test solution for 20 sec, expectorating and taking another 25 mL repeatedly over a 10 min period, prior to ingesting the solution fully	Single exposure per test beverage	Healthy; 12 (4 M & 8 F)	26.2 ± 1.2 years	1
Orku et al. (2023)	HCT (Randomised controlled trial)	330 mL water +0.066 g sucralose	Daily intake for 4 weeks	48 (female), 42 completed the intervention period	21.82 ± 3.16 (sucralose)	1
Ye et al. (2024)	HCT (Randomised controlled trial)	0.200 g sucralose and 0.500 g citric acid in water (150 mL) after undergoing sensory tests and conditioned pain modulation paradigm	Single exposure per test beverage	64 participants (25 M & 39 F; grouped based on adiposity and HbAc1 level)	37.5 ± 10–63.00 ± 28 years	1

TABLE 22 (Continued)

Authors, year	Type of study design	Dose (g/person or g/kg bw)[a]	Intervention/exposure	Number of subjects	Population (mean age in years)	RoB tier
Gallagher et al. (2016)	HCT (Randomised controlled trial)	Splenda® (10 g) containing Sucralose (0.1 g)	Study period not stated, 1-day single exposure per test material Overnight fast (except for water) on 3 occasions separated by at least 1 week. All test items delivered as sucrose sweet taste-balanced muffins with a total fat load (66 g). Participants were asked to consume the muffin within 15 min, and could also have a drink without sweeteners, recorded and replicated during the visits.	27 (11 M & 18 F)	44.3 ± 19 years (19–81 years old)	1
Toepp et al. (2019)	HCT (Randomised controlled trial)	5g Splenda® in 300 mL solution	Single exposure per test beverage	18 M	22.8 ± 2.4 years	1
Debras, Chazelas, Sellem, et al. (2022)	Cohort study (Co)	1.59 ± 16.17 mg per day mean (lower consumers: 1.08 ± 1.94 mg per day; higher consumers: 7.49 ± 36.95 mg per day)	Study from 2009 to 2021; median follow-up: 7.7 years. Sucralose was assessed by multiple 24 h recall.	103,388 (20,903 M and 82,485 F)	Mean: 42.22 ± 14.41 years	1
Debras, Chazelas, Srour, et al. (2022)	Cohort study (Co)	1.59 ± 16.21 mg per day mean (lower consumers: 1.09 ± 1.98 mg per day; higher consumers: 7.52 ± 37.08 mg per day)	Study from 2009 to 2021; median follow-up: 7.8 years. Sucralose was assessed by multiple 24h recall.	102,865 (22,154 M and 80,711 F)	Mean: 42.22 ± 14.5 years	1
Debras et al. (2023)	Cohort study (Co)	1.59 ± 16.1 mg/day (lower consumers 1.08 ± 1.95 mg/day, higher consumers 7.44 ± 36.7 mg/day)	Study from 2009 to 2022; median follow-up: 9.1 years. Sucralose was assessed by multiple 24h recall.	105,588 (83,625 F and 21,963 M)	Mean 42.5 ± 14.6	1
Zafrilla et al. (2021), Villaño et al. (2021)[b]	HCT (Randomised controlled trial)	0.0132 g sucralose in a maqui/citrus beverage (330 mL) daily for 60 days	Duration of exposure: 60 days. FFQ every 15 days	45 (27 M & 18 F) for sucralose (136 in total)/45 (27 M & 18 F) for sucralose (138 in total)/45 for sucralose (138 in total)	42 ± 8 years	2
Ma et al. (2009)	HCT (Randomised controlled trial)	All subjects received an intragastric infusion, over 3 min of either 1) 50 g sucrose dissolved in water to a total volume of 500 mL 2) 80 mg sucralose (Tate & Lyle, Decatur, IL) in 500 mL, normal saline, 3) 800 mg sucralose in 500 mL normal saline or 4) 500 mL normal saline, in randomised, single-blind fashion.	Each subject attended the Discipline of Medicine at the Royal Adelaide Hospital at approx 8.30 h after an overnight fast (14 h for solids, 12 h for liquids) on four occasions, each separated by 3–7 days.	7 healthy subjects	24 ± 2 years	2

(Continues)

TABLE 22 (Continued)

Authors, year	Type of study design	Dose (g/person or g/kg bw)[a]	Intervention/exposure	Number of subjects	Population (mean age in years)	RoB tier
Han et al. (2018)	HCT (Randomised controlled trial)	0.012 g Sucralose twice daily, provided bottled in the form of a grapefruit flavoured non-carbonated drink (30 mL)	Duration of exposure: 12 weeks	121 participants (17 M & 23 F in sucralose group analysed). Also high and low-dose D-allulose groups analysed	20–40 years	2
Hess et al. (2018)	HCT (Randomised controlled trial)	0.006 g sucralose from diet only	Duration of exposure: 2 weeks. Sucralose was assessed by 24-h dietary recall (visit or unannounced phone call)	125 participants (29 M & 34 F in the non-nutritive sweetener (NNS) consumers group; 25 M & 37 F in the NNS non-consumers group)	38.8 years in the NNS consumers group, 34.5 years in the NNS non-consumers group	2
Higgins and Mattes (2019)	HCT (Randomised controlled trial)	0.16 g sucralose daily	Exposure duration: 12 weeks 24-h appetite log	123 participants; 28 exposed to sucralose (42.9% M & 57.1% F)	25.9 ± 9.0 years in exposed group; 25.8 ± 6.9–29.5 ± 12.0 years for groups exposed to other sweeteners	2
Thomson et al. (2019)	HCT (Randomised controlled trial)	One sucralose-containing capsule 3x daily (Vitasweet containing 260 mg sucralose & 70 mg calcium carbonate)	One sucralose-containing capsule 3x daily (Exposure duration: 7 days)	30 M (16 exposed; 14 control)	22.8 ± 3.0 years sucralose group; 23.5 ± 2.9 years control group	2
Dalenberg et al. (2020)	HCT (Randomised controlled trial)	0.06 g sucralose beverage (355 mL; 0 Kcal)	Consumption of 7 beverages within 2-week period. A timeline follow back questionnaire was used to estimate sugar beverage and LCS beverage intake	Exp 1: Healthy young adults; 13 (6 M & 7 F) exposed to sucralose and 13 (7 M & 6 F) exposed to sucralose plus maltodextrin (39 in total) Exp 2: Adolescents; 11 (3 M & 8 F) (11 in total)	Exp 1: 26.54 ± 3.78 years for sucralose group; 29.15 ± 3.76 years for sucralose plus maltodextrin group; Exp 2: 15.95 ± 1.37 years	2
Ajami et al. (2020)	HCT (Randomised controlled trial)	A single tablet of sucralose sweetener consumed in tea daily	A single tablet of sucralose sweetener consumed in tea daily (duration exposure: 8 weeks)	19 T2D patients exposed to sucralose (38.1% M & 61.9% F) (34 in total)	52.1 ± 7.6 years	2
Bueno-Hernandez et al. (2020)	HCT (Randomised controlled trial)	48 mg/day sucralose or 96 mg/day sucralose	10 weeks daily sucralose consumption. Sucralose was assessed by 24-h recalls and a validated Food Frequency Questionnaire with Intense Sweeteners used.	95 participants (30 for the 48 mg sucralose group and 31 for the 96 mg sucralose group)	18–35 years (22.9 ± 3.5 years for 48 mg sucralose and 22.6 ± 2.8 years for 96 mg sucralose)	2

TABLE 22 (Continued)

Authors, year	Type of study design	Dose (g/person or g/kg bw)[a]	Intervention/exposure	Number of subjects	Population (mean age in years)	RoB tier
Mendez-Garcia et al. (2022)	HCT (Randomised controlled trial)	0.048 g pure liquid sucralose in sterile water (60 mL), daily	Duration exposure: 10 weeks	Healthy; 20 (6 M & 14 F) for sucralose (40 in total)	22.9 ± 3.4 years	2
Suez et al. (2022)	HCT (Randomised controlled trial)	Aspartame, saccharin, sucralose and stevia. All NNS were given as commercially available sachets containing glucose as a bulking agent (2 sachets/3 times a day), corresponding to 34%, of the acceptable daily intake (ADI) of sucralose, equal to. 102 mg/day of sucralose	Exposure duration: 2 weeks	Total of 120 participants were included in the analysis of which 65% were female, 21 in sucralose group, 20 analised	29.95 (IQR 26.93–35.23), whole population	2
Brown et al. (2011)	HCT (Randomised controlled trial)	6 g of granular sucralose in the form of Splenda brand sweetener dissolved in 355 mL of water	4 days. Each treatment administered to each participant on 4 separate days of participation	8 Females	21.75 ± 2.25 years	2
Sylvetsky et al. (2016)	HCT (Randomised controlled trial)	0.068 g, 0.170 g or 0.250 g sucralose in water prior to an OGTT in study arm 1	Single exposure per test beverage	30 (14 M and 16 F) study arm 1	29.7 ± 7.6 years study arm 1	2
Sanchez-Delgado et al. (2021)	HCT (Randomised controlled trial)	41 g packets/day in commercial presentation containing 0.012 g of sucralose each packet	Exposure duration: 6 weeks	38 (13 for sucralose, 3 M & 10 F)	18 to 30 years (22.3 ± 4.4 years for sucralose)	2
Temizkan et al. (2015)	HCT (Randomised controlled trial)	0.024 g of sucralose in 200 mL of water	Single exposure on 3 different days	16 participants: 8 healthy volunteers (4 M & 4 F) +8 type 2 diabetic patients (4 M & 4 F)	45.0 ± 4.1 years (healthy volunteers) & 51.5 ± 9.2 years (type 2 diabetic patients)	2
Oliynyk (2021)	HCT (Randomised controlled trial)	15 mg/kg/day sucralose (StarkPharm)	Exposure duration: 1 month of daily intake of sucralose	50 F	24.5 ± 5.2 years	2
Chien et al. (2023)	Cohort study (Co)	Absolute intake: 0.0021 mg (F) and 0.0040 mg (M)	Food frequency questionnaire in the 3 months preceding the time of examination	1893 (1239 F and 654 M)	Range 6–15 years. Mean 9.69 ± 1.82 (F) and 11.78 ± 1.93 (M)	2
Steffen et al. (2023)	Cohort study (Co)	–	CARDIA Diet History questionnaire (of previous month) at 0, 7 & 20 years. 25-year study.	Young adults from CARDIA study; 618 (40–50% approx. M & 50–60% approx. F) for sucralose (3088 in total)	25.0 ± 0.1 - 25.3 ± 0.1 years	2
Zhu et al. (2025)	Cross-sectional (CrSe)	–	Sucralose was assessed by 24 h dietary recalls (≤ 6).	49 for sucralose (257 for non-consumers; 572 in total; 210 M & 362 F)	52.5 ± 9.4 years (data for entire study population)	2

Abbreviations: F, females; HCT, human controlled trial; M, males; NA, Not applicable; RoB, risk of bias.

[a]As reported by the authors.

[b]These two references relate to the same study but report the findings on different outcomes and were therefore considered jointly.

(Continues)

The outcomes addressed in the studies listed in Table 22 were aggregated into different health outcome categories (HOCs, Table 23). The human HOCs were constructed to align, to the extent possible, with the HOCs defined for the animal studies (Table 15). Given the differences in outcomes assessed in the human and animal studies the health outcomes within each category may not always be directly comparable.

TABLE 23 Health outcome categories and related outcomes of the appraised human studies subjected to WoE evaluation.

Health outcome categories (HOCs)	Outcomes
Cardiovascular risk factors and disease	Blood adipokines, blood cholesterol/HDL cholesterol/LDL cholesterol, blood lipid profile, blood triglycerides, blood pressure, body composition, BMI/WC CVD, Metabolic syndrome (MetS), Homocysteine, Incident obesity, plasminogen activator inhibitor-1 (PAI-1), type II diabetes
Glucose/insulin homeostasis	B-cell response (based on C-peptide) (during OGTT), Blood C-peptide, blood GIP, blood GLP-1, blood glucagon, blood glucose, blood haemoglobin A1c (HbA1c), blood haemoglobin A1c (HbA1c), blood insulin, insulin sensitivity
Liver effects	Blood alanine aminotransferase (ALT), blood albumin, blood alkaline phosphatase (ALP), blood aspartate aminotransferase (AST), blood bilirubin total bilirubin, blood gamma-glutamyl transpeptidase (GGT), GGT
Kidney effects	Blood creatinine
Cancer	All cancer, which were then divided into breast cancer, obesity-related cancer, prostate cancer
Inflammation/immune function	C-reactive protein, cytokine, IL-10, IL-1β, IL-6, monocyte chemoattractant protein 1 (MCP-1), serum flagellin (IgA and IgG), serum LPS (IgA and IgG), TNF-α, tumour necrosis factor-alpha (TNF-α), total antibodies
Thyroid effects	TSH, fT3, fT4, T3, T4

Abbreviations: GLP-1, glucagon-like peptide-1; OGTT, oral glucose tolerance test.

Based on the included human data, the Panel considered the confidence in the body of evidence for all health outcome categories, see Table 24, Appendix A and Annex F2).

TABLE 24 Rating of the confidence in the body of evidence for each health outcome category investigated in the human studies.

		Elements for downgrading[d]				Elements for upgradingd					
Health outcome categories (HOCs) investigated	**Initial rating (no. of studies)[a,b]**	**Concern for risk of bias across studies**	**Concern for unexplained inconsistency across studies**	**Concern related to relevance of studies**	**Concern for imprecision**	**Residual confounding**	**Magnitude of effect**	**Dose–response**	**Consistency across Study population/ study design**	**Final rating of confidence**	**Association observed (yes/no)**
Glucose/insulin homeostasis	High (30)	Downgrading: concern for imprecision/relevance/RoB				Upgrading: no				Moderate	No
Cardiovascular risk factors and diseases	High (17)	Downgrading: concern for relevance/RoB across studies				Upgrading: no				Moderate	No
Liver effects	High (2)	Downgrading: concern for RoB across studies				Upgrading: no				Moderate	No
Kidney effects	High (2)	Downgrading: no				Upgrading: no				High	No
Cancer	Moderate (1)	Downgrading: no				Upgrading: no				Moderate	No
Inflammation/immune function	High (4)	Downgrading: no				Upgrading: no				High	No
Thyroid effects	Moderate (1)[c]	Downgrading: concern for relevance				Upgrading: no				Low	Yes

[a]For human studies, it is generally expected that: Human controlled trials: high (++++), cohort studies: moderate (+++), Case–control studies: moderate (+++), Cross-sectional studies: low/moderate (++/+++). Here the overall initial rating of confidence is reported which accounts for all the available studies and their respective initial confidence rating (see Annex F2).

[b]The total number of studies assessed was 38. The number in parentheses refers to studies considered under the specific HOC.

[c]This interventional study has no randomised intervention and therefore the initial rating was considered as moderate.

[d]Please refer to Appendix A and Annex F2 and to the protocol (EFSA FAF Panel, 2023) for further explanations on what is assessed under each element.

In this section, the confidence in the body of evidence and the translation of confidence ratings into level of evidence for conclusions of adverse effects on health or no adverse effect on health for each HOC are discussed. Further consideration on the overall conclusion is addressed in Sections 3.5.4.3 and 3.7.

Glucose and insulin homeostasis

A total of 30 studies, including 29 intervention studies and 1 cross-sectional study, examined the effect or association between intake of sucralose and different measures of glucose homeostasis (see Table 20 and Annex F2). Of note is the fact that many of these studies were not designed to assess any potential adverse effect of sucralose on glucose homeostasis. Instead, sucralose was used as placebo or reference when assessing the effects of carbohydrate-rich food on blood glucose (and associated measures). Therefore, the doses used in many of the studies were low. Furthermore, many of the studies were only assessing effects on post prandial glucose response or effects on glucose homeostasis in the short-term, while few studies assessed changes in glucose homeostasis over few weeks. Overall, no consistent effects related to intake of sucralose were observed on measures of glucose homeostasis in these studies.

Considering the final rating of confidence in the body of evidence for the HOC 'glucose and insulin homeostasis' as 'moderate' (Downgraded for imprecision/relevance/RoB) and the absence of effects (see Table 24 and Annex F2), the Panel considered that there is moderate confidence in the body of evidence that exposure to sucralose (E 955) does not adversely affect glucose and insulin homeostasis.

Cardiovascular risk factors and diseases

A total of 17 studies, comprising 12 intervention studies, 4 cohort studies and 1 cross-sectional study, examined the effect or association of exposure to sucralose with markers of cardiovascular disease including blood lipids and anthropometric measures (see Table 20 and Annex F2). One large (n ~ 100.000) prospective cohort study examined the association between quantified intake of sucralose and the risk of total cardiovascular disease (CVD), cerebrovascular disease and coronary heart disease (Debras, Chazelas, Sellem, et al., 2022). Similarly to the studies on glucose homeostasis, the human intervention studies examining effects of sucralose on CVD and cardiovascular risk factors had short duration. Overall, the human intervention studies examining sucralose did not report any effects on cardiovascular risk factors. These results are in contrast with findings reported in a large prospective cohort study suggesting an association with coronary heart disease (Debras, Chazelas, Sellem, et al., 2022) and type 2 diabetes (Debras et al., 2023). However, the intervention studies on cardiovascular disease risk factors along with the intervention studies on glucose homeostasis assessed in this opinion, as described above, do not support the findings of (Debras et al., 2023; Debras, Chazelas, Sellem, et al., 2022).

Considering the final rating of confidence in the body of evidence for the HOC 'Cardiovascular risk factors and diseases' as 'moderate' (downgraded for relevance/RoB across studies) and the fact that associations between sucralose intake and relevant outcomes (type 2 diabetes, coronary heart disease) were only observed in a single cohort study with no mechanistic support from interventional studies (see Table 24 and Annex F2), the Panel considered that there is moderate confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse effects in the cardiovascular system.

Liver effects

Two intervention studies examined the effect of sucralose intake over few weeks on liver enzymes, blood albumin (measured only in one study (Han Y et al., 2018)) and bilirubin (see Table 20 and Annex F2). In both studies, no effects were observed.

Considering the final rating of confidence in the body of evidence for the HOC 'liver effects' as 'moderate' (downgraded for RoB across studies) and the absence of effects (see Table 24 and Annex F2), the Panel considered that there is moderate confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse effects in the liver.

Kidney effects

Two intervention studies examined the effect of sucralose intake over few weeks on blood creatinine level. In both studies, no effect was observed (see Table 20 and Annex F2).

Considering the final rating of confidence in the body of evidence for the HOC 'kidney effects' as 'high' and the absence of effects (see Table 24 and Annex F2), the Panel considered that there is high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse effects in the kidneys.

Cancer

The same prospective cohort study that examined the association between sucralose intake and CVD and diabetes type 2, reported no association with cancer i.e. total cancer as well as breast, prostate and obesity-related cancer[63] (Debras et al., 2023; Debras, Chazelas, Srour, et al., 2022).

Considering the final rating of confidence in the body of evidence for the HOC 'cancer' as 'moderate' (no downgrading since initial confidence rating moderate) and the fact that no associations between sucralose intake and certain cancers were observed (see Table 24 and Annex F2), the Panel considered that there is moderate confidence in the body of evidence that exposure to sucralose (E 955) is not associated with cancer.

Inflammation/immune function

Four intervention studies of duration ranging between 6 weeks to 12 weeks and a cross-sectional study examined the effects of sucralose intake on markers of inflammation. No effects were observed in these studies (see Table 24 and Annex F2).

Considering the final rating of confidence in the body of evidence for the HOC 'inflammation/immunotoxicity' as 'high' and the absence of effects (see Table 22 and Annex F2), the Panel considered that there is high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse effects on the immune system.

Effects on thyroid

One intervention study examined the effect of 15 mg sucralose intake over a period of 1 month on thyroid hormones in 50 young women (mean age: 25 y) (see Table 20 and Annex F2). After 1 month the thyroid-stimulating hormone (TSH) levels were statistically significantly increased from baseline (1.25 vs. 5.52 mIU/L) while free T4 levels were weakly (not significantly) decreased (1.10 vs. 0.81 ng/dL) and free T3 levels were statistically significantly reduced (3.16 vs. 1.56 pg/mL). The main limitations of this study were a lack of comparison group and lack of information on the blood sampling time. Given these limitations and the lack of replication, the Panel considered that there is low confidence (initial confidence rating was moderate; downgraded for relevance) in the body of evidence (see also Table 24 and Annex F2), which at this time consists of a single study, that exposure to sucralose (E 955) is associated with altered thyroid function (Table 25).

TABLE 25 Translation of confidence ratings into level of evidence for conclusions of adverse effects or no adverse effects on health for the included human studies for each of the HOC considered in the assessment.

HOC	Final rating of confidence	Level of evidence
Glucose and insulin homeostasis	Moderate	**Moderate** There is moderate confidence in the body of evidence that exposure to sucralose (E 955) does not adversely affect glucose and insulin homeostasis.
Cardiovascular risk factors and diseases	Moderate	**Moderate** There is moderate confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse effects in the cardiovascular system.
Liver effects	Moderate	**Moderate** There is moderate confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse effects in the liver.
Kidney effects	High	**High** There is high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse effects in the kidneys.
Cancer	Moderate	**Moderate** There is moderate confidence in the body of evidence that exposure to sucralose (E 955) is not associated with cancer.
Inflammation/immune function	High	**High** There is high confidence in the body of evidence that exposure to sucralose (E 955) is not associated with adverse effects on the immune system.
Effects on thyroid	Low	**Low** There is low confidence in the body of evidence, which at this time consists of a single study, that exposure to sucralose (E 955) is associated with altered thyroid function.

[63]Defined in Debras et al. (2022b) as: colorectal, stomach, liver, mouth, pharynx, larynx, oesophageal, breast, ovarian, endometrial and prostate cancers.

Additional studies

Additional studies measuring specific outcomes and/or performed in specific populations were retrieved in the literature. Therefore, these studies or some of the outcomes measured in these studies (cortisol levels, cognitive effects) were not considered in the WoE but are summarised narratively below.

Aguayo-Guerrero et al. (2023) conducted a cross-sectional study comparing birth weight, glucose and insulin cord blood levels, monocyte subsets and inflammatory cytokine profile in neonates from mothers with light sucralose intake (LSI; < 60 mg sucralose/week) or heavy sucralose intake (HIS; > 36 mg sucralose/day) during pregnancy. Neonates from HSI mothers had higher birth weight, higher insulin levels and more inflammatory nonclassical monocytes than neonates from LSI mothers. Selection bias and potential type 1 error limit the validity of the study and make the interpretation of the findings challenging.

Liu et al. (2022) conducted a nested case–control study comparing sucralose levels among cases with gestational diabetes mellitus (GDM) and non-GDM controls. No differences were observed.

Eckstein et al. (2021) conducted a double-blind randomised crossover placebo-controlled trial to assess the impact of glucose, fructose, glucose and fructose, and sucralose as 'placebo' on several parameters in healthy individuals, these were all considered in the WoE with the exception of cortisol levels since this parameter did not fit in any of the established HOCs. There were no changes in cortisol levels from timepoint 0 until any timepoint of each trial arm, within and in-between groups.

Five intervention studies (Deng et al., 2021; Ginieis et al., 2018; Keesing et al., 2019; López-Meza et al., 2022; Toepp et al., 2019) where sucralose was used as placebo or comparator reported effects on neurobehavioral outcomes (cognitive effects). These studies were primarily aimed at examining the effects of carbohydrate ingestion on cognitive performance after fasting and were not designed to assess effects of sucralose per se. It is well established that ingestion of carbohydrates is, after fasting, known the be beneficial for cognitive performance so no conclusions of sucralose effects on neurobehavioral outcomes can be drawn from such design.

3.5.4.3 | *Integration of the evidence from animal and human studies*

Human and animal evidence streams were integrated for similar HOCs, in accordance with Appendix A and with the definition given in the protocol (EFSA, 2020a; EFSA FAF Panel, 2023), taking into consideration the assessment in Sections 3.5.4.1 and 3.5.4.2. The conclusions from the integration were expressed in terms of likelihood of an association between the intake of sucralose (E 955) and an adverse effect on human health. Consideration was given to the conclusions of previous evaluations (SCF, 2000) to assess whether the new data support them. In the case of the HOCs with data available only from animal studies (namely 'haematotoxicity', 'reproductive toxicity', 'maternal and developmental toxicity' and 'neurotoxicity') the integration of evidence was done considering the option 'missing data' from Figure A.2, Appendix A. 'Missing data' pertains to data missing in the body of evidence in the current re-evaluation in accordance with the revised protocol (EFSA FAF Panel, 2023) (Table 26).

TABLE 26 Overview of the HOCs for which human and animal evidence streams were integrated.

HOC human studies	HOC animal studies
Glucose/insulin homeostasis	Glucose/insulin homeostasis
Inflammation/immune function	Inflammation/immunotoxicity
Effects on liver	Liver toxicity
Effects on kidney	Nephrotoxicity
Cancer	Carcinogenicity
Effects on thyroid	Thyroid toxicity
Body weight[a]	Body weight
Cardiovascular risk factors and diseases	Additional clinical chemistry

[a]Please refer to the explanation in the text below under 'body weight'.

Body weight

Since the effects observed in the rat studies indicated an adverse weight reduction (see Sections 3.5.4.1) and the outcomes related to body weight changes in the human studies, which are part of the HOC 'cardiovascular risk factors and diseases', were analysed separately in this section. The other outcomes included in the HOC 'cardiovascular risk factors and diseases' are considered in the following section.

Effects on body weight or related endpoints were assessed both in animals and humans. The level of evidence was rated as 'moderate' for human and 'high' for animals. In the available animal studies decreases in body weight were observed, in particular, in the combined chronic and carcinogenicity study in rats (see Section 3.5.4.1). No effect or association between the exposure to sucralose and changes in body weight (or in related outcomes, see Annex F2) were observed in human

studies at doses up to 3 mg/kg bw per day (see Table 27). Integrating the stream of evidence from human and animal data, the Panel concluded that it is likely that sucralose (E 955) exposure is associated with an adverse decrease in body weight at doses well above those reported in the human studies.

TABLE 27 Doses in the available human studies assessing effects on outcomes related to body weight changes (BMI/WC/waist-to-hip ratio, hip circumference, waist-to-height ratio), see also Annex F2.

Reference	Type of study	Dose (mg/kg per day)	Duration	Number of participants
Higgins and Mattes (2019)	Randomised controlled trial	3	12 weeks	28
Steffen et al. (2023)	Cohort study	2.87	25 years	617
Bueno-Hernandez et al. (2020)	Randomised controlled trial	1.37	10 weeks	31
Orku et al. (2023)	Randomised controlled trial	1.2	4 weeks	11
Sanchez-Delgado et al. (2021)	Randomised controlled trial	0.8	6 weeks	13
Mendez-Garcia et al. (2022)	Randomised controlled trial	0.75	10 weeks	20
Han et al. (2018)	Randomised controlled trial	0.34	12 weeks	121

Additional clinical chemistry, cardiovascular risk factors and diseases

The Panel considered it appropriate to integrate aspects of the HOC 'cardiovascular risk factors' addressed in human studies with aspects of the HOC 'additional clinical chemistry' from animal studies. The Panel noted that the included animal studies did not cover fully the outcomes related to cardiovascular risk factors and diseases addressed in the human studies since not all outcomes measured in human were assessed in the animal studies. Therefore, the integration has to account for the differences across the two datasets.

Clinical chemistry parameters (total cholesterol, HDL, LDL, vLDL and triglycerides) were assessed in animals and total cholesterol, HDL, LDL, blood lipid profile and triglycerides were also measured in human studies. The level of evidence for no effect was rated as 'moderate' for both human and animals. The Panel concluded that it is unlikely that sucralose (E 955) exposure is associated with adverse changes in clinical chemistry parameters in humans.

The human studies showed no association between sucralose exposure and cardiovascular risk factors (plasminogen activator inhibitor-1 (PAI-1), homocysteine levels, adipokines levels, blood pressure, metS, increase in BMI, WC) and obesity. One prospective cohort study reported an increased risk of coronary heart disease (Debras, Chazelas, Sellem, et al., 2022) and diabetes type 2 (Debras et al., 2023). Replication of these findings is lacking. The level of evidence for no effect was rated as 'moderate'. Therefore, the Panel concluded that it is unlikely that sucralose (E 955) exposure is associated with an increased incidence of cardiovascular diseases in humans.

Glucose and insulin homeostasis

The HOC 'glucose and insulin homeostasis' was evaluated in human and animal studies. The level of evidence for the lack of adverse effects was rated as 'moderate' in human and 'high' in animals. Therefore, the Panel concluded that it is unlikely that exposure to sucralose (E 955) is associated with disturbances of glucose/insulin homeostasis in humans.

Inflammation/immunotoxicity/immune function

The HOC 'Inflammation/immunotoxicity' and 'inflammation/immune effects' were evaluated in human and animal studies. The level of evidence for the lack of adverse effects was rated as 'high' for human and as 'moderate' for animals. Therefore, the Panel concluded that it is very unlikely that exposure to sucralose (E 955) is associated with effects on the immune system in humans.

Liver toxicity/effects

The HOC 'liver effects' and 'liver toxicity' were evaluated in human and animal studies. The level of evidence for the lack of adverse effects was rated as 'moderate' in human and 'moderate to high' in animals. Therefore, the Panel concluded that it is unlikely that exposure to sucralose (E 955) is associated with effects on liver in humans.

Nephrotoxicity/Kidney effects

The HOCs 'nephrotoxicity' and 'kidney effects' were evaluated in human and animal studies. The level of evidence was rated as 'high' for the lack of adverse effects in human and as 'moderate' for the lack of adverse effects in animal studies. Therefore, the Panel concluded that it is very unlikely that exposure to sucralose (E 955) is associated with effects on kidneys in humans.

Carcinogenicity/Cancer

The HOCs 'cancer' and 'carcinogenicity' were evaluated in human and animal studies. The level of evidence for the lack of adverse effects was rated as 'moderate' in human and 'high' in animals. The Panel further noted that no concern for genotoxicity was identified for sucralose (see Section 3.5.3). Further support for the lack of carcinogenicity comes from the integration of key characteristics of carcinogens (KCCs). KCC scores for sucralose allow the quantitative integration of data to indicate consistency of activity and facilitate a weight of evidence analysis. Although some weak activity was noted in relation to certain parameters such as oxidative stressor, chronic inflammation, cell proliferation and cell death, overall, there was a lack of activity for sucralose across the KCCs with no strong activity observed for any individual KCC (Chappell et al., 2020). Overall, the Panel concluded that it is unlikely that exposure to sucralose (E 955) is associated with cancer in humans.

Thyroid toxicity/thyroid effects

The HOCs 'thyroid toxicity' and 'thyroid effects' were evaluated in human and animal studies. The level of evidence was rated as 'high' for the lack of adverse effects in animals and 'low' for the presence of adverse effects in humans. The Panel concluded that it is unlikely that exposure to sucralose (E 955) adversely affects thyroid function in humans.

Haematotoxicity

No adverse effects were observed in a number of haematological parameters in the animal studies. The level of evidence was evaluated as high. Haematological parameters were not reported in human studies. The Panel concluded that it is unlikely that sucralose (E 955) exposure is associated with haematological effects in humans.

Reproductive toxicity

No adverse effects on reproduction were observed in the animal studies. The level of evidence was evaluated as high. Reproductive toxicity was not assessed in the human studies. The Panel concluded that it is unlikely that sucralose (E 955) exposure is associated with reproductive effects in humans.

Maternal and developmental toxicity

No maternal and developmental toxicity was observed in the animal studies. The level of evidence was evaluated as high. Maternal and Developmental toxicity were not assessed in the human studies. The Panel concluded that it is unlikely that sucralose (E 955) exposure is associated with maternal and developmental effects in humans.

Neurotoxicity

No adverse effects on the nervous system were observed in the animal studies. The level of evidence was rated as 'high' for lack of adverse effects. Neurobehavioral effects were not assessed in the human studies. Therefore, the Panel concluded that it is unlikely that sucralose (E 955) exposure is associated with neurobehavioral effects in humans.

3.5.5 | Hazard characterisation and identification of a reference point

Table 28 provides an overview of the conclusions on the level of likelihood for the association or absence of association between the intake of sucralose (E 955) and an adverse effect on health for each group of integrated HOCs (see Section 3.5.4.3).

TABLE 28 Overview of the conclusions on the level of likelihood of an association or absence of association between the exposure to sucralose (E 955) and an adverse effect on human health for each HOC.

Evidence stream	HOC (endpoint)	Effect or association observed (yes/no)	Level of evidence	Integration of the evidence (likelihood)
Human	Cardiovascular risk factors and disease (only BMI, WC, body composition)	No	Moderate	Integrating the stream of evidence from human and animal data, the Panel concluded that it is **likely** that sucralose (E 955) exposure is associated with a decrease in body weight[64] which was considered as adverse at doses well above those reported in the human studies.
Animal	Body weight	Yes	High	
Human	Cardiovascular risk factors and disease (clinical chemistry)	No	Moderate	It is **unlikely** that sucralose (E 955) exposure is associated with adverse changes in clinical chemistry parameters in humans.
Animal	Additional clinical chemistry	No	Moderate	
Human	Cardiovascular risk factors and disease	No	Moderate	It is **unlikely** that sucralose (E 955) exposure is associated with cardiovascular diseases in humans.
Animal	–	–	Missing data	
Human	Glucose/insulin homeostasis	No	Moderate	It is **unlikely** that sucralose (E 955) exposure is associated with disturbances of glucose or insulin homeostasis.
Animal	Glucose/insulin homeostasis	No	High	
Human	Inflammation/immunotoxicity	No	High	It is **very unlikely** that sucralose exposure (E 955) is associated with effects on the immune system/allergenicity.
Animal	Immunotoxicity/allergenicity	No	Moderate	
Human	Liver effects	No	Moderate	It is **unlikely** that sucralose (E 955) exposure is associated with effects on liver.
Animal	Liver toxicity	No	Moderate to high	
Human	Kidney effects	No	High	It is **very unlikely** that sucralose (E 955) exposure is associated with effects on kidney.
Animal	Nephrotoxicity	No	Moderate	
Human	Cancer	No	Moderate	It is **unlikely** that sucralose (E 955) exposure is associated with cancer.
Animal	Carcinogenicity	No	High	
Human	–	–	Low	It is **unlikely** that sucralose (E 955) exposure is associated with neurotoxicity.
Animal	Neurotoxicity	No	High	
Human	–	–	Missing data	It is **unlikely** that sucralose (E 955) exposure is associated with haematotoxicity.
Animal	Haematotoxicity	No	High	
Human	–	–	Missing data	It is **unlikely** that sucralose (E 955) exposure is associated with reproductive toxicity
Animal	Reproductive toxicity	No	High	
Human	–	–	Missing data	It is **unlikely** that sucralose (E 955) exposure is associated with developmental effects.
Animal	Maternal and developmental toxicity	No	High	
Human	Thyroid effects	Yes	Low	It is **unlikely** that sucralose (E 955) exposure) is associated with thyroid effects
Animal	Thyroid toxicity	No	High	

(Continues)

[64]Defined as percentage of change with respect to the control of the mean final body weight

For the selection of a RP, considering the outcome of the assessment above, the Panel considered those HOCs for which adverse effects were considered likely. The Panel considered it likely that the exposure to sucralose (E 955) is associated with decrease in body weight in animals which was considered as adverse. The Panel selected this endpoint for the identification of a reference point.

Decreases in body weight higher than 10% were observed, particularly in the combined chronic and carcinogenicity study in rats (Documentation provided to EFSA No 1). Therefore, the dose-effect data from this study were used to perform a Benchmark dose (BMD) analysis (see Annex G).

A decrease in body weight equal or higher than 10% was considered as adverse in earlier assessments (EFSA FAF Panel, 2024b; WHO, 2015). For sucralose, the Panel noted that the decrease in body weight was partly due to poor palatability and reduced feed intake and that about half of this response (decrease in body weight) could be attributable to reduced palatability of the test diet (see Appendix G). Following EFSA BMD guidance (EFSA Scientific Committee, 2022), applying a tiered approach to BMR setting (accounting for palatability), the Panel considered a BMR set at 15% as biologically relevant. The Panel additionally noted that this is further supported by the observation that the variation in the body weight of the control group in the combined chronic and carcinogenicity study in rats (expressed as standard deviation from the mean) at the end of the experiment was larger than 10% (see Appendix G). Sex was considered in the analysis as covariate. For females, the calculated BMDL was 55 mg/kg bw per day (BMD 117 mg/kg bw per day; and BMDU 240 mg/kg bw per day), for males the BMDL was 61 mg/kg bw per day (BMD 110 mg/kg bw per day and BMDU 221 mg/kg bw per day), see Annex H.

Based on this analysis, the Panel considered the BMDL of 55 mg/kg bw per day as the reference point.

In 2005, the WHO/ICPS proposed a framework indicating how chemical-specific TK and/or TD data can be used to replace the default factors or its subfactors (WHO/ICPS, 2005). Accordingly, EFSA Scientific Committee also recommends using chemical-specific data on kinetics and/or dynamics instead of the overall default assessment factor of 100 (EFSA Scientific Committee, 2012). In line with these suggestions, and in the context of the re-evaluation of already permitted food additives, the Panel has already developed chemical-specific assessment factors (CSAFs) for a number of food additives (e.g. EFSA FAF Panel 2019, 2022, 2024b, 2024c).

The Panel considered whether the available data would allow modifying the default assessment factor for sucralose (E 955).

The default assessment factor of 100 is composed of a factor of 10 to account for interspecies differences between experimental animal species and humans, and a second factor of 10 for interindividual differences in the human population. These two factors allow for interspecies differences and human variability and consider both toxicokinetics and toxicodynamics. For the TK component of the interspecies factor, a value of 4 is used when the extrapolation is made from rat to human (EFSA Scientific Committee, 2012). This factor 4 is based on allometric scaling from rat to human. The other factor of 2.5 accounts for interspecies differences in TD. For interindividual differences, the factor 10 is subdivided into two factors of 3.2 each, to account for TK and TD differences.

In the weight of evidence analysis, only a reduction of body weight was identified, and the Panel considered that this effect is likely to result from a direct effect on the GIT, noting also the increase in caecum weight in the available rat studies (see Section 3.5.4.1 and Appendix D). Since this effect is not likely to be dependent on the systemic availability of sucralose, no kinetic differences have to be considered and the interspecies and intraspecies kinetic factors could be set both to 1.

For intraspecies (human) variability, no data are available that would justify deviating from the default toxicodynamic factor of 3.2. If the default toxicodynamic interspecies factor of 2.5 is used, the resulting CSAF would be 8. Given the effects on the gastrointestinal tract, it is reasonable to assume that humans are not more sensitive than rats. Thus, the toxicodynamic interspecies factor (2.5) could be reduced to 1. Under these assumptions, the resulting CSAF would be 3.2.

For the current assessment of sucralose, a systematic appraisal of updated evidence since the SCF evaluation (SCF, 2000) and of the studies considered in the setting of the previous ADI was performed, including risk of bias evaluation and a weight of evidence. Based on the available data, BMD analysis (EFSA Scientific Committee, 2022) was conducted for the endpoint of reduced body weight, resulting in a BMDL of 55 mg/kg bw per day. The Panel noted that human studies have shown that intakes up to 3 mg/kg bw per day of sucralose were well tolerated without adverse effects in studies with duration from months to years (see Table 27).

In view of the range of values resulting from the application of either of the possible CSAFs (3.2 or 8) to the BMDL of 55 mg/kg bw per day, which would result in ADI values encompassing the current ADI, and considering that no new adverse effects have been identified, the Panel considered that there is no need to revise the ADI of 15 mg/kg bw per day for sucralose (E 955) established by the SCF in 2000 (SCF, 2000).

3.6 | Environmental considerations

The applicable EU legislation on food additives establishes that the approval of food additives should consider, among other factors, also the protection of the environment. In the framework of the re-evaluation of sucralose under Regulation (EU) No 257/2010, EFSA has not received any information from IBOs or any other interested party in relation to any environment risks of sucralose (E 955), however, it became aware of a large amount of data and information available in the public domain.

A systematic review collating published data on sucralose (E 955) to identify evidence of potential adverse effects on the environment resulting from the use of sucralose (E 955) as a food additive is available (Agriculture and Environment

Research Unit, University of Hertfordshire (AERU), 2021). This review was complemented by additional papers retrieved in the updated literature search in the present assessment (see Appendix A).

As reported in Section 3.5.1, absorption of sucralose in humans is low (not more than approximately 20%) and the majority of the consumed sucralose is excreted unchanged in the faeces and in urine. Therefore, sucralose, when consumed as food additive has the potential to reach the environment via wastewater. It is expected that the main receiving compartment will be the aquatic environment. Sucralose, used as food additive, may also reach the terrestrial environment (e.g. via fertilisation with contaminated sewage sludge or by flood events), however, these routes are expected to be less relevant than the direct emission from wastewater facilities into surface water. Considering the physicochemical properties of sucralose (i.e. high solubility in water, low log K_{ow}, low adsorption coefficient and low vapour pressure; see AERU, 2021), it is expected that sucralose mainly partitions to water. Bioaccumulation along the food chain is not expected.

The amount of sucralose (E 955) that may reach the environment depends on (i) how efficiently wastewater treatment plants can remove it from their influent and (ii) on the subsequent (bio)degradation in the environmental compartments.

According to AERU (2021) the efficiency of wastewater treatment systems in removing sucralose from wastewater is generally poor and depends on the applied treatment. It is noted that due its recalcitrance, sucralose was identified as a biomarker of domestic pollution in several studies (e.g. Blackstock, 2019; Oppenheimer et al., 2011; Perkola, 2014; Van Stempvoort, Robertson, & Brown, 2011; Van Stempvoort, Roy, et al., 2011; Yang et al., 2018; Tran, Hu, & Ong, 2013; Tran, Hu, Li, & Ong, 2013; Scheurer et al., 2014). In the updated literature search, several additional studies investigating the removal of sucralose from (waste) water, including laboratory scale experiments, were retrieved (see Annex H). These studies confirmed what was reported in AERU (2021) and reiterated generally low and variable removal efficiencies depending on the applied method and conditions.

A number of retrieved papers investigated the formation of transformation products of sucralose following water treatment and proposed transformation pathways (Sang et al., 2014; Fu et al., 2019; Hu et al., 2017; Batchu et al., 2013; Calza et al., 2013; Wang et al., 2019; Xu et al., 2016, 2017; Yang et al., 2021). The potential toxicity of sucralose transformation products was investigated in a number of studies with screening assays such as Microtox (Calza et al., 2013; Sang et al., 2014; Lin et al., 2017; Ferreira et al., 2015), biomarkers of toxicity assessment in fish (Fu et al., 2019), activated sludge inhibition test (Xu et al., 2016, 2017) and acute ecotoxicity test (*Daphnia magna* immobilisation test (Xu et al., 2017). For those studies, in which a transient increase in toxicity followed by a recovery was observed, this was interpreted by authors as indicating a potential increase in the toxicity of the transformation products with respect to the parent compound. The fact that a recovery was observed was considered indicative that detoxification could occur with the prolongation of the treatment (Calza et al., 2013; Lin et al., 2017, Ferreira et al., 2015; Fu et al., 2019; Xu et al., 2016, 2017).

Following sucralose release into natural surface water via, e.g. wastewater treatment plants effluents, sucralose can undergo some transformation or (bio)degradation, but this seems to be a very slow process under environmentally-relevant conditions of temperature and pH (AERU, 2021; OECD, 2025). Some studies addressing the (bio)degradability and transformation of sucralose were retrieved in the review (AERU, 2021). Bergheim et al. (2015) performed a biodegradability test in line with OECD TG 301 D, F and 302B (OECD, 1992a, 1992b) and found that sucralose could be classified as 'not readily biodegradable' (degradation percentage 0%–4%). This was confirmed in the papers retrieved in the updated literature search in which sucralose was reported as not readily biodegradable (Gatidou et al., 2020; Jahani et al., 2020). According to the REACH registration dossier, sucralose is not readily biodegradable. Bergheim et al. (2015) tested the stability of sucralose under irradiation with a xenon lamp (300–800 nm; no information on flux, simulating sunlight conditions) and a mercury lamp (200–600 nm; 47 W). No major changes in the concentration of sucralose following the irradiation were observed. In Perkola et al. (2016) the photolysis of sucralose under simulated sunlight was assessed. The photolytic half-life (DT50) was determined to be > 1300 days depending on the conditions (source of the water and in presence of ferric iron). In Batchu et al. (2013), retrieved in the updated literature search, photodegradation experiments were performed using canal and sea water, sampled in South Florida, simulating natural light conditions (UV 350 nm or 'solar simulator') and sucralose was persistent to photolysis in both types of water (< 20% degradation).

In AERU (2021), several studies addressing the fate and behaviour of sucralose in soil were reviewed. The degradation of sucralose in soils was reported as dependent on different conditions and half-life ranging between 8 and 124 days were reported. The degradation of sucralose seems to be more rapid in high organic matter media where the growth of microbial communities is enhanced (Biel-Maeso et al., 2019; Buerge et al., 2011; Labare & Alexander, 1993, 1994; Ma et al., 2017; Soh et al., 2011). Soil half-lives ranging from 69 to > 700 days (depending on the testing conditions) were reported in Regnery et al. (2015), retrieved in the updated search.

Several studies retrieved in AERU (2021) and in the updated literature search reported analytical results of the measurement of sucralose in environmental matrices (see Annex H). In studies measuring sucralose in both wastewater influent and effluent, the concentrations in the effluent were generally similar to those in the influent. This indicates that sucralose is not, or is only poorly, removed during wastewater treatment. In some studies, sucralose was reported as not detected in surface water and in other studies the concentrations of sucralose in surface water were highly variable ranging from 0.0001 to 600 µg/L. The concentration of sucralose in sediment from surface and marine water was reported in nine studies from non-EU countries (Dong et al., 2015; Fu et al., 2020; Gvozdić et al., 2020, 2023; Hain et al., 2023; James et al., 2023; Picard et al., 2021; Yin et al., 2025; Yue et al., 2023). Sucralose was detected in five studies at a maximum concentration of 115 µg/kg dry weight (Yin et al., 2025). Sucralose was reported in groundwater at concentrations above 0.1 µg/L in some cases (see Annex H), such levels would trigger further assessment in other regulatory areas (see EFSA FEEDAP Panel, 2019). Sucralose was measured in soil in three studies with concentrations ranging from 0.16 to 35 µg/kg (see Annex H).

The international platform of chemical monitoring (IPCHEM)[65] includes data on the concentration of sucralose in different environmental compartments from several EU and non-EU countries from EMPODAT.[66] The maximum reported concentration for surface water was 25 µg/L (range 0.001 to 25 µg/L) and the maximum concentration in groundwater was 1.1 µg/L (range 0.02 to –1.1 µg/L). According to the same database, sucralose was not detected in sediment.

Several studies retrieved in AERU (2021) and in the updated literature search assessed the ecotoxicological effects of sucralose (see Annex H). Stolte et al. (2013) assessed the toxicity of sucralose towards green algae (*Scenedesmus vacuolatus*, chronic toxicity), water fleas (*Daphnia magna*, acute toxicity) and duckweed (*Lemna minor*, chronic toxicity) and in an activated sewage sludge microbial community. The NOEC for all tested organisms/communities was the highest concentration tested, 1000 mg/L. Several studies addressed the toxicity of sucralose in aquatic invertebrates covering different species and both acute and chronic toxicity (see Annex G for details). When the chronic toxicity is considered, the NOEC for *D. magna* and *Americamisis bahia* was reported as 1800 mg/L and 93 mg/L, respectively (Huggett & Stoddard, 2011). In Stoddard and Huggett (2014), an early life stage (ELS) toxicity test in fathead minnows, *Pimephales promelas*, was reported and a NOEC of 98 mg/L was identified. In Colin-Garcia et al. (2022), an embryotoxicity test on *Danio rerio* (zebrafish) was performed. After 96 h of exposure, mortality and malformation rates showed a statistically significant concentration-dependent increase and LC50 and EC50 (malformations) were calculated as 123 µg/L and 4.9 µg/L, respectively. Regarding aquatic plants (macrophytes), no effects on growth were observed in Soh et al. (2011), Kerberová et al. (2021) and Stolte et al. (2013). In Amy-Sagers et al. (2017) a large range of concentrations of sucralose were tested with duckweed (*Lemna minor*; 6 to 6000 mg/L). Sucralose significantly increased the leaf area (approx. 30%) and the photosynthetic capacity of *L. minor*. In Kobetičová et al. (2018), sucralose was found to inhibit growth parameters (frond number, LOEC = 0.1 mg/L and frond area, NOEC = 5 mg/L) but not the chlorophyll content (NOEC = 500 mg/L). Only one study addressing effects on soil organisms was retrieved (Lin et al.,2024), an increased juvenile reproduction at 100 µg/kg was observed. Some studies measured alterations in biomarkers or non-apical endpoints in aquatic invertebrates (Eriksson Wiklund et al., 2014, 2023; Rizzi et al., 2023; Sladkova et al., 2016), fish (Chiclana-Rodríguez et al., 2023; Colin-Garcia et al., 2022; Heredia-Garcia et al., 2019; Saucedo-Vence et al., 2017) and amphibia (Abbott & Helbing, 2021). The ecotoxicological relevance of such changes is unclear.

The REACH registration dossier[67] for sucralose reports the summaries of several ecotoxicological studies. Regarding the aquatic organisms, the following acute toxicity endpoints[68] are reported: LC50 for fish of 1800 mg/L and EC50 for aquatic invertebrates (*D. magna*) higher than 1800 mg/L. Studies on algae were also available: EC50 > 1800 mg/L (growth rate and biomass). Studies on macrophytes, sediment dwellers or terrestrial organisms were not reported.

In summary, sucralose is not fully metabolised in humans. Its removal in wastewater treatment is variable and can be poor (depending on the treatment method) and there are uncertainties on the possible formation of environmentally relevant transformation products of sucralose during water treatment. Further investigation on the possible formation of environmentally relevant transformation products of sucralose and on their ecotoxicological effects might be necessary. Sucralose has been reported to be not readily biodegradable and was not transformed under simulated sun light. Variable concentrations of sucralose have been found in the aquatic environment. The Panel noted that the available data on the environmental concentrations of sucralose are based on isolated monitoring studies and are not part of systematic monitoring programmes. The available studies included data from both EU and non-EU countries and may not be fully representative of the European situation. These data therefore provide only a rough indication of sucralose concentrations and may not reflect worst-case exposure levels for aquatic organisms. Sucralose measured concentrations in surface waters (ranging from 0.0001 to 600 µg/L) are generally lower than the available effect concentrations for aquatic organisms. However, the lowest reported effect concentrations (Colin-Garcia et al., 2022; Kobetičová et al., 2018) fall within the range of the measured concentrations in surface water.

Overall, considering (i) that sucralose is not extensively metabolised in human, (ii) that sucralose may not be fully degraded in a sewage treatment plant and is not readily degradable and (iii) the available evidence from the literature described above, further considerations may be needed to address the possible impact of sucralose to the environment.

[65] https://ipchem.jrc.ec.europa.eu/#databaseConsole/EMPODAT/sucralose/56038-13-2/none/Water;%20Soil, accessed on 1.12.2025.

[66] EMPODAT compiles data from monitoring, survey, research / technical studies provided by NORMAN member organisations (https://www.normandata.eu/?q=Home). The reason of data collection therefore may vary by dataset and/or data provider.

[67] https://chem.echa.europa.eu/100.054.484/dossier-list/reach/dossiers/active?searchText=sucralose.

[68] Intended here as the ecotoxicological values resulting from the available ecotoxicological tests (e.g. LC50, EC50, NOEC), see also section 'Glossary and/or abbreviations and/or acronyms for detailed definitions'.

3.7 | Discussion

The present opinion deals with the re- evaluation of sucralose (E 955), authorised as a food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No. 1333/2008 on food additives and with the assessment of the safety of the proposed extension of use for sucralose (E 955) in energy-reduced or without added sugar fine bakery wares (FC. 7.2).

Sucralose is 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside and is produced in a three-step chemical process that selectively replaces three hydroxyl groups on the sucrose molecule with chlorine. Sucrose is first converted to sucrose-6-acetate by reaction with a tin-containing catalyst and then chlorinated to form sucralose-6-acetate which is de-acylated to produce sucralose. The resulting sucralose undergoes several purification steps. In the EU specifications, the content of sucralose must be more than 98% and the analytical data provided indicate a content higher than 99%.

According to the sucralose monograph of the European Pharmacopoeia (Ph. Eur. 11.8), 8 organic impurities can be present in sucralose. In the EU specifications for sucralose (E 955) there is a limit of 0.5% for 'other chlorinated disaccharides', 0.1% for 'chlorinated monosaccharides' and of 150 mg/kg for TPPO. Analytical data on the quantification of individual chlorinated monosaccharides and chlorinated disaccharides in samples of E 955 have been submitted. Based on the available genotoxicity studies and in silico analysis of the potential organic impurities identified in sucralose (E 955), the Panel considered that there is no concern for their genotoxicity (Section 3.5.3.2).

The maximum reported amount for the sum of the chlorinated disaccharides was 0.42%. Chlorinated disaccharides share the same basic structure as sucralose but contain two, three or four chlorine atoms at different positions within the molecule. The Panel noted that these potential impurities of sucralose may have been present in the test material used in the toxicity studies available in the database. Consequently, their potential toxicity is expected to have been covered by the studies considered in the safety assessment of sucralose.

Chlorinated monosaccharides, 4-CG and 1,6-DCF, are also degradation products as result of the hydrolysis of sucralose under acidic conditions. Reported concentrations of 1,6-DCF in analysed samples range from 25 to 185 mg/kg, while levels of 4-CG range from 23 to 169 mg/kg. The Panel performed the risk assessment that would result if they were present in sucralose (E 955) at the rounded up highest measured value of 200 mg/kg (Appendix F). When comparing the potential exposure to 4-CG and 1,6-DCF, as impurities, from the use of sucralose (E 955) (Table F.3) with the NOAEL of 10 mg/kg bw per day for 1,6-DCF and of 9 mg/kg bw per day for 4-CG, identified from toxicological studies performed with an equimolar mixture of both substances, the Panel noted that the MOE would be higher than 100 and no safety concern is raised (Appendix F). Since these impurities can be quantified individually, the Panel recommended including individual limits for 4-CG and 1,6-DCF in the specifications of sucralose (E 955) and accordingly to delete the limit for chlorinated monosaccharides.

The Panel also assessed the risk that would result if TPPO was present in E 955 at the existing limit of 150 mg/kg in EU specifications (Appendix F). When comparing the potential exposure to TPPO from the use of sucralose (E 955) (Table F.3) with the NOAEL of 5 mg/kg bw per day, the Panel noted that the MOE would be higher than 100 and no safety concern is raised.

Analytical results for lead, arsenic, cadmium, mercury and tin in commercial samples of sucralose (E 955) were submitted. The Panel noted that among these potential inorganic impurities, only lead has a limit in the EU specifications. Based on the additional data provided for the other toxic elements, as well as the production process involving chemical synthesis followed by purification steps including crystallisation (which minimise systematic contamination), the Panel considered there is no need for additional specification limits for arsenic, cadmium, mercury or tin.

The Panel assessed the risk that would result if lead was present in sucralose (E 955) at: (i) the existing limit in EU specifications; (ii) considering the reported limit of 0.02 mg/kg modulated by the Panel by applying a factor of 10, to allow flexibility with respect to representativeness and homogeneity. Taking into account the calculations performed by the Panel, the fact that the food additive is not the only potential dietary source of lead, and that the maximum limits should be established based on actual levels in the commercial food additive, the Panel recommended lowering the specification limit for lead. If the European Commission decides to revise the current limit in the EU specifications, the estimates of exposure to lead intake could be considered.

Based on the submitted microbiological data and the manufacturing process, the Panel considered microbiological contamination unlikely and considered there is no need to include microbiological criteria in the EU specifications for sucralose (E 955).

Regarding the potential presence of small particles including nanoparticles in sucralose (E 955), the Panel noted that the ultrafiltration step recommended in EFSA Guidance particles-TR (EFSA Scientific Committee, 2021a) to remove small particles was not included in the solubility test provided. However, the reported solubility of sucralose (E 955) in water (> 330 g/L), measured according to European Commission Regulation (EC) No. 440/2008 and OECD TG 105 methods, is substantially higher than the 33.3 g/L threshold requiring additional assessment for the fraction of small particles including nanoparticles. Thus, the Panel considered that sucralose (E 955) is fully dissolved when consumed as a food additive, and the potential presence of small particles, including nanoparticles, does not pose a concern. Sucralose can therefore be assessed following the conventional risk assessment, i.e. EFSA Guidance for submission for food additives evaluations (EFSA ANS Panel, 2012).

Based on the available studies, the Panel noted that sucralose undergoes glycosidic bond cleavage under acidic conditions, degrading into 1,6-DCF and 4-CG. Under alkaline conditions, sucralose undergoes intramolecular dehydrochlorination, with the degradation rate again influenced by pH and temperature. Therefore, sucralose can degrade to 4-CG and

1,6-DCF in flavoured drinks, especially at low pH, high temperatures and long storage times, though reported stability data are inconsistent (see Section 3.1.4). Using a worst-case assumption of 10% degradation, estimated exposure to each degradation product could lead to MOE below 100, which could indicate a concern. However, the actual level of hydrolysis is likely lower and varies by beverage and storage conditions (Appendix F), introducing uncertainty regarding the actual extent of degradation under normal conditions of use and consequently the level of concern.

Regarding thermal stability, model studies conducted at high temperatures (120°C–400°C) and under unrealistic conditions of use (e.g. the amount of sucralose tested, sucralose in oils such as olive oil) reported the formation of PCDDs, PCDFs or chloropropanols. In contrast, cooking studies mimicking realistic baking conditions suggest that the potential formation of 3-MCPD and PCDD/PCDF is low and does not give rise to concern. However, a recent study (Hellwig, 2024) indicates that baking at temperatures above 180°C may result in chlorination of organic compounds present in the food matrix, with the formation of chlorinated dicarbonyl compounds being reported. According to current authorised uses (Table 5), food processing at temperatures above 180°C is only expected for certain food categories (FCs 6.3, 7.2 (only cornets and wafers, for ice-cream or essoblaten – wafer paper), 15.1 and 15.2). In these cases, sucralose is expected to be exposed to high temperatures only for short durations (a few minutes), thereby limiting the potential risk of chlorination.

Although the transfer of chlorine from sucralose to organic molecules under high-temperature conditions, leading to the formation of chlorinated compounds, as suggested by a recent study (Hellwig, 2024, Section 3.1.4), requires further investigation, the uncertainty in the risk associated with the proposed extension of use of sucralose for the whole FC 7.2 'Fine bakery wares' cannot be disregarded unless restrictions on baking temperature and time are applied.

High temperature together with time and quantity of addition of sucralose are critical factors in the formation of thermal degradation products. As these factors cannot be assessed for the preparation of home-made foods using sucralose, a risk associated with the potential formation of chlorinated compounds in such home-made foods prepared by e.g. baking, frying or roasting cannot be excluded.

The biological and toxicological dataset available to the Panel comprised evidence from animal toxicological studies and human data, both published and unpublished, made available to EFSA in response to calls for data and related clarification requests and/or also identified from the published literature. The selection, appraisal and integration of the evidence were performed according to the principles outlined in the revised protocol on hazard identification and characterisation of sweeteners (EFSA, 2020a; EFSA FAF Panel, 2023) and reported in Appendix A.

The kinetics of sucralose was investigated in mice, rats, rabbits, dogs and man. In all species the absorption following oral administration was variable and not more than approximately 20%. Absorption following oral administration in humans varied between 8.8% and 21.7% of the dose. Elimination of systemically available sucralose was mainly by urinary excretion; below 10% of the urinary excreted radioactivity was associated with metabolites with characteristics of glucuronide conjugates. As faecal excretion was observed following intravenous administration in rats and dogs, this indicates that biliary excretion and/or secretion into the gut lumen also occurs. After oral administration, the decrease in both unlabelled and radioactively labelled plasma sucralose showed a fast first phase (within about the first 8 h) and thereafter a second slower phase in rats, dogs and man. The half-lives were 4–6 h in the first phase and 12 h in the second phase, in all species (except for rabbits in which the decay was characterised by a single phase with a half-live of 24 h). Urinary excretion data confirm the existence of two phases in rats, dogs and in humans. The mechanism underlying these two phases and the observed interspecies differences remains unclear. Apart from rabbits, where an accumulation factor of 2 was estimated, no accumulation was observed in rats or in humans. Concerning 1,6-DCF and 4-CG, studies in rats demonstrate extensive metabolism and renal and biliary excretion.

Sucralose and 1,6-DCF gave negative results in in vitro and in vivo assays covering different apical genetic endpoints (gene mutation, clastogenicity and aneugenicity). However, with both compounds a distinct positive response, suggestive of the induction of primary DNA damage, was observed in several tissues in a comet assay in rats. Considering (i) the negative results obtained in mutagenicity studies in vivo on apical genotoxicity endpoints (gene mutation and chromosomal aberrations), also including the same tissues that revealed positive effects in comet assay and (ii) the plausible indirect genotoxic mode of action secondary to induced oxidative stress, the Panel concluded that there is no concern for the genotoxicity of sucralose (E 955). The Panel considered that, based on read-across, the same conclusions apply to impurities and degradation products structurally related to sucralose, including 1,6-DCF for which adequate in vivo mutagenicity data are available, including in tissues tested positive in the comet assay. When sucralose is heated in its dry form in sealed tubes, the formation of chlrorinated-furan-3-one as a thermal degradation product has been reported, however, this compound has not been detected under cooking (baking) conditions (Hellwig, 2024, Section 3.1.4). Although in silico data indicates a potential genotoxicity concern for chlrorinated-furan-3-one, the experimental conditions under which it forms do not reflect food processing. Therefore, this finding is not considered to raise a safety concern.

An evaluation of the risk of bias (RoB) in each relevant study was performed (Annex E), and a Weight of Evidence approach across studies considered of low (tier 1) or moderate (tier 2) RoB was applied for each of the identified health outcome categories (HOCs) for both human and animal studies (Appendix A, Annexes F1 and F2).

Concerning the animal data, the Panel concluded that there is high confidence in the body of evidence that the exposure to sucralose (E 955) is associated with a decrease in body weight which was considered as adverse at doses well above those reported in the human studies. Apart from this, in the absence of treatment-related and/or toxicologically relevant effects on any endpoints investigated, the Panel concluded that sucralose exposure is not associated with adverse haematological changes (high level of confidence), impairment of glucose/insulin homeostasis (high level of confidence), carcinogenicity (high level of confidence), reproductive toxicity (high level of confidence), maternal and developmental

toxicity (high level of confidence), neurotoxicity (high level of confidence) and thyroid toxicity (high level of confidence), additional clinical chemistry (moderate level of confidence), inflammation/immunotoxicity (moderate level of confidence), nephrotoxicity (moderate level of confidence) and liver toxicity (moderate to high level of confidence).

On the basis of the evidence from the included human studies, the Panel concluded that sucralose does not adversely affect kidneys (high level of confidence), the immune system (high level of confidence), the glucose and insulin homeostasis (moderate level of confidence), the cardiovascular system (moderate level of confidence), liver (moderate level of confidence) and that sucralose exposure is not associated with cancer (moderate level of confidence). There is low confidence in the body of evidence, which consists of a single study, that exposure to sucralose (E 955) is associated with altered thyroid function.

Following an integration of the evidence from human and animals data as described in the revised protocol on hazard identification and characterisation of sweeteners (EFSA FAF Panel, 2023), the Panel concluded that it is likely that sucralose (E 955) exposure is associated with a decrease in body weight which was considered as adverse at doses well above those reported in the human studies. The Panel concluded that it is unlikely that exposure to sucralose (E 955) is associated with adverse changes in clinical chemistry parameters, cardiovascular diseases, disturbances of the glucose or insulin homeostasis, liver effects, cancer, neurotoxicity, haematotoxicity, reproductive and developmental effects and thyroid effects in humans. The Panel concluded that it is very unlikely that sucralose exposure is associated with effects on the immune system and on kidneys in humans.

On the basis of the outcome of the WoE, the Panel considered the decrease in body weight observed in rats as the relevant endpoint for the derivation of a reference point and, following the 2014 ANS Panel conceptual framework approach for the re-evaluation of food additives (EFSA ANS Panel, 2014), considered it appropriate to set a numerical ADI. The Panel noted that also in the previous evaluation from the SCF, the pivotal effect on which the ADI was based on was the consistent reduction in body weight gain observed in several rats' studies. The same studies were considered in the current assessment. The Panel's analysis revealed that the effect size depends on two factors, the dose level tested and the duration of the study. The Panel decided to select the study which showed the highest effect size, which was the study with high doses and the longest duration of exposure i.e. the 104-week combined chronic and carcinogenicity study in rats (in contrast to the decision of the SCF which selected the 26-week gavage study as the key study). The Panel used the data from this study to perform a BMD analysis with a BMR that accounts for the contribution of reduced feed intake due to poor palatability of sucralose. A decrease in bodyweight equal or higher than 10% was considered as adverse in earlier assessments (EFSA FAF Panel, 2024b; WHO, 2015). In the case of sucralose, the Panel noted that the observed response is in part driven by poor palatability and consequent reduced feed intake. The Panel estimated, from the results of the pair fed dietary restriction study and a palatability study, that about half of the response (decrease in body weight) could be attributable to reduced palatability of the test diet. The BMR was therefore set at 15% for sucralose. The Panel additionally noted that the variation in the body weight of the control group in the combined chronic and carcinogenicity study (expressed as standard deviation from the mean) at the end of the experiment was larger than 10%. The resulting lowest BMDL was 55 mg/kg bw per day.

The Panel considered the possibility to derive a chemical-specific assessment factor (CSAF) as proposed in the Guidance document of the IPCS (2005) and recommended by EFSA Scientific Commitee (EFSA Scientific Commitee, 2012).

In the weight of evidence analysis, only a reduction of body weight was identified, and the Panel considered that this effect is likely to result from a direct effect on the gastrointestinal tract, noting also the increase in caecum weight in the available rat studies (see Section 3.5.4.1 and Appendix D). Since this effect is not likely to be dependent on the systemic availability of sucralose, no kinetic differences have to be considered and the interspecies and intraspecies kinetic factors could be set both to 1.

Concerning the toxicodynamic subfactors the Panel considered two alternatives (i) to use the default toxicodynamic interspecies factor of 2.5 (ii) to assume that humans are not more sensitive than rats and therefore that the toxicodynamic interspecies factor (2.5) could be reduced to 1. Both the alternatives support the opinion of the Panel that there is no need to revise the current ADI of 15 mg/kg bw per day of sucralose (E 955) previously derived by the SCF (SCF, 2000).

Dietary exposure to sucralose (E 955) was estimated according to different exposure scenarios based on consumers only as described in Section 3.4.1. The Panel noted that the exposure to sucralose (E 955) from its use as a food additive according to Annex II to Regulation (EC) No 1333/2008 was overestimated in all scenarios (see Section 3.4.2). The Panel considered the refined brand-loyal exposure assessment scenario the most appropriate exposure scenario for the risk assessment. In this scenario, among consumers only of at least one food category containing sucralose (E 955), mean exposure to sucralose (E 955) ranged from 0.1 mg/kg bw per day in infants, children, adolescents and elderly to 4.7 mg/kg bw per day in toddlers. The 95th percentile of exposure ranged from 0.4 mg/kg bw per day in infants to 14.3 mg/kg bw per day in toddlers. The Panel noted that none of the exposure estimates (mean and P95 in any population groups) in this scenario exceeded the ADI of 15 mg/kg bw per day for sucralose (E 955).

In addition, the Panel considered a proposed extension of use of sucralose (E 955) in FC 07.2 'Fine bakery wares' (no added sugar or energy-reduced products) at a maximum level of 700 mg/kg. The overall exposure did not increase substantially when considering this proposed extension of use. Only in the refined maximum level scenario the highest P95 exposure of 16 mg/kg bw per day in toddlers exceeded the ADI. However, the uncertainty regarding the potential transfer of chlorine from sucralose to organic molecules under high-temperature conditions is of concern for the proposed extension of use in FC 7.2. This food category includes a wide range of baking processes that may involve high temperatures and variable baking times, depending on the specific food application. Without additional data covering all relevant processing

conditions, the Panel cannot conclude on the safety of the proposed extension of use of E 955 in energy-reduced or no added sugar fine bakery wares (FC 7.2).

4 | UNCERTAINTY

The uncertainties, and the direction of the uncertainty, related to the exposure assessments are summarised in Table 8 of Section 3.4.2. Overall, the Panel considered the exposure to sucralose (E 955) from its use as food additive to be overestimated by all exposure assessment scenarios.

Concerning genotoxicity, the Panel noted that there is uncertainty on the mechanism responsible for the positive response obtained with both sucralose and 1,6-DCF in in vivo comet assays in rats. Although several lines of evidence indicate sucralose-induced oxidative stress as a plausible indirect mechanism for DNA damage, there is no definitive proof of its involvement in the observed results. However, the Panel noted that there was no evidence of mutagenicity or other adverse effects related to genotoxicity, such as carcinogenicity, observed in all other studies, also covering the same tissues tested positive in comet assays in vivo. Therefore, the Panel considered it unlikely that this uncertainty would lead to an underestimation of risk.

The actual extent of hydrolysis of sucralose in beverages remains uncertain, as it varies with storage conditions and therefore the level of concern is also uncertain. However, a long-term consumption of a beverage with the specific pH and prolonged high-temperature storage conditions required to induce hydrolysis is considered an unlikely scenario. For this reason, the Panel considered it unlikely that this uncertainty would lead to an underestimation of risk.

Uncertainty also exists regarding the potential transfer of chlorine from sucralose to organic molecules under high-temperature conditions, as suggested in a recent study. These conditions, however, do not reflect the currently authorised uses of sucralose. The Panel, therefore, considered it unlikely that this uncertainty would lead to an underestimation of risk.

However, there are uncertainties regarding the potential transfer of chlorine from sucralose to organic molecules under high-temperature conditions that could result from the use of sucralose by consumers for the preparation of home-made foods via heating processes, such as baking, frying and roasting.

For the proposed extension of use of sucralose, the uncertainty cannot be dismissed unless restrictions on baking temperature and time are applied to the different potential uses under FC 7.2.

For the BMD modelling the Panel had to select a BMR for decrease in body weight. The Panel noted that a BMR of 10% is usually considered adequate for this endpoint. In the case of sucralose, the Panel had to take into consideration that the observed effect size is in part driven by poor palatability of sucralose and related reduced feed intake, which contribution was estimated from the results of the pair fed dietary restriction study to account for about half. The Panel was therefore of the view that considering the data available, the BMR should be set at 15%. The selection of a BMR is depending on the amount of available informative data. However, when based on substance- and scenario-specific information, the uncertainty is smaller than using a fixed default value, calculated according to the Guidance on the use of the benchmark dose approach in risk assessment (EFSA Scientific Committee, 2022). An uncertainty remains in this case, because the information is obtained from two studies with a shorter duration than the study from which the data for the BMD modelling was taken. Selecting a higher BMR would result in higher BMDLs with the same uncertainty, so that the Panel considered a conservative approach when selecting a BMR of 15% (see also Appendix G). Therefore, the Panel considered it unlikely that this uncertainty would lead to an underestimation of risk.

5 | CONCLUSIONS

Taking into account the available dataset, the Panel concluded that there is no need to change the current ADI of 15 mg/kg bw per day of sucralose (E 955).

The exposure estimates at the mean and P95 in all population groups for all scenarios considering the currently authorised uses did not exceed the ADI of 15 mg/kg bw per day for sucralose (E 955). Therefore, the Panel concluded that there is no safety concern at the reported uses and use levels for sucralose (E 955) as a food additive.

Based on the available data and the identified uncertainties regarding the potential formation of chlorinated compounds under the wide range of baking processes that may be applicable for FC 7.2 'Fine bakery wares', the Panel could not conclude on the safety of the proposed extension of use of E 955 in this FC.

6 | RECOMMENDATIONS

The Panel recommended the European Commission to consider:

- including individual limits for 4-CG and 1,6-DCF in the specifications of sucralose (E 955) and accordingly to delete the limit for chlorinated monosaccharides
- lowering the limit of lead in the EU specifications.
- including the CAS number 56038-13-2 in the EU specifications.

- the issue of potential formation of unwanted degradation products during the preparation of home-made products using sucralose during domestic uses that require high temperature such as frying and baking.

7 | DOCUMENTATION AS PROVIDED TO EFSA

1. International Sweeteners Association (ISA). Submission of data in response to the EFSA call for technical and toxicological data on sweeteners authorised as food additives in the EU (EFSA-Q-2017-00500). Submitted on 21 June 2018.
2. International Sweeteners Association (ISA). Additional information submitted in response to a request from EFSA. Submitted on 23 March 2021.
3. Calorie Control Council (CCC). Submission of data in response to the EFSA call for technical data on sucralose (E 955) (EFSA-Q-2011-00724). Submitted on 4 April 2022.
4. International Sweeteners Association (ISA). Submission of data response to the EFSA call for technical data on sweeteners (EFSA-Q-2019-00318). Submitted on 5 December 2019–20 March 2020.
5. International Sweeteners Association (ISA). Additional information submitted in response to a request from EFSA. Submitted on 26 July 2021.
6. International Sweeteners Association (ISA). Additional information submitted in response to a request from EFSA. Submitted on 15 May 2024.
7. International Sweeteners Association (ISA). Clarification sent via email on the 11 June 2024.
8. Calorie Control Council (CCC). Additional information submitted in response to a request from EFSA. Submitted on July 2024.
9. International Sweeteners Association (ISA). Additional information submitted in response to a request from EFSA. Submitted on 18 April 2024.
10. International Sweeteners Association (ISA). Additional information submitted in response to a request from EFSA. Submitted on 21 January 2022.
11. International Sweeteners Association (ISA). Additional information submitted in response to a request from EFSA. Submitted on 25 February 2022.
12. International Sweeteners Association (ISA). Additional information submitted in response to a request from EFSA. Submitted on 6 January 2023.
13. International Sweeteners Association (ISA). Spontaneous submission of data. Submitted on 15 December 2020.
14. International Sweeteners Association (ISA). Spontaneous submission of data. Submitted on 2 October 2025.
15. International Sweeteners Association (ISA). Submission of data response to the EFSA call for technical data on sucralose (E 955) (EFSA-Q-2011-00724). Submitted on 18 February 2022.
16. Kanbo. Submission of data response to the EFSA call for technical data on sweeteners. Submitted on 1 March 2019.
17. Sweetness Technologies. Submission of data response to the EFSA call for technical data on sweeteners. Submitted on 30 July 2019.
18. International Sweeteners Association (ISA). Spontaneous submission of data. Submitted on 28 January 2020.
19. Association of the European Self-Medication Industry. Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 1 October 2018.
20. Association of Italian Sweets and Pasta Industries. Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 28 September 2018.
21. European Fruit Juice Association. Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 1 October and 23 November 2018.
22. BioGaia. Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 20 November 2018.
23. Cloetta Suomi Oy. Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 1 October 2018.
24. European Dairy Association (EDA). Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 28 September 2018.
25. Food Drink Europe. Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 26 September and 19 October 2018.
26. Food Supplement Europe. Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 10 July 2019.
27. International Chewing Gum Association. Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 1 October 2018.
28. International Sweeteners Association (ISA). Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 28 September 2018.

29. Dr. Loges Naturheilkunde neu entdecken. Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 16 April 2018.
30. Produlce. Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 30 September 2018.
31. Specialised Nutrition Europe. Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 28 September 2018.
32. Total Diet & Meal Replacements Europe. Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 1 October 2018.
33. Susan Schiffmann. Submission of data response to the EFSA call for data on genotoxicity data on sweeteners. Submitted on 22 July 2022 (draft report) and complemented on 17 August 2022.
34. Susan Schiffmann. Spontaneous submission- final manuscript of the publication 'Toxicological and Pharmacokinetic Properties of Sucralose-6-Acetate and Its Parent Sucralose: In Vitro Screening Assays' (Schiffmann et al., 2023, see Reference list).
35. International Sweeteners Association (ISA). Submission of data and information response to the EFSA call for data on genotoxicity data on sweeteners. Submitted on 20 October 2022, 24 February 2023 and 29 January 2024.
36. International Sweeteners Association (ISA). Submission of data response to the EFSA call for data on genotoxicity data on sweeteners: final reports of the Ames test with sucralose and 1,6-DCF and of the ToxTracker with sucralose and 1,6-DCF and statement of the vitro mammalian MN assay in human lymphocytes on sucralose and 1,6-DCF. Submitted on 12 May 2023.
37. International Sweeteners Association (ISA). Submission of data response to the EFSA call for data on genotoxicity data on sweeteners: review of toxicokinetic data. Submitted on 6 July 2023.
38. International Sweeteners Association (ISA). Submission of data response to the EFSA call for data on genotoxicity data on sweeteners: final report of the in vitro mammalian MN assay in human lymphocytes on sucralose. Submitted on 27 July 2023.
39. International Sweeteners Association (ISA). Submission of data response to the EFSA call for data on genotoxicity data on sweeteners: final report of the in vitro mammalian MN assay in human lymphocytes on 1,6-DCF. Submitted on 24 August 2023.
40. International Sweeteners Association (ISA). Submission of data response to the EFSA call for data on genotoxicity data on sweeteners: final reports of the Comet assay on sucralose and 1,6 DCF and draft report of the transgenic gene mutation assay in MutaTM mice on sucralose and expert review of the genotoxicity studies. Submitted on 29 September 2023.
41. International Sweeteners Association (ISA). Submission of data response to the EFSA call for data on genotoxicity data on sweeteners: final report of the transgenic gene mutation assay in MutaTM mice on sucralose. Submitted on 1 December 2023.
42. International Sweeteners Association (ISA). Submission of data response to the EFSA call for data on genotoxicity data on sweeteners: final report of the transgenic gene mutation assay in MutaTM mice on 1,6-DCF and of the rat bone marrow micronucleus assay on sucralose and 1,6-DCF and expert review of the genotoxicity studies. Submitted on 25 March 2024.
43. International Sweeteners Association (ISA). Submission of data response to the EFSA call for data on genotoxicity data on sweeteners: amended final report of the transgenic gene mutation assay in MutaTM mice on 1,6-DCF. Submitted on 12 April 2024.
44. Calorie Control Council (CCC). Submission of data response to the EFSA call for data on genotoxicity data on sweeteners. Submitted on 4 April 2022.
45. Application for an extension of use for sucralose (E 955) in energy-reduced or without added sugar fine bakery wares. Technical Dossier. Zùsto; submitted on 25 September 2017 by Aegis Holding NV.

ABBREVIATIONS

1,2-DCP	1,2-dichloropropanols
1,3-DCP	1,3-dichloropropanols
1,6-DCF	1,6-dichloro-1,6-dideoxy-β-D-fructofuranose
3-MCPD	3-monochloropropandiol
4-CG	4-chloro-4-deoxy-α-D-galactopyranose
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, excretion
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
AOAC	Association of Official Analytical Collaboration
AOCS	American Oil Chemists' Society
As	arsenic
AUC	area under the curve
BAM	Bacteriological Analytical Manual
BfR	German Federal Institute for Risk Assessment
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit

BMDU	benchmark dose upper confidence limit
BMR	benchmark response
Bw	bodyweight
Cd	cadmium
CFU	colony forming units
CLEA	cross-linked enzyme aggregate
C_{max}	peak plasma concentration
CONTAM	Scientific Panel on Contaminants in the Food Chain
dl-PCB	dioxin-like polychlorinated biphenyls
DSC/ TGA-FTIR	differential scanning calorimetry/thermogravimetric analysis coupled with Fourier transform infrared spectroscopy
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
FAF	EFSA Panel on Food Additives and Flavourings
FC	food category
FCE	Food conversion efficiency
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FTase	fructosyltransferase
FTIR	Fourier transform infrared
GC–MS/MS	gas chromatography–tandem mass spectrometry
GD	gestational days
GNPD	Global New Products Database
Hg	mercury
HPLC	high-performance liquid chromatography
HPLC/ESI–MS	high-performance liquid chromatography with electrospray ionisation mass spectrometry detection
HPLC–ELSD	high-performance liquid chromatography with evaporative light scattering detection
HPLC–RID	high-performance liquid chromatography with refractive index detection
HPLC–UV	high-performance liquid chromatography with ultraviolet detection
HPTLC	high-performance thin layer chromatography
HRCG–HRMS	High-resolution gas chromatography and high-resolution mass spectrometry
HSGC–FID	headspace gas chromatography with flame ionisation detection
HSGC–MS	headspace gas chromatography–mass spectrometry
HSM	hot stage microscopy
IBO	interested business operator
ICP–MS	Inductively Coupled Plasma-Mass Spectrometry
IQ	intelligence quotient
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC/TOF–MS	liquid chromatography/time-of-flight mass spectrometry
LC–MS	liquid chromatography–mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MOE	Margin of exposure
MOS	Margin of Safety
MPL(s)	maximum permitted level(s)
NMR	nuclear magnetic resonance
NMT	not more than
NOAEL	no observed adverse effect level
NTP	US National Toxicology Program
OECD TG	Organisation for Economic Co-operation and Development Test Guideline
OM	Official Method
P95	95th percentile
PAH	polycyclic aromatic hydrocarbons
Pb	lead
PCDD	polychlorinated dibenzodioxins
PCDF	polychlorinated dibenzofurans
PCN	polychlorinated naphthalenes
Py-GC/MS	pyrolysis-gas chromatography–mass spectrometry
QS	*quantum satis*
RI	refractive index
RoB	Risk of bias
RP	reference point
RP-HPLC/RID	reverse phase high-performance liquid chromatography with refractive index detection

RP-HPLC/UV	reverse phase high-performance liquid chromatography with ultraviolet detection
RP-UHPLC/MS	reverse phase ultra high-performance liquid chromatography–mass spectrometry
SC	Scientific Committee
SCF	Scientific Committee on Food
SD	standard deviation
SEM	scanning electron microscopy
SPE	solid-phase extraction
TD	toxicodynamic
TDI	tolerable daily intake
TEF	toxicity equivalency factor
TEQ	toxic equivalent
TG-DTA	thermogravimetry and differential thermal analysis
TK	toxicokinetic
TLC	thin layer chromatography
T_{max}	time to reach the peak plasma concentration
TPPO	triphenylphosphine oxide
TWI	tolerable weekly intake
UB	upper bound
UHT	ultra-high temperature
USP-NF	United States Pharmacopeia–National Formulary
UV	ultraviolet
WHO	World Health Organization
WHO-TEF	World Health Organization-Toxic Equivalency Factor
WHO-TEQ	World Health Organization-toxic equivalent

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APPENDIX A

Tailored protocol for the hazard identification and characterisation of sucralose (E 955)

The assessment of sucralose (E 955) followed the protocol on hazard identification and characterisation of sweeteners (EFSA, 2020a; EFSA FAF Panel, 2023). For details on the hazard identification and characterisation, please refer to steps 1.11–1.15.

The decisions specific to the assessment of sucralose (E 955) are outlined below.

A.1 | Extensive literature search – sucralose (E 955) and its degradation products

Methodology

For step 1.11, open-ended literature searches were conducted in the three selected databases with the search strings and criteria applied as listed below. The last update of the sucralose searches was performed on March 04, 2025; the search included relevant literature published since the previous evaluation by the SCF (SCF, 2000), allowing 1 year of overlap.

PubMed

Sucralose

Set	Query	Results	Notes
#1	"trichlorosucrose" [Supplementary Concept] OR sucralose[tiab] OR trichlorosucrose[tiab] OR E955[tiab] OR "E 955"[tiab] OR "56038–13-2"[tiab]	1247	Sucralose
#2	("1999"[Date - Publication]: "3000"[Date - Publication])	25,391,574	Published from 1999 and in English
#3	#1 AND #2	1191	Sucralose Published from 1999 and in English

1,6-DCF and 4-CG

Set	Query	Results	Notes
#1	78508–21-1[tiab] OR "1 6 DCF"[tiab:~1] OR "1 6DCF"[tiab:~1] OR 16DC[tiab] OR ((dichloro[tiab] OR "di chloro"[tiab]) AND dideoxy[tiab] AND fructo*[tiab]) OR (("di chloro"[tiab] OR dichloro[tiab]) AND dideoxyfructose[tiab])	20	1,6-DCF
#2	848642–13-7[tiab] OR "4 CG"[ti] OR 4CG[ti] OR (("4-CG"[tiab] OR 4CG[tiab]) AND (chloro[tiab] OR deoxy[tiab] OR galactopyranose[tiab] OR "galacto pyranose"[tiab:~3] OR deoxyglactose[tiab] OR sweetener*[tiab] OR impur*[tiab] OR hydrolysis[tiab] OR hydrolyses[tiab] OR metabolite*[tiab] OR sucralose[tiab])) OR (chloro[tiab] AND deoxy[tiab] AND (galactopyranose[tiab] OR "galacto pyranose"[tiab:~3])) OR (chloro[tiab] AND deoxygalactose[tiab]) OR (chloro[tiab] AND "deoxy galactose"[tiab])	11	4-CG
#3	#1 OR #2	29	1,6-DFC OR 4-CG
#4	#3 Filters: English, from 1999 to 2025	19	Published from 1999 and English

Chlorinated furan 3-one

Set	Query	Results
#1	Chlorin* [Title/Abstract] AND "furan*"[Title/Abstract] AND "3 one"[Title/Abstract]	2
#2	("chlorine furan"[Title/Abstract:~3] OR "chlorinated furan"[Title/Abstract:~3] OR "chlorine furans"[Title/Abstract:~3] OR "chlorinated furans"[Title/Abstract:~3])	106
	#1 OR #2 Filters: English, from 1999 to 2025	79

Web of Science (SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC)

Sucralose

Set	Query	Results	Notes
#1	TS = (sucralose OR E955 OR "E 955" OR "56038–13-2" OR trichlorosucrose)	1936	Sucralose
#2	PY = (1999–2100)	65,942,543	Published from 1999 and in English
#2	#1 AND #2 and English (Languages)	1806	Sucralose Published from 1999 and in English

1,6-DCF and 4-CG

Set	Query	Results	Notes
#1	TS = (78508–21-1 OR "1 6 DCF" OR "1 6DCF" OR 16DC OR ((dichloro OR "di chloro") AND dideoxy AND fructo*) OR ((dichloro OR "di chloro") AND dideoxyfructose))	28	1,6-DCF
#2	TS = (848642–13-7 OR (("4-CG" OR 4CG) AND (chloro OR deoxy OR galactopyranose OR "galacto pyranose" OR deoxyglactose OR sweetener* OR impur* OR hydrolysis OR hydrolyses OR metabolite* OR sucralose)) OR (chloro AND deoxy AND (galactopyranose OR "galacto pyranose")) OR (chloro AND deoxygalactose) OR (chloro AND "deoxy galactose")) OR TI = ("4-CG" OR 4CG)	23	4-CG
#2	#1 OR #2	48	1,6-DFC OR 4-CG
#4	#3 AND PY = (1999–2100) and English (Languages)	23	Published from 1999 and English

Chlorinated furan 3-one

Set	Search query	Results
1	TS = (chlorin* NEAR/2 furan*)	269
2	TS = ("chlorin*" AND "furan*" AND "3 one")	4
6	#1 OR #2 AND LA = (English)Timespan: 1999-01-01 to 2025-03-04 and Article or Proceeding Paper or Review Article (Document Types)	123

Scifinder-n

Sucralose

Search	Query
56038–13-2 - > References Filter by: Document type: journal, review, clinical trial, commentary, preprint, report Language: English Publication year: 1999–2025	2147

1,6-DCF and 4-CG

Search	Query
78508–21-1- > References Filter by: Document type: journal *Language*: English Publication year: 1999–2025	1

Search	Query
848642–13-7 - > References Filter by: Document type: journal *Language*: English Publication year: 1999–2025	5

Chlorinated furan 3-one

Set	Query	Results
#1	Title: "chlorinated furan 3-one" OR "chlorinated furan 3 one" OR Abstract/Keywords: "chlorinated furan 3-one" OR "chlorinated furan 3 one"	1
#2	Chlorin* AND furan AND "3-one" in Title	17
#3	Chlorin* AND furan AND "3-one" in Abstract	0
#4	Chlorin* AND furan* AND "3-one" in All fields	7
5	#1 OR #2 OR #4	24
#6	#5 AND 1999–2025 AND English AND publication type	13

Results

An overview of the results of the literature search for sucralose and its impurities and/or degradation products is presented in Figure A.1.

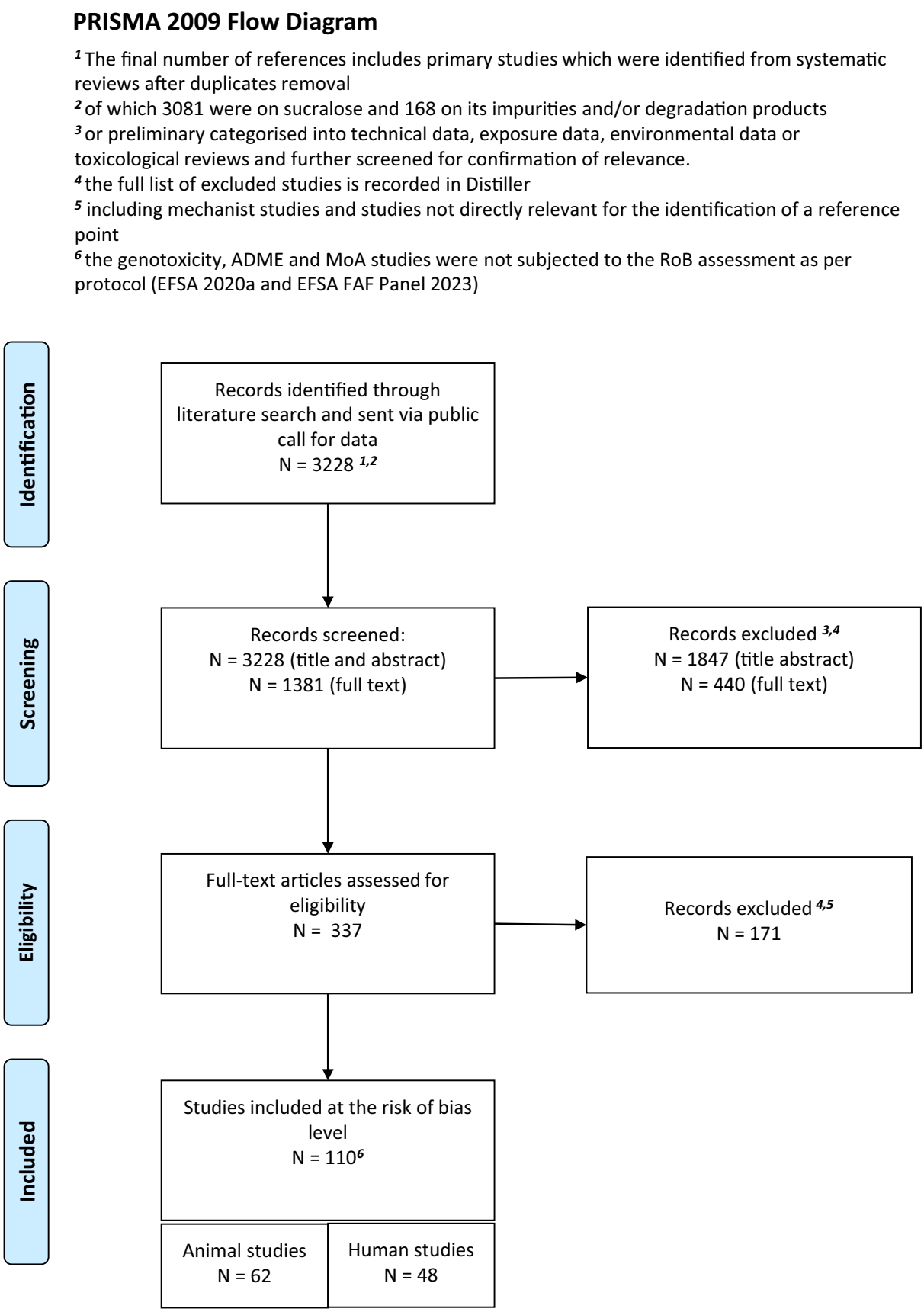


FIGURE A.1 PRISMA flow chart (adapted from Moher et al., 2009).

A.2 | Evaluation of the risk of bias (RoB)

Methodology

For step 1.12, the criteria outlined in the revised protocol for the risk of bias evaluation of studies have been applied (EFSA FAF Panel, 2023), including the rules for tier allocation.

The studies evaluated for the RoB were allocated to a tier (from 1 to 3 corresponding to decreasing levels of internal validity) based on the rules as reported in step 1.12 of the revised protocol.

The evaluation of the RoB was conducted in parallel independently by two reviewers and in case a conflict on tier allocation of a study was identified, ad-hoc discussion between the reviewers took place prior to a final agreed tier allocation that was reached by consensus. Any potential conflict between reviewers' assessments is being addressed solely at the tier level, rather than at the level of individual scoring.

The tier was automatically generated by the DistillerSR tool, after input of the ratings for the individual elements of the study considered for the RoB. At the end of the evaluation, the reviewers were requested to express their agreement or disagreement on the tier generated by the tool based on their expert judgement. In case of disagreement, a justification is to be provided.

As reported in the revised protocol (EFSA FAF Panel, 2023), studies on which the derivation of the ADI was based or studies used for identification of a NOAEL/no observed effect level (NOEL) (in case of ADI not specified) were included and evaluated according to the protocol together with any relevant literature published since the previous evaluation by the SCF, allowing 1 year of overlap. If studies that were previously considered as critical were evaluated as having moderate (Tier 2) or low risk of bias (Tier 1), other non-critical studies from previous assessments, including those received by the interested business operators, would not have to be re-evaluated and subjected to the different steps of the protocol (e.g. RoB, WoE).

In addition, the Panel considered it relevant to include in the assessment a palatability study (Documentation provided to EFSA No 1; see Appendix G for a short summary). This study was already assessed by the SCF (2000) but was considered relevant for inclusion since it provides complementary information to the pair fed dietary restriction study (Documentation provided to EFSA No 1) with respect to the palatability of sucralose.

Results

Relevant studies retrieved from the literature, both in humans and in animals, were considered and evaluated for the RoB together with the critical studies above-mentioned. The results of this assessment are reported in Annex E. Disagreements with the tier generated by the tool were identified by the reviewers and the tier was adjusted based on expert judgement, including the reasoning behind.

27 animal studies and 37 human studies were evaluated as having moderate (tier 2) or low risk of bias (tier 1) and therefore considered further in the assessment, while those having a high RoB (tier 3) were not included.

A.3 | Data extraction

Methodology

For step 1.13, information and data from the assessed unpublished animals studies as well as from the included genotoxicity studies were extracted and reported in tabular form. The data extraction forms outlined in the revised protocol (EFSA FAF Panel, 2023) were used.

Results

The data extraction forms of the studies are reported in Annex C and Annex D of the current opinion.

A.4 | Weighing the body of evidence and synthesis of the evidence

Methodology

For step 1.14 (Weighing the body of evidence), a WoE analysis for different health outcome categories, grouped by endpoint as appropriate, was performed on eligible animal and human studies. The WoE analysis was performed using Excel tables. The detailed methodology is reported in the revised protocol (EFSA FAF Panel, 2023).

Results

The result of the WoE analysis for sucralose (E 955) is presented in Annex F1 and F2 and summarised in Section 3.5.4 on 'Synthesis of systematically appraised evidence and weight of evidence'.

A.5 | Integration of animal and human evidence

Methodology

The integration of animal and human evidence was performed following the Figure A.2.

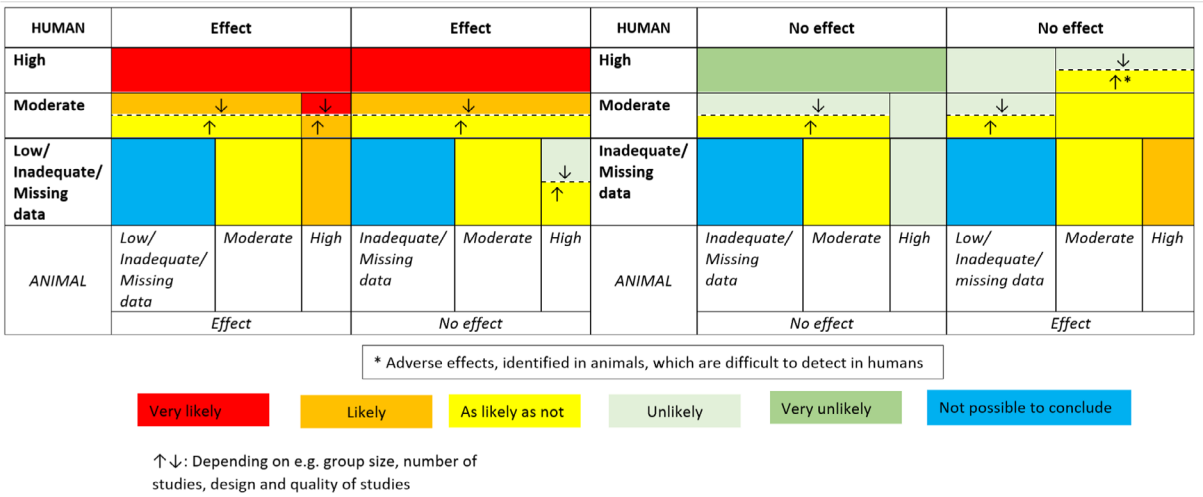


FIGURE A.2 Matrix for integration of animal and human body of evidence based on identification of effect and level of evidence.

The integration, as reported in the above-mentioned figure, was complemented by additional considerations from the Panel on the biological plausibility of each effect and MoA considerations. Consideration was given to the conclusions of previous evaluations (SCF, 2000) to assess whether the new data support them. The results of the integration are reported in Section 3.5.4.3.

APPENDIX B

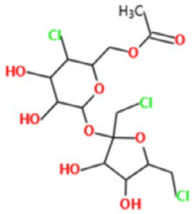
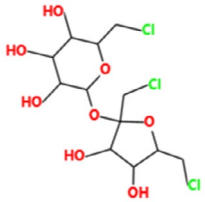
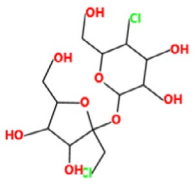
In silico assessment of sucralose (E 955) impurities/degradation products

The genotoxicity and Cramer class of the impurities/degradation products of sucralose (E 955) were evaluated using in silico tools. A suite of VEGA statistical models, and the rule-based OECD QSAR Toolbox profilers were interrogated. For comparative purpose, also sucralose (Compound 12) and 1,6-DCF (Compound 7) were included in the set of analysed compounds.

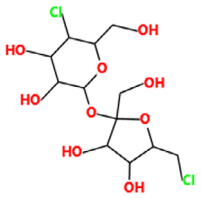
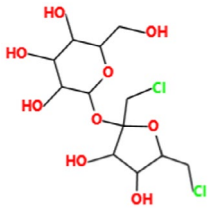
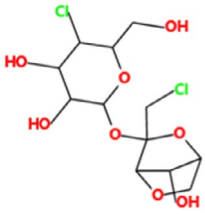
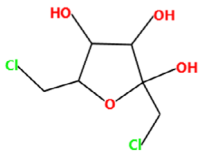
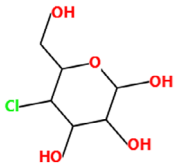
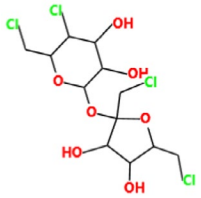
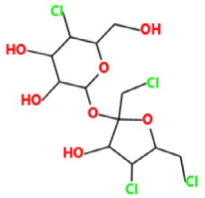
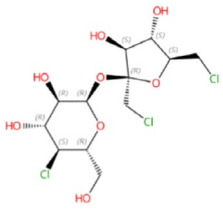
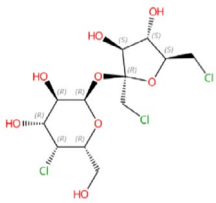
B.1 | Name and code of analysed compounds

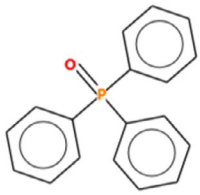
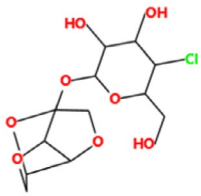
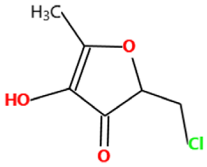
- Compound 1 (Impurity A) - 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl 6-O-acetyl-4-chloro-4-deoxy-α-D-galactopyranoside (6-O-acetylsucralose)(CAS 105066-21-5)
- Molecule 2 (Impurity B) - 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl 6-chloro-6-deoxy-α-D-glucopyranoside (1′,6,6′-trichloro-1′,6,6′-trideoxysucrose) (CAS 40631-75-2)
- Compound 3 (Impurity D) - 1-chloro-1-deoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside (1′,4-dichloro-1′,4-dideoxygalactosucrose) (CAS 64644-65-1)
- Compound 4 (Impurity E) - 6-chloro-6-deoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside (4,6′-dichloro-4,6′-dideoxygalactosucrose) (CAS 55832-24-1)
- Compound 5 (Impurity F) - 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl α-D-glucopyranoside (1′,6′-dichloro-1′,6′-dideoxysucrose) (CAS 61854-83-9)
- Compound 6 (Impurity G) - 3,6-anhydro-1-chloro-1-deoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside (3′,6′-anhydro-1′,4-dichloro-1′,4-dideoxygalactosucrose) (CAS 105066-20-4)
- Compound 7 (Impurity H, also degradation product) - 1,6-dichloro-1,6-dideoxy-β-D-fructofuranose (1,6-DCF) (CAS 78508-21-1)
- Compound 8 (Impurity I) - 4-chloro-4-deoxy-α-D-galactopyranose (4-CG) (CAS 848642-13-7)
- Compound 9–4,6,1′,6′ tetrachlorogalactosucrose (CAS 59343–74-7)
- Compound 10–4,1′,4′,6′-tetrachlorogalactosucrose (CAS 82950–43-4)
- Compound 11–4,1′,6′-trichlorosucrose[69] (CAS 69414-04-6)
- Compound 12 -sucralose (CAS 56038-13-2)
- Compound 13 -triphenylphosphine oxide (CAS 791-28-6)
- Compound 14 (degradation product resulting from the loss of two HCl molecules)
- Compound 15 – chlorinated furan-3-one

B.2 | Chemical structures

No	Name	CAS	Canonical SMILES	Structure
1	Impurity A - 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl 6-O-acetyl-4-chloro-4-deoxy-α-D-galactopyranoside (6-O-acetylsucralose) (sucralose-6-acetate)	105066-21-5	O=C(OCC1OC(OC2(OC(CCl)C(O)C2O)CCl)C(O)C(O)C1Cl)C	
2	Impurity B - 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl 6-chloro-6-deoxy-α-D-glucopyranoside (1′,6,6′-trichloro-1′,6,6′-trideoxysucrose)	40631-75-2	ClCC1OC(OC2(OC(CCl)C(O)C2O)CCl)C(O)C(O)C1O	
3	Impurity D - 1-chloro-1-deoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside (1′,4-dichloro-1′,4-dideoxygalactosucrose)	64644-65-1	ClCC1(OC2OC(CO)C(Cl)C(O)C2O)OC(CO)C(O)C1O	

[69]The difference between this impurity and sucralose is the configuration at the 4-position; the chlorine is oriented 'down' instead of 'up,' as in sucralose.

No	Name	CAS	Canonical SMILES	Structure
4	Impurity E - 6-chloro-6-deoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside	55832-24-1	ClCC1OC(OC2OC(CO)C(Cl)C(O)C2O)(CO)C(O)C1O	
5	Impurity F - 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl α-D-glucopyranoside (1′,6′-dichloro-1′,6′-dideoxysucrose)	61854-83-9	ClCC1OC(OC2OC(CO)C(O)C(O)C2O)(CCl)C(O)C1O	
6	Impurity G - 3,6-anhydro-1-chloro-1-deoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside (3′,6′-anhydro-1′,4-dichloro-1′,4-dideoxygalactosucrose)	105066-20-4	ClCC1(OC2OC(CO)C(Cl)C(O)C2O)OC3COC1C3O	
7	Impurity H[b] - 1,6-dichloro-1,6-dideoxy-β-D-fructofuranose (1,6-DCF)	78508-21-1	ClCC1OC(O)(CCl)C(O)C1O	
8	Impurity I - 4-chloro-4-deoxy-α-D-galactopyranose (4-CG)	848642-13-7	ClC1C(O)C(O)C(O)OC1CO	
9	4,6,1′,6′-tetrachlorogalactosucrose	59343-74-7	ClCC1OC(OC2(OC(CCl)C(O)C2O)CCl)C(O)C(O)C1Cl	
10	4,1′,4′,6′-tetrachlorogalactosucrose	82950-43-4	ClCC1OC(OC2OC(CO)C(Cl)C(O)C2O)(CCl)C(O)C1Cl	
11	4,1′,6′-trichlorosucrose	69414-04-6	ClCC1OC(OC2OC(CO)C(Cl)C(O)C2O)(CCl)C(O)C1O	
12	**Sucralose**	**56038-13-2**	**ClCC1OC(OC2OC(CO)C(Cl)C(O)C2O)(CCl)C(O)C1O**	

No	Name	CAS	Canonical SMILES	Structure
13	Triphenylphosphine oxide	791-28-6	O=P(C=1C=CC=CC1)(C=2C=CC=CC2)C=3C=CC=CC3	
14	Degradation product resulting from the loss of two HCl molecules	–	OCC4OC(OC23COC1C(COC12)O3)C(O)C(O)C4Cl	
15	Chlorinated furan-3-one	–	CC1OC(CCl)C(=O)C=1O	

B.3 | Summary of results

The results obtained using the VEGA predictions models are reported in Table B.1 and the results from the OECD QSAR toolbox are reported in Table B.2.

TABLE B.1 VEGA Models predictions.

Compound n. M/M	1	2	3	4	5	6	7 (1,6) DCF	8
Ames consensus 1.0.4	*M (0.15)*	*M (0.15)*	*M (0.15)*	*M (0.15)*	*M (0.15)*	*M (0.15)*	**M (0.6)**	*NM (0.3)*
Ames CAESAR 2.1.14	*SM/L*[1,2,3,4]	*SM/L*[1,2,3.4]	*SM/L*[1,2,3.4]	*SM/L*[1,2,3.4]	*SM/L*[1,2,3,4]	*SM/L*[1,2,3,4]	SM/M[2,3,4]	*M/L*[1,2,3]
Ames ISS 1.0.3	*M/L*[2,3,4]	*M/L*[2,3,4]	*M/L*[2,4]	*M/L*[2,4]	*M/L*[2,4]	*M/L*[2,3,4]	M/M[4]	NM/M[2,3]
Ames SarPy-IRFMN 1.0.8	*M/L*[1,2,3]	*M/L*[1,2,3]	*M/L*[1,2,3]	*M/L*[1,2,3]	*M/L*[1,2,3]	*M/L*[1,2,3]	M/M[1,2,3]	M/M[2,3]
Ames KNN-Read-across 1.0.1	*NM/L*[1,2]	*NM/L*[1,2]	*NM/L*[1,2]	*NM/L*[1,2]	*NM/L*[1,2]	*NM/L*[1,2]	M/M[1,2,3]	NM/M[1,2]
CAs CORAL 1.0.1	A/M[3]	A/M[3]	A/M[3]	A/M[3]	A/M[3]	A/M[3]	*A/L*[2,3]	I/M
In vitro MN IRFMN-VEERMER 1.0.1	*A/L*[2,3]	*A/L*[2,3]	*A/L*[2,3]	*A/L*[2,3]	*A/L*[2,3]	*A/L*[2,3]	A/M[2,3]	**A/G**[3]
In vivo MN IRFMN 1.0.2	*NP*[6]	*NP*[6]	*N6*[5]	*N6*[5]	*N6*[5]	*NP*[6]	*NP*[6]	*NP*[6]
Cramer TOXTREE 1.0.1	*Class III*	*Class III*	*Class III*	*Class III*	*Class III*	*Class III*	*Class III*	*Class III*

Compound n. M/M	9	10	11	12 sucralose	13	14	15
Ames consensus 1.0.4	*M (0.15)*	*M (0.15)*	*M (0.15)*	*M (0.15)*	*NM (0.15)*	*M (0.3)*	**M (0.75)**
Ames CAESAR 2.1.14	*SM/L*[1,2,3,4]	*SM/L*[1,2,3,4]	*SM/L*[1,2,3,4]	*SM/L*[1,2,3,4]	*NM/L*[2,3]	M/M[2,3]	**M/G**[4]
Ames ISS 1.0.3	*M/L*[2,3,4]	*M/L*[2,3,4]	*M/L*[2,3,4]	*M/L*[2,3,4]	*NM/L*[3]	NM/M[2,3]	M/M[4,5]
Ames SarPy-IRFMN 1.0.8	*M/L*[1,2,3]	*M/L*[1,2,3]	*M/L*[1,2,3]	*M/L*[1,2,3]	*NM/L*[2]	M/M[1,2,3]	M/M[1]
Ames KNN-Read-across 1.0.1	*NM/L*[1,2]	*NM/L*[1,2]	*NM/L*[1,2]	*NM/L*[1,2]	*M/L*[1,2]	NM/L[1,2]	**M/G**
CAs CORAL 1.0.1	A/M[3]	A/M[3]	A/M[3]	A/M[3]	*I/L*[3]	A/M[3]	**A/G**
In vitro MN IRFMN-VEERMER 1.0.1	*A/L*[2,3]	*A/L*[2,3]	*A/L*[2,3]	*A/L*[2,3]	*NP/L*[1,2,3]	*A/L*[2,3]	A/M[1,2]
In vivo MN IRFMN 1.0.2	*NP*[6]	*NP*[6]	*NP*[6]	*NP*[6]	*A/L*[2,3]	NP[6]	A/M[1]
Cramer TOXTREE 1.0.1[6]	*Class III*	*Class III*	*Class III*	*Class III*	*Class III*	*Class III*	*Class III*

Note: In the table, prediction/reliability codes. In bold the predictions with good reliability; in italics predictions with low reliability.

Code of predictions: NM: non-mutagenic; M: mutagenic; SM: suspect mutagen; I: inactive; A: active; NP: not predicted (not possible to perform an assessment).

Reliability code: G: good reliability (The predicted compound is into the Applicability Domain of the model); M: moderate reliability (The predicted compound could be out of the Applicability Domain of the model); L: low reliability (The predicted compound is outside the Applicability Domain of the model).

[1]Accuracy of prediction for similar compounds found in the training set is not optimal.

[2]Similar compounds found in the training set have experimental values that disagree with the predicted value.

[3]Only moderately similar compounds with known experimental value in the training set have been found.

[4]The following relevant fragment has been found: SA8 aliphatic halogen.

[5]The following relevant fragment has been found: SA10 alpha-beta unsaturated carbonyls.

[6]Impossible to perform an assessment (unable to perform the applicability domain check).

TABLE B.2 OECD QSAR Toolbox.

Compound	1	2	3	4	5	6	7	8
DNA binding by OASIS	AN2[4] Radical[5] SN1[6] SN2[7,8,9]	NA	Radical[5] SN2[8]	Radical[5] SN2[8]	NA	Radical[5] SN2[8]	NA	Radical[5] SN2[8]
DNA binding by OECD	SN2[10]	SN2[10]	SN2[10]	SN2[10]	SN2[10]	SN2[10]	SN2[10]	SN2[10]
Carcinog. Genotox/ non-genotox by ISS	Aliphatic halogen[1]	Aliphatic halogen[1]	Aliphatic halogen[1]	Aliphatic Halogen[1]	Aliphatic Halogen[1]	Aliphatic Halogen[1]	Aliphatic Halogen[1]	NA
DNA alerts for Ames, CA, MN vit by OASIS	NA	NA	NA	NA	NA	NA	NA	NA
Ames test alert by ISS	Aliphatic halogen[2]	Aliphatic halogen[2]	Aliphatic halogen[2]	Aliphatic halogen[2]	Aliphatic halogen[2]	Aliphatic halogen[2]	Aliphatic halogen[2]	NA
In vivo mutagenicity by ISS (MN)	Aliphatic halogen[2] H-acceptorpath3-h-acceptor[3] Oxolane[11]	Aliphatic halogen[2] H-acceptorpath3-h-acceptor[3] Oxolane[11]	Aliphatic halogen[2] H-acceptorpath3-h-acceptor[3] Oxolane[11]	Aliphatic halogen[2] H-acceptorpath3-h-acceptor[3] Oxolane[11]	Aliphatic halogen[2] H-acceptorpath3-h-acceptor[3] Oxolane[11]	Aliphatic halogen[2] H-acceptorpath3-h-acceptor[3] Oxolane[11]	Aliphatic halogen[2] H-acceptorpath3-h-acceptor[3] Oxolane[11]	H-acceptorpath3-H-acceptor[3]
Protein binding alerts for chromosomal aberrations by OASIS	NA	NA	NA	NA	NA	NA	NA	NA
Toxic hazard classification by Cramer	Class III	Class III	Class III	Class III	Class III	Class III	Class III	Class III

Compound	9	10	11	12	13	14	15
DNA binding by OASIS	Radical[5] SN2[8]	Radical[5] SN2[8]	Radical[5] SN2[8]	Radical[5] SN2[8]	NA	Radical[5] SN2[8]	SN2[9]
DNA binding by OECD	SN2[10]	SN2[10]	SN2[10]	SN2[10]	NA	SN2[10]	SN2[10]
Carcinog. Genotox/ non-genotox by ISS	Aliphatic halogen[1]	Aliphatic halogen[1]	Aliphatic halogen[1]	Aliphatic halogen[1]	NA	NA	Aliphatic halogen[12]

TABLE B.2 (Continued)

Compound	9	10	11	12	13	14	15
DNA alerts for Ames, CA, MN vit by OASIS	NA	NA	NA	NA	NA	NA	AN2[13] SN2[9]
Ames test alert by ISS	Aliphatic halogen[2]	Aliphatic halogen[2]	Aliphatic halogen[2]	Aliphatic halogen[2]	NA	NA	Aliphatic halogen[12]
In vivo mutagenicity by ISS (MN)	Aliphatic halogen[2] H-acceptor path 3-h-acceptor[3] Oxolane[11]	Aliphatic halogen[2] H-acceptor path 3-h-acceptor[3] Oxolane[11]	Aliphatic halogen[2] H-acceptor path 3-h-acceptor[3] Oxolane[11]	Aliphatic halogen[2] H-acceptor path 3-h-acceptor[3] Oxolane[11]	NA	H-acceptor path 3-H-acceptor[3]	Aliphatic halogen[12]
Protein binding alerts for chromosomal aberrations by OASIS	NA	NA	NA	NA	NA	NA	NA
Toxic hazard classification by Cramer	Class III	Class III	Class III	Class III	Class III	Class III	Class III

Abbreviation: NA: no alert.

[1]Aliphatic halogen **–** structural alert for genotoxic carcinogenicity.

[2]Aliphatic halogen – structural alert for genotoxicity via different mechanisms (direct alkylation, formation of reactive carbonyl compounds by Cyp450 oxidation, glutathione conjugation, depending on the haloalkane).

[3]H-acceptor-path3-H-acceptor3 – non-covalent binding with DNA and/or proteins, such as DNA intercalation or groove-binding.

[4]AN2 – Schiff base formation after aldehyde release (specific acetate esters).

[5]Radical – ROS formation after GSH depletion (indirect, haloalcohols).

[6]SN1 – nucleophilic attack after carbenium ion formation (specific acetate esters).

[7]SN2 – acylation (acetate esters).

[8]SN2 – alkylation by epoxide metabolically formed after E2 reaction (haloalcohols).

[9]SN2 – nucleophilic substitution at sp3 carbon atom.

[10]SN2 – reaction at a sp3 C atom (aliphatic halides).

[11]Oxolane – Oxolane (tetrahydrofuran) moiety represents the chemical skeleton of the furanose structure of biologically important aldopentoses, such as ribose. The oxolane substructure is a common chemical feature present in an important class of antimetabolites, the nucleoside-analogues drugs. Induction of micronuclei was reported for various nucleoside-analogues, presumably through the inhibition of DNA polymerase function and/or to be misincorporation into DNA.

[12]Aliphatic halogen – alpha-beta unsaturated carbonyl.

[13]AN2 – Schiff base formation.

B.4 | Evaluation of in silico predictions

Vega models

A consistent prediction of mutagenicity was provided by VEGA models for the alpha-beta unsaturated carbonylic compound # 15 (the sucralose thermal degradation product chlorinated furan-3-one). This compound was predicted to be mutagenic in the Ames test by all four models, and active in the in vitro and in vivo cytogenetic assays.

For the other compounds, most predictions provided by VEGA models were of low reliability, as the compounds analysed were out of the applicability domain of the models, or because the accuracy and concordance of predictions for substances in the training set, for which experimental data were available, were inadequate. For mutagenicity in the Ames test, a positive prediction with a moderate level of reliability was only made for compound #7 (1,6-DCF), evaluated as mutagenic/suspect mutagenic by all models. All other compounds were out of the applicability domain, except for # 8 (4-CG) for which contrasting predictions were made with no final reliable consensus. For this compound, however, negative experimental data in the Ames test are available.

For the activity in the in vitro chromosomal aberration assay, 12 out of the 15 compounds were predicted as active with moderate reliability. Compound #8 (4-CG) was the only one predicted as inactive, while #7 and #13 (triphenylphospine oxide) were out of the applicability domain of the model. For the activity in the in vitro micronucleus test, two compounds (#7 and #8) were predicted as active with moderate and good reliability, respectively, while all the other compounds were out of the applicability domain of the model. No prediction for the activity in the in vivo micronucleus test could be made.

Overall, the application of Vega models indicated a genotoxic concern in vitro and in vivo for #15 (chlorinated furan-3-one) and confirmed the activity of #7 (1,6-DCF) in the Ames test. For the remaining compounds, no reliable prediction could be formulated. These substances were out of the applicability domain of Ames test models, and provided inconsistent results when evaluated with other models: most compounds were predicted as clastogenic in vitro, including #7 (1,6-DCF) and #12 (sucralose) for which negative experimental data are available; conversely only the non-clastogenic #8 (4-CG) was predicted to be active in the in vitro micronucleus test based on the presence of structural alerting fragments also present in #7 and #12, for which reliable negative experimental data in the micronucleus test are available.

OECD QSAR Toolbox

The presence of the alpha-beta unsaturated carbonylic structure was identified by all profilers as an alert for DNA binding and in vitro and in vivo genotoxicity and genotoxic carcinogenicity in compound # 15.

For the other compounds, the general mechanistic profilers indicated a potential DNA binding capacity associated with the presence of the haloalcohol and aliphatic halide substructures in all compounds examined (including sucralose), except for #13. The aliphatic halogen substructure was also identified by the ISS endpoint specific profilers as an alert of potential genotoxic carcinogenicity and in vitro (Ames test) and in vivo (micronucleus) mutagenicity in most compounds (#1-#7, #9-#12). This alert relies on general knowledge on aliphatic halogen toxicology, where different mechanisms are involved depending on the chemical structure, and it should be interpreted with caution and in association with other data.

An additional alert for in vivo mutagenicity (micronucleus test) was identified by the ISS profiler in all compounds with the single exception of #13 and 15. This alert (H-acceptor path3-H-acceptor) is related to the unspecific capacity of non-covalent interaction with DNA and proteins: the alert was previously shown to lack positive predictivity (Aljallal et al., 2024; Ring et al., 2021) and it was disregarded in previous EFSA opinions.

Overall, the presence of the alpha-beta carbonylic structure is highlighted by all profilers as an alert of potential genotoxicity in the thermal degradation product of sucralose # 15. For the other impurities and degradation products structurally related to sucralose, a similar pattern of predictions was provided by both the mechanistic and endpoint specific profilers. The presence of the same potentially alerting substructures in sucralose and these impurities and degradation products supports the read-across with sucralose for their genotoxic evaluation. No genotoxic activity is anticipated for the structurally unrelated #.

APPENDIX C

Other endpoints measured in the available studies

C.1 | ANIMAL STUDIES

Clinical condition

Clinical condition was monitored in four studies in rats: a sub-chronic study with dietary administration and pair fed dietary restriction study, a sub-chronic study with administration by gavage to investigate palatability influence on feed intake and body weight, a combined chronic toxicity and carcinogenicity study and in reproductive toxicity study (Documentation provided to EFSA No 1: see also Annex C). No clinical signs were reported in these studies up to the highest dietary concentrations/dose tested.

Overall, the Panel considered that oral exposure to sucralose has no adverse effect on clinical condition.

Survival

Two studies reported survival of animals after a long-term dietary exposure to sucralose: one study in rats (104-week combined toxicity and oncogenicity study in CD rats with 'in utero' exposure, (Documentation provided to EFSA No 1; see also Annex C) and one study in mice (carcinogenicity study with in utero exposure from the 12th day of gestation, through weaning and until the termination at moribund condition (Soffritti et al., 2016)). Furthermore, survival was also reported in one reproductive toxicity study in rats (Documentation provided to EFSA No 1; see Also Annex C).

In the combined chronic and carcinogenicity study in rats (Documentation provided to EFSA No 1), the survival of F0 breeders was not affected by the treatment up to 3% sucralose in the diet equivalent to 2700 mg/kg bw per day. A total three F0 animals died: two control males in week 8 and 11, respectively and one high-dose female in week 1. These deaths were considered by the Panel as not related to the treatment. The survival of F1 rats in the carcinogenicity phase was higher than that of the controls with a statistically significant increase in low- and high-dose males and mid- and high-dose females. Thus, sucralose at dietary concentration up to 3% in the diet for 104 weeks (equal to 1005 mg/kg bw per day for males and 1395 mg/kg bw per day for females) in rats which were also exposed in utero and during the weaning had no adverse effect on the survival.

In the mouse study, survival of males was statistically significantly decreased at the medium-low dose 273 mg/kg bw per day (($p \leq 0.04$) and at the high dose of 2184 mg/kg bw per day ($p \leq 0.009$) while there was no statistically significant difference in survival in the sucralose-exposed groups compared to the controls.

In the reproductive toxicity study in rats (Documentation provided to EFSA No 1) survival of the F0 and F1 males and females was not affected by the treatment up to 3% sucralose in the diet (equal to 1580 and 2090 mg/kg bw per day or 1680 and 2100 mg/kg bw per day for males and females from F0 and F1 generations, respectively). The cases of three animals euthanised in extremis i.e. one low-dose F0 male in week 26, one mid-dose F0 female in week 23 and one mid-dose F1 female in week 25 were considered not treatment related.

Overall, the Panel considered that oral exposure to sucralose has no adverse effect on the survival in laboratory rodents.

Organ toxicity

Organ weight and histopathology were assessed for several organs not considered in the weight of evidence under the HOCs liver toxicity, nephrotoxicity, reproductive toxicity, neurotoxicity, inflammation/immunotoxicity and thyroid toxicity. Data on caecum are reported in Appendix D.

In the **26-week oral (by gavage) toxicity study** in CD rats (Documentation provided to EFSA No 1) with sucralose doses of 750, 1500 or 3000 mg/kg bw per day, the following organs were weighed: heart, lungs, adrenals, pituitary gland. Microscopic examination was performed on aorta (thoracic), colon, duodenum, eye and optic nerve, ileum, jejunum, mesenteric and mandibular lymph nodes, oesophagus, pancreas, pituitary gland, lung, rectum, submandibular left salivary gland, skeletal muscle, spinal cord, sternum, stomach (non- and glandular bodies), trachea, urinary bladder. No statistically significant changes in absolute and relative weights of the weighed organs were recorded, and no macroscopic and histopathological changes attributable to the treatment were found in the above-mentioned tissues from rats exposed to 3000 mg sucralose/kg bw per day, the highest dose tested.

In the **combined chronic and carcinogenicity study** in CD rats kept on diets with 0%, 0.3%, 1% or 3% sucralose to 104 (equal to 0, 94, 323, 1005 mg/kg bw per day in males and to 0, 135, 458 and 1395 mg/kg bw per day) (Documentation provided to EFSA No 1) the following organs were weighed: heart, lungs, adrenal and pituitary gland. Microscopic examination of other organs was performed on: spinal cord, Zymbal glands, turbinates, eyes (both with optic nerves, lachrymal gland, Harderian gland, salivary gland, trachea, oesophagus, aorta, smooth muscle (diaphragma), pancreas, stomach (phundus and pyrolus), duodenum, jejunum, colon, rectum, lymph nodes (cervical and mesenteric, urinary bladder, fallopian tube, lung, skin, sciatic nerve, femur with marrow, skeletal muscle, pituitary gland and presumptive tumours). No toxicologically relevant changes in organ weights were observed. No macroscopic abnormalities or changes attributable to the treatment

with sucralose dietary concentrations up to 3% (equal to 1005 mg/kg bw per day in males and to 1395 mg/kg bw per day in females for a period of 104 weeks) in toxicity and oncogenicity phases were found.

In the **reproductive toxicity study** in CD rats exposed to 0.3%, 1% or 3% sucralose in the diet, the following organs were weighed: lungs, liver, kidneys, adrenals, pituitary gland (Documentation provided to EFSA No 1). No toxicologically relevant changes in organ weights were observed.

Exposure of female C57BL6J mice to 6.3 mg sucralose/kg bw per day in drinking water during gestation and lactation had no effect on the absolute heart weight of the offspring not exposed to sucralose from the end of weaning until 11 weeks of age (Azad et al., 2020).

C.2 | EFFECTS ON HUMAN GUT MICROBIOTA

A number of studies (including: Thomson et al., 2019; Gerasimidis et al., 2020; Ahmad et al., 2020; Shahriar et al., 2020; Markus et al., 2021; Shil & Chichger, 2021; Ghadimi et al., 2023; Gonza et al., 2024; Park et al., 2024; Reyes-López et al., 2024), examined gut microbiota changes in response to oral exposure to sucralose. The Panel noted that there is currently no consensus on when changes in intestinal microbiota should be considered adverse and on what the potential health consequences of changes in bacterial composition would be. In this respect it is noted that EFSA has recently published the external report of an Art. 36 grant[70] proposing a roadmap for action to (i) strengthen the nascent evidence relating to effects on/by human and animal gut microbiomes; and (ii) identify key knowledge gaps and provide guidance on how to experimentally approach these urgent research needs.

C.3 | IMPACT OF SWEETENERS ON ANTIMICROBIAL RESISTANCE (AMR)

Yu et al. (2021), Yu & Guo (2022), Yu et al. (2023) deal with four artificial sweeteners (acesulfame potassium, saccharin, sucralose, aspartame) and are showing about the same effect of the four sweeteners analysed:

Yu et al. (2021) demonstrated a significant increase in transformation efficiency of the model organism *Acinetobacter baylyi* in the presence of sweeteners. They also show in an in vitro experiment (fitness cost experiment based on serial culturing) that the plasmids would persist more stably in the bacterial cells in the presence of the sweetener in the medium. Only doubling in transformation frequency compared to control was confirmed which is, although statistically relevant, a low effect and it is doubtful if it is of any biologically relevance. The increased persistence of a plasmid is shown in an in vitro system which would need further investigation and confirmation in a biological setup closer to practical conditions.

Yu and Guo (2022) demonstrated a significant antimicrobial effect of the four sweeteners tested against both Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) and positive (*Bacillus subtilis*) strains. This effect was, although statistically relevant, quite limited and for most of the results lower than sub-MIC concentrations of antibiotics; not a one log reduction which would be expected to be reached for considering it as biologically relevant.

Yu et al. (2023) demonstrated that the four sweeteners may promote plasmid-mediated conjugative transfer to the gut microbiome. This effect was, although statistically significant, quite limited and it is questionable if this is of biological relevance.

In De Dios et al., 2023 in vitro experiments showed a minor but significant inhibitory impact of sucralose on the growth of a clinical strain of *Acinetobacter baumannii*, and on a lab strain of Pseudomonas aeruginosa.

Qu et al., 2017 showed that a concentration of about 15.6 mg/mL sucralose increased the survival rate of *E. coli* in an in vitro experiment to oxolinic acid (a synthetic quinolone antibiotic); the effect to moxifloxacin was significant but very low. A significant but minor increase (up to 4 times) in mutation rate was observed in the presence of sucralose. This increase is not considered of high biological relevance in an in vivo situation.

Overall, the Panel noted that the effects seen are in general rather limited, dependent on the sweetener and bacterial strain tested, their relevance for the development of AMR is unclear.

[70] https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/sp.efsa.2024.EN-8597

APPENDIX D

Caecum weights

Caecum weight was addressed in three studies in CD rats (Documentation provided to EFSA No 1).

In the **sub-chronic 26-week toxicity study by oral gavage** in CD rats, absolute and relative full and empty caecum weights were statistically significantly increased relative to controls in males and females at all doses (i.e. 750, 1500 and 3000 mg/kg bw per day) except for the absolute and relative empty caecum weight in the low-dose females. The increases in the absolute full caecum weight were +67%, +118% and 164% in males, and +22%, +46% and +81% in females at the low, mid and high doses, respectively. The increases in the absolute empty caecum weight were +40%, + 42% and +83% in males at the low, mid and high doses, and +22% and +24% in females at the mid and high doses. The increases in the relative full caecum weight were +63%, +130% and +166% in males, and +20%, +46% and +87% in females at the low, mid and high doses, respectively. The increases in the relative empty caecum weight were +34%, +49% and +84% in males at the low, mid and high doses, and +21% and +28% in females at the mid and high doses (Table D.1).

TABLE D.1 Caecum weights in the sub-chronic 26-week toxicity study by oral gavage (Documentation provided to EFSA No 1).

	Males				Females			
	C	750 mg/kg bw per day	1500 mg/kg bw per day	3000 mg/kg bw per day	C	750 mg/kg bw per day	1500 mg/kg bw per day	3000 mg/kg bw per day
Relative full caecum weight	–	+63%	+130%	+166%	–	+20%	+46%	+87%
Relative empty caecum weight	–	+34%	+49%	+84%	–	ns	+21%	+28%

Abbreviations: C, control; ns, not statistically significant.

In the **reproductive toxicity study** in rats, statistically significant increases in full caecum absolute, absolute weight adjusted to allow for body weight covariate analysis and relative weights were present in F0 males at sucralose dietary concentrations of 1% and 3% (equal to 514 and 1580 mg/kg bw per day) and in F0 females, F1 males and F1 females at 0.3%, 1% and 3% sucralose dietary concentrations (equal to 194, 654 and 2090 mg/kg bw per day for F0 females, to 154, 524 and 1680 mg/kg bw per day in F1 males and to 192, 642 and 2100 mg/kg bw per day in F1 females). For the empty caecum of F0 generation males and females, absolute, absolute weight adjusted to allow for body weight covariate analysis and relative weights were statistically significantly increased relative to the controls only at the 3% sucralose dietary concentration. In F1 generation males and females, statistically significant increases in absolute empty caecum weights were present only at the 3% sucralose dietary concentration, in the absolute weight adjusted to allow for body weight covariate analysis at the 3% sucralose dietary concentration in F1 females and at 1% and 3% sucralose dietary concentration in F1 males. The relative empty caecum weights were statistically significantly increased in F1 males and F1 females at 1% and 3% sucralose dietary concentration.

The statistically significant increases relative to controls for the full caecum weight were in F0 males: +15% and +80% for the absolute weight, +17% and +84% for the adjusted weight and +27% and +108% for the relative weight at mid and high concentrations, respectively; in F0 females: +13, +36% and +91% for the absolute weight, +17%, +41% and +101% for the adjusted weight and +22%, +48% and +125% for the relative weight at low, mid and high concentrations, respectively; in F1 males: +10%, +15% and +67% for the absolute weight, +14%, +17% and +74% for the adjusted weight and +19%, +19% and +86% for the relative weight at low, mid and high concentrations, respectively; in F1 females: +20%, +45% and +77% for the absolute weight, +24%, +51% and +88% for the adjusted weight and +25%, +56% and +93% for the relative weight at low, mid and high concentrations, respectively.

The statistically significant increases relative to controls for the empty caecum weight were in F0 males: +21% for the absolute weight, +27% for the adjusted weight and +38% for the relative weight at the high concentration; in F0 females: +26% for the absolute weight, +31% for the adjusted weight and +48% for the relative weight at the high concentration; in F1 males: +24%, for the absolute weight at the high concentration, +14% and +34% for the adjusted weight and +14% and +37% for the relative weight at mid and high concentrations, respectively; in F1 females: +54% for the absolute weight at the high concentration, +53% for the adjusted weight at the high concentration and +24% and +70% for the relative weight at mid and high concentrations, respectively (Table D.2).

TABLE D.2 Caecum weights in the reproductive toxicity study (Documentation provided to EFSA No 1).

	F0 males				F0 females			
	C	3000 ppm sucralose[a]	10,000 ppm sucralose[b]	30,000 ppm sucralose[c]	C	3000 ppm sucralose[a]	10,000 ppm sucralose[b]	30,000 ppm sucralose[c]
Relative full caecum weight	–	ns	+27%	+108%	–	+22%	+48%	+125%
Relative empty caecum weight	–	ns	ns	+38%	–	ns	ns	+48%
	F1 males				F1 females			
Relative full caecum weight	–	+19%	+19%	+96%	–	+25%	+56%	+93%
Relative empty caecum weight	–	ns	+14%	+37%	–	ns	+24%	+70%

Abbreviations: C, control; ns, not statistically significant.

[a]Dose equal to 155 (males) and 194 (females) mg/kg bw per day.

[b]Dose equal to 514 (males) and 656 (females) mg/kg bw per day.

[c]Dose equal to 1580 (males) and 2090 (females) mg/kg bw per day.

In the **combined chronic and carcinogenicity study** in CD rats of the duration up to 104 weeks statistically significant increases in full and empty caecum weights were present at sucralose dietary concentrations of 1% and 3% (equal to 323 mg/kg bw per day and 1005 mg/kg bw per day for males and to 458 mg/kg bw per day and 1395mg/kg bw per day for females for the entire study period. The absolute weights of the full caecum were statistically significantly increased in mid- and high-dose males (+18% and 87%, respectively) and females (+18 and +105%, respectively) after 52 weeks, in high-dose males and females (+79% and 43%, respectively) after 78 weeks, and in high-dose males and females (+72% and +63%, respectively) after 104 weeks. The absolute weights of the empty caecum were also statistically significantly increased in high-dose males and females (+21% and +25%, respectively) after 52 weeks, in high-dose females (+20%) after 78 weeks and in high-dose males and females (+28% and +21%, respectively) after 104 weeks. Similar statistically significant increases relative to controls were recorded for the relative full caecum weight in mid- and high-dose males (+35% and +118%, respectively and in all treated females (+43%, +54% and +171%, respectively) after 52 weeks, in high-dose males (+123%) and in mid-and high-dose females (+29% and +73%, respectively) after 78 weeks, in mid- and high-dose males (+35% and +114%, respectively) and females (+22% and 101%, respectively) after 104 weeks. The relative empty caecum weights were statistically significantly increased relative to the controls in high-dose males (+41%) and in mid- and high-dose females (+28% and +65%) after 52 weeks, in high-dose males and females (+42% and +44%, respectively after 78 weeks, and in mid- and high-dose males (+20% and 57%, respectively) and females (+20% and 50%, respectively) after 104 weeks (Table D.3).

TABLE D.3 Caecum weights in the combined chronic and carcinogenicity study (Documentation provided to EFSA No 1).

	Males					Females				
	C1	C2	3000 ppm sucralose[a]	10,000 ppm sucralose[b]	30,000 ppm sucralose[c]	C1	C2	3000 ppm sucralose[a]	10,000 ppm sucralose[b]	30,000 ppm sucralose[c]
52 weeks										
Relative full caecum weight	–		ns	+35%	+118%	–		+43%	+54%	+171%
Relative empty caecum weight	–		ns	ns	+41%	–		ns	+28%	+65%
78 weeks										
Relative full caecum weight	–		ns	ns	+123%	–		ns	+29%	+73%
Relative empty caecum weight	–		ns	ns	+42%	–		ns	ns	+44%
104 weeks										
Relative full caecum weight	–		ns	+35%	+114%	–		ns	+22%	+101%
Relative empty caecum weight	–		ns	+20%	+57%	–		ns	+20%	+50%

Abbreviations: C, control; ns, not statistically significant.

[a]Dose equal to 94 (males) and 135 (females) mg/kg bw per day.

[b]Dose equal to 323 (males) and 458 (females) mg/kg bw per day.

[c]Dose equal to 1005 (males) and 1395 (females) mg/kg bw per day.

Overall, oral intake of sucralose increases full and empty caecum weights in rats. This effect was present already from a dietary concentration of 0.3% sucralose in the diet in F0 females, F1 males and F1 females in the reproductive toxicity study in rats. The lowest sucralose dose in this study was 154 mg/kg bw recorded for F1 males. The effect appeared stronger in female rats in the three available studies and was more pronounced for the full than the empty caecum.

APPENDIX E

Toxicological studies on 1,6-DCF and 4-CG

Studies on the toxicological properties of 1,6-DCF and 4-CG or their equimolar mixture were provided by the IBO (Documentation provided to EFSA No 1). An open-ended literature search was conducted but no additional relevant references were found addressing the toxicological properties of these substances were retrieved (see Appendix A). In this Appendix a narrative assessment of the available studies is provided along with an overview Table of the NOEALs derived by the FAF Panel (Table E.1).

In assessing these results of tests on the equimolar mixture, the Panel made the generally accepted assumptions in the calculations that the effects observed are either due to one of the two components of the mixture, considering each of the two in turn or that the two components of the mixture were acting equally in a simple concentration (dose)-addition manner. Numerically, the same outcome is obtained.

The derivation of doses as mg/kg bw per day was done as reported in Section 2.2 and the derivation of the NOAEL for 1,6-DCF and 4-CG was done using conversion factors based on the molecular weight of these two substances.

E.1 | 13-week oral toxicity study in rats with in utero exposure to an equimolar mixture of 1,6-DCF and 4-CG

Sprague–Dawley rats (20/sex/group) were given an equimolar mixture of 1,6-DCF and 4-CG at dietary concentrations of 0, 0.02%, 0.06% or 0.2% (equal to 12.9, 38.3 and 132 mg/kg bw per day for males and 17, 50.1 and 174.5 mg/kg bw per day for females) from the end of weaning for 26 weeks. The rats were obtained from F1A generation in a two generation study from parents exposed to the same concentrations of the test compound; thus, the rats in this study were also exposed in utero and during weaning to the same dietary doses of the tested compound. The study design and procedures were as described in OECD TG 408 (version from 1981) (OECD, 1981) Clinical condition and survival were not affected by the treatment. No effects were found in ophthalmoscopic examinations. There were no statistically significant differences from controls in body weight, but the final body weights of high-dose males and high-dose females were 12% and 11% lower than the controls (not statistically significant). The body weight gain of the high-dose males and high-dose females was statistically significantly lower from the control by 14%. The feed intake was reduced by 10% and 4% in high-dose males and high-dose females but the difference from the controls was not statistically significant. Statistically significant differences between the test and control groups were recorded in haematology and clinical chemistry parameters. These changes were considered by the Panel as not toxicologically relevant as they were either small, observed in one sex, without dose–response relationship nor consistency between the changes in males and females, and there were no changes in other relevant parameters including histopathological correlates. Statistically significant changes from controls detected in organ weights were a reduction in absolute heart weight in high-dose males (–11%) and females (–10%) groups, an increase in relative brain weight in high-dose males and females (+15% in both), an increase in relative testes weight in high-dose males, an increase in liver absolute weight in mid-dose females (+9%), in relative liver weight in all female treated groups (+6% at the low dose, and +11% at mid and high doses) and in liver weight adjusted for terminal body weight by analysis of covariance in all treated females (+7%, +11% and +10% at the low, mid and high doses, respectively), an increase in relative kidney weight in mid- and high dose males (+8% and +10%) and mid- and high-dose females (+10% and +11%) and in adjusted kidney weight in mid- and high dose males (+6% at both dose levels) and mid- and high-dose females (+10% and +5%). The Panel considered the changes of no toxicological concern as they were either small or lacked dose–response relationship and no histopathological correlates. No treatment-related gross or microscopic changes were observed in the examined organs and tissues.

The Panel identified a no observed adverse effect level (NOAEL) of 0.06% in the diet of the equimolar mixture of 1,6-DCF and 4-CG based on a statistically significant reduction in body weight gain at 0.2% in the diet, the highest dietary concentration tested which is considered by the Panel as adverse. This NOAEL is equal to 38.3 mg/kg bw per day in males and to 50.1 mg/kg bw per day in females of the equimolar mixture of 1,6-DCF and 4-CG. A dose of 38.6 mg/kg bw per day of the equimolar mixture of 1,6-DCF and 4-CG in males corresponds to 20.0 mg 1,6-DCF/kg bw per day and to 18.32 mg 4-CG/kg bw per day. A dose of 50.1 mg/kg bw per day of the equimolar mixture of 1,6-DCF and 4-CG in females corresponds to 26.25 mg 1,6-DCF/kg bw per day and to 23.9 mg 4-CG/kg bw per day.

E.2 | 26-week oral toxicity study in dogs with an equimolar mixture of 1,6-DCF and 4-CG

Dose range finding/toxicity study

One male and one female beagle dogs were given orally in feed an equimolar mixture of 1,6-DCF and 4-CG in doses of 30, 90, 180 or 270 mg/kg bw per day. Each dose was administered for 7 consecutive days with the dose level increasing every 9 days. No effects on clinical condition, body weight, feed intake, haematology, clinical chemistry and urinalysis were detected at any of the dose tested. The NOAEL was 270 mg/kg bw of the mixture, the highest dose tested, corresponding to approximately 141 mg 1,6-DCF/kg bw per day and 129 mg 4-CG/kg bw per day.

Main study

Beagle dogs (4/sex/group) were given orally in feed an equimolar mixture of 1,6-DCF and 4-CG in doses 0, 10, 50 or 250 mg/kg bw per day for 26 weeks. Detailed physical examination including neurological evaluation, body weight and feed intake records were performed weekly ophthalmological examination, haematology, clinical chemistry and urinalysis were performed before the treatment and at 3 and 6 months in the study. At necropsy, all gross changes and organ weights were recorded, and samples of organs and tissues were taken and examined by microscopy. There were no effects on clinical condition and no neurological effects were seen. There were no statistically different changes in feed intake during the study although a decrease was observed in the early part of the study. This decrease corresponded with a lower body weight gain in the high-dose group in the first month of the study. There were no significant differences from controls in body weight and body weight gain although the final body weights of mid- and high-dose males and high dose females were 6% lower than the controls while the difference from controls in body weight gain was –10% and –12% in mid- and high-dose males and –14% in high-dose females at the end of the study. A number of statistically significant differences between the test and control groups were recorded in haematology (MCV, MCHC, MCH) and clinical chemistry (glucose, chloride, sodium, AST, albumin, pyruvic acid) but they were considered not toxicologically relevant as the changes were either small, observed only in one sex, the changes were present only at 3 months, there was no dose–response relationship, or the values were not statistically significantly different from the concurrent control when pre-dose values were subtracted from interval values. There were no treatment-related changes in urinalysis parameters. There were no statistically significant changes in organ weights, and no gross or microscopic changes which could be attributed to the treatment with sucralose. The NOAEL identified by the authors of the study was 250 mg/kg bw of the mixture, i.e. the highest dose tested, corresponding to approximately 130.55 mg 1,6-DCF/kg bw per day and 119,45 mg 4-CG/kg bw per day.

The Panel noted a lower body weight and a reduction in body weight gain in both sexes at the end of the treatment, although a difference from controls was not statistically significant. The Panel identified a NOAEL of 50 mg/kg bw per day of the equimolar mixture of 1,6-DCF and 4-CG, based on body weight and body weight gain changes. This NOAEL corresponds to 26.1 mg/kg bw per day of 1,6-DCF and 23.9 mg/kg bw per day of 4-CG.

E.3 | 104-week carcinogenicity study in rats with an equimolar mixture of 1,6-DCF and 4-CG

Sprague–Dawley rats (CD strain) (50/sex/group) received 0%, 0.02%, 0.06% or 0.2% an equimolar mixture of 1,6-DCF and 4-CG in the diet for 104 weeks (equal to doses of 0, 6, 19 and 64 mg/kg bw per day for males and 0, 8, 25 and 87 mg/kg bw per day for a period 0–104 weeks). The following parameters were monitored: clinical condition and mortality (twice daily), ophthalmoscopic changes (ophthalmoscopy before the start, at wees 13, 26, 38, 51, 65, 78, 91 and 104 occurrence of masses (palpation once a week), body weight (once a week for the first 14 weeks and every second week thereafter), feed intake (weekly), water intake (over 24 h period once a week for the first 13 week of the treatment), haematology (RBC total count, WBC total and differential counts at final termination), organ weights macro-and microscopic abnormalities.

There were no statistically significant changes in survival between the control and all male groups. In high dose females the survival was statistically significantly increased relative to the control group and there was a statistically significant trend towards higher survival with increasing dietary concentrations of the tested item. No changes attributable to the treatment with test item were recorded in clinical condition and ophthalmoscopic examination. The number of rats bearing palpable masses was not statistically significantly different between the treated and control groups but multiplicity of masses (i.e. number of masses per rat bearing masses) was lower in mid-and high dose groups, the difference being statistically significant for males only. Body weight in the treated groups was lower from controls in week 78 (males: –5%, –7% and –13%, females: –5%, –1% and –18% at the low, mid and high doses). Body weight gain for a period 0–78 weeks was statistically significantly lower from controls in mid- and high dose males (–8% and –13%) and in high dose females (–23%). After 104 weeks of the treatment, body weight of the treated males was slightly lower from the controls (–2%, –7% and –8% at the low, mid and high doses) while the difference from control for the treated females was greater especially at the high dose (–10%, –2%, –20% at the low, mid and high doses). Body weight gain for a period 0–104 weeks was reduced in the treated males (–2%, –8% and –9% at the low, mid and high doses) but the difference to the control group was not statistically significant. The reduction in body weight gain in the treated female groups attained statistical significance in the high dose group (–12, –2% and –24% at the low, mid and high doses, respectively). Feed intake was statistically significantly lower in high-dose males and females during the study, on several occasions also in mid-dose males and in low- and mid-dose females. No treatment-related changes in RBC and WBC counts were recorded in all treated groups of both sexes although a number of statistically significant differences between the test and control groups were recorded in RBC and WBC total and differential counts in the treated male groups. However, these were not found after an outlier was excluded from the control group. In females, the only statistically significant difference from controls was an increase in neutrocytes (+65%) in the low-dose females, which was considered not toxicologically significant as it lacked a dose–response relationship. A number of statistically significant differences between the test and control groups were recorded in organ weights: a decrease in absolute and adjusted for terminal body weight by analysis of covariance weights of adrenals in high-dose males (–26% for both), a decrease in absolute and adjusted weights of heart (–18% and –9%) and kidneys (–22% and –16%) in high-dose females, a decrease in absolute kidney weigh in low-dose males (–15%) and high-dose females (–22%), and in adjusted kidney weight in a high-dose males (–14%) and high-dose females (–16%), a decrease in absolute liver weights in high-dose females (–19%), a decrease in absolute spleen weight in high-dose females (–29%) and in adjusted spleen

weight in low-and high -dose males (−32% and −29%. The absolute weight of uterus was statistically significantly lower in low-dose females (−21%). Testes weights were statistically significantly increased: the absolute (+24%, +24%, +26%) and adjusted (+24%, +24%, +27%) at low, mid and high doses and the relative at mid and high doses (36% in both). According to the authors of the report the control males had lower testes weight compared to the background data for this strain of rats in this laboratory, while the weight in the treated males was within these background data. Microscopic examination revealed increased incidence of degeneration of the testicular germinal epithelium in the control group while no treatment-related changes were seen in the testes of the treated males. The Panel noted that relative weights of adrenals, heart, kidneys, liver, spleen and uterus were not statistically different from the controls and that there were no treatment-related microscopic changes in these organs. No adverse treatment-related 'non-neoplastic changes' were found in the treated groups. No treatment-related tumours were found in the treated groups.

The Panel concluded that an equimolar mixture of 1,6-DCF and 4-CG was not carcinogenic to rats at dietary concentration amounting to 0.02% equal to 64 mg/kg bw per day for males and to 87 mg/kg bw per day for females following 104 weeks of administration. The Panel further concluded that a NOAEL for other endpoints than carcinogenicity is 0.06% in the diet of the equimolar mixture of 1,6-DCF and 4-CG based on a reduction in the final body weight relative to control at 0.02% in the diet particularly in females and on a reduction in body weight gain in both sexes (statistically significant for female only) at the same dose. This NOAEL is equal to 19 mg/kg bw per day in males and to 25 mg/kg bw per day in females of the equimolar mixture of 1,6-DCF and 4-CG. A dose of 19 mg/kg bw per day of the equimolar mixture of 1,6-DCF and 4-CG corresponds in males to 9.9 mg 1,6-DCF/kg bw per day and to 9.1 mg 4-CG/kg bw per day. A dose of 25 mg/kg bw per day of the equimolar mixture of 1,6-DCF and 4-CG corresponds in females to 13.1 mg 1,6-DCF/kg bw per day and to 11.9 mg 4-CG/kg bw per day.

E.4 | Two generation reproductive study in rats on an equimolar mixture of 4-CG and 1,6-DCF

A GLP study was performed to assess the influence of 4-CG and 1,6-DCF upon the reproductive capacity of two successive generations of rats. An approximately equimolar mixture of 4-CG and 1,6-DCF, was supplied as a 50%w/w aqueous solution and containing 23.8% of 1,6-dichloro-1,6-dideoxyfructose and 24.6% of 4-chloro-4-deoxygalactose. The test material was stable for the duration of the treatment period. The dietary route was selected in line with the intended human exposure. Dose levels of 200, 600 and 2000 ppm of the equimolar mixture in the diet were selected. Control animals received untreated diet for the same time-period as the treated animals.

30 Animals/sex/treatment group were in the F0 and F1 parental generation. The animals received the experimental diets for 71 days before pairing for mating. Treatment continued throughout mating and until males and females were killed when their F1 litters are weaned. F1 animals received the experimental diets from the time of weaning for a minimum of 71 days. They were then be paired for mating and treatment continued until termination at the same time as for the P animals. The first pairing produced the F1A litters which were assigned for use on a 13-week toxicity study. The remainder were examined for macroscopic abnormalities and the discarded at weaning. After the second pairing 30 male and 30 female offspring from the F1B litters were selected to form F1 generation and were treated for 10 weeks before being paired twice in succession to produce F2A and F2B offspring. These offspring were reared to day 25 post-partum at which point they were examined for macroscopic abnormalities and then discarded. Parental F0 and F1 animals were subjected to a detailed necropsy procedure including organ weight determinations.

Body weights, food and water consumption were documented for all animals.

In F1, body weight, sex ratio, physical development, auditory and visual function were investigated. Parental F0 and F1 animals were subjected to a detailed necropsy procedure including organ weight determinations. The following organs were investigated: adrenals, pituitary, epididymides, prostate, kidneys, seminal vesicles, liver, spleen, lungs, testes, ovaries, uterus.

No treatment-related effects on clinical condition were observed. Death of in total 4 animals was not treatment-related.

Body weight

Body weight and body weight gain was influenced by the treatment. Body weight was significantly increased and the effect size greater than 10% in both sexes at 2000 ppm. No influence of treatment was noted on food intake, food conversion efficiency and water consumption.

No adverse treatment-related effects were observed on mating performance and fertility. Gestation length and gestation index were not influenced by treatment.

In the litters initial litter size was marginally reduced in the 2000ppm treated F1A and F1B groups. Live birth index and survival were fund unaffected by treatment. Initial litter size and live birth index for F2A and F2B matings were also unaffected by treatment. Offspring viability to Day 4 post-partum showed no treatment-related effects. Viability after Day 4 post-partum was similar in all groups, The ratio of male to female offspring was unaffected by treatment.

Bodyweight of offspring

Initial group mean bodyweights of offspring in all treated groups were similar to their respective controls for A and B litters of both the F0 and F1 generations; however, body weight gain to Day 25 was significantly reduced at 2000 ppm and exceeds 10%. Animals receiving 200 or 600 ppm were unaffected.

Development of offspring

The rate of physical development and the responses to auditory and visual stimuli were similar in all groups of F1B and F2B animals.

In the necropsy of F0 and F1 adults, no macroscopic examination revealed no treatment-related effects. Reactions to auditory and visual stimuli were similar in all groups of F1B and F2B animals.

The following not adverse effects were noted: a dose-related increase (up to 18%) in relative kidney weights in both sexes and generations, a dose-related increase (up to 22%) in relative spleen weights in females of both generations, a dose-related increase (up to 18%) in relative lung weights in F0 males and F1 females. The absolute testes weights were statistically significant, however not adversely reduced in F0 animals at 2000 ppm (6%; $p < 0.05$).

In summary, there were no adverse effects upon reproductive performance throughout two successive generations at 2000 ppm or at lower dosage levels. At the dose level of 2000 ppm, reductions in food intake and bodyweight gain during maturation, a reduction in weight gain during pregnancy and a reduction in bodyweight gain of offspring up to the time of weaning were observed. At 600 ppm, bodyweight gain in females was consistently reduced during both maturation phases and during F1A and F1B pregnancies, however not more than 10%. The bodyweight gains of F0 and F1 male parents and of offspring in both generations were unaffected. The only adverse weight gain reduction was seen in females at a dose level of 2000 ppm.

Therefore, the Panel identified a NOAEL of 600 ppm[71] equal to 38 mg/kg bw per day of the equimolar mixture of 1,6-DCF and 4-CG. This NOAEL corresponds to 20 mg/kg bw per day of 1,6-DCF and 18 mg/kg bw per day of 4-CG.

E.5 | Studies on the neurotoxic potential of an equimolar mixture of 4-CG and 1,6-DCF in mice and marmoset

Neuropathological studies were performed in mice of both sexes of sucralose and an equimolar mixture of hydrolysis products of sucralose (1,6-DCF and 4-CG), and 6-chloro-6-deoxy-glucose, 6-CG as positive control as well as a control group. The same substance and mixture were used in neuropathological studies in marmosets of both sexes. The neuropathological studies were focusing on morphological changes in the central nervous system. 6-Chloro-6-deoxy-glucose, previously reported to produce neurotoxic effects, served as positive control. The administered daily doses of sucralose and the sucralose hydrolysis products (sucralose-HP) were 50, 150, 500 and 1000 mg/kg for 21 days in mice (5 males, 5 females per group) and 1000 mg/kg for both treatments, sucralose and sucralose hydrolysis products, for 28 days in marmosets (3 male marmosets per groups). The daily dose of 6-chloro-6-deoxy-glucose was 500 mg/kg. Administration was by gavage. In mice, muscle tone, activity, gait, stance, tremors, righting and placement reflexes were controlled three times per day. In marmosets, additional clinical endpoints were studied (i) cranial nerve reflexes with pupillary light and consensual light; blink; optic righting (ii) general examination of the head and tongue (iii) segmental reflexes. flexor; plantar and extensor tone (iv) postural reactions. Placing, visual and tactile (v) extensor thrust (vi) righting, (vii) hopping reflex and involuntary grip. The animals were observed for behaviour; gait and stance; tremor or other dyskinesias. In the histopathological investigation particular attention was given to histopathologic and ultrastructural changes in the following neural tissues: posterior cerebrum; anterior colliculi; anterior cerebrum; posterior colliculi; posterior colliculi/cerebellum; medulla/cerebellum; medulla oblongata; cervical spinal cord (transverse and longitudinal sections); mid-thoracic spinal cord; lumbar spinal cord (transverse and longitudinal section); sacral spinal cord; sciatic nerve (left and right). Deaths in mice were recorded during the study: one female (150 mg sucralose-HP/kg); and two males (1000 mg sucralose-HP/kg). It should be noted that the LD 50 is 3.500 mg/kg for this species. Mice that received 6-CG as positive control exhibited reduced muscle tone, delayed righting reflex and changes in overall activity. No such effects were noted in animals that received sucralose or its hydrolysis products. Two marmosets receiving 6-CG were euthanised after 7 days because of severe and debilitating neurological effects. A third monkey in the 6-CG group was killed in extremis on day 28. All three animals showed inappetence, body weight loss and evidence of neurotoxicity. Tremors and a convulsion were other neurological signs exhibited in the 6-CG group. Animals treated with sucralose, or its hydrolysis products did not exhibit any similar neurological signs. No changes were detected in the central nervous system by light or electron microscopy in mice and marmosets that received sucralose or its hydrolysis products. 6-Chloro-6-deoxyglucose, applied as positive control, induced symmetrical lesions in the deep nuclei of the cerebellum, brain stem and spinal cord. According to this study, no histopathological changes in the CNS and no effects on behaviour and some neurological tests were observed following the administration of sucralose and an equimolar mixture of sucralose hydrolysis products of sucralose (6-DCF and 4-CG) up to 1000 mg/kg bw daily for 21 and 28 days in mice and marmosets, respectively.

The Panel concluded that the NOAEL is 1000 mg/kg bw per day of the equimolar mixture of 1,6-DCF and 4-CG (highest dose tested) both for mice and marmosets. This NOAEL corresponds to 520 mg/kg bw per day of 1,6-DCF and 480 mg/kg bw per day of 4-CG.

[71] 600 mg/kg in the diet, with a food consumption of about 130 g/female rat/week, a body weight of 290g the body dose is about 38 mg/kg bw per day based on the data reported here, mean values as well as substance intake data are not reported, though formular for substance intake is given.

TABLE E.1 Overview of the NOAEL derived by the FAF Panel from the available toxicological studies on 1,6-DCF, 4-CG and their equimolar mixture.

Reference	Test item	NOAEL derived for 1,6-DCF	NOAEL derived for 4-CG
13-week oral toxicity study in rats with in utero exposure to an equimolar mixture of 1,6-DCF and 4-CG	Approx. equimolar mixture of 4-CG and 1,6-DCF	20 mg/kg bw per day (males) 26 mg/kg bw per day (females)	18 mg/kg bw per day (males) 24 mg/kg bw per day (females)
26-week oral toxicity study in dogs with an equimolar mixture 1,6-DCF and 4-CG	Approx. equimolar mixture of 4-CG and 1,6-DCF	26 mg/kg bw per day	24 mg/kg bw per day
104-week carcinogenicity study in rats with an equimolar mixture of 1,6-DCF and 4-CG	Approx. equimolar mixture of 4-CG and 1,6-DCF	10 mg/kg bw per day (males) 13 mg/kg bw per day (females)	9 mg/kg bw per day (males) 12 mg/kg bw per day (females)
Two generation reproductive study in rats on an equimolar mixture of 4-CG and 1,6-DCF	Approx. equimolar mixture of 4-CG and 1,6-DCF	20 mg/kg bw per day	18 mg/kg bw per day
Study on the effects of oral admin. upon pregnancy of an approx. equimolar mixture of 4-CG and 1,6-DC on rat	Approx. equimolar mixture of 4-CG and 1,6-DCF	47 mg/kg bw per day	43 mg/kg bw per day
Study on effects of 1,6-DCF on male fertility in the rat	1,6-DCF	65mg/kg bw per day	–
Antifertility screen on 4-CG	4-CG	–	60 mg/kg bw per day
Study on the neurotoxic potential of an equimolar mixture of 4-CG and 1,6-DCF in mice	Approx. equimolar mixture of 4-CG and 1,6-DCF	520 mg/kg bw per day	480 mg/kg bw per day
Study on the neurotoxic potential of an equimolar mixture of 4-CG and 1,6-DCF in marmoset monkey	Approx. equimolar mixture of 4-CG and 1,6-DCF	520 mg/kg bw per day	480 mg/kg bw per day

APPENDIX F

Exposure calculations to impurities from the use of sucralose as a food additive

The potential exposure to impurities from the use of E 955 was calculated by assuming that they are present in the food additive up to a certain limit value, and then by calculation pro-rata to the estimates of exposure to the food additive itself.

For the current assessment, the refined brand-loyal exposure scenario was considered as the most representative and the highest exposure levels for the mean and 95th percentile among the different population groups were considered, i.e. 5 and 14 mg/kg bw per day, for toddlers, respectively (Table 7).

F.1 | Inorganic impurities

Analytical data on the analysis by inductively coupled plasma-mass spectrometry (ICP-MS) of a wide range of elements including lead, arsenic, cadmium, mercury and tin in six samples of E955, and of aluminium in five samples were submitted (Documentation provided to EFSA No 2a). In all samples the elements were reported below the reporting limits of 0.02 mg/kg for arsenic, lead and tin, 0.01 mg/kg for cadmium and mercury, and 0.5 mg/kg for aluminium, except for a sample in which a level of 0.04 mg/kg for arsenic was reported.

Upon a request from EFSA, the same IBO provided analytical results for tin in 10 other samples of E 955 (five batches of micronised sucralose and five of granular sucralose), as evidence of the absence of potential impurities resulting from the use of a tin-containing reagent in the manufacturing process of sucralose (Documentation provided to EFSA No 3). In all analysed samples, tin was reported as below the LOQ of 0.05 mg/kg. Tin was also analysed by ICP-MS in 2 additional samples and the results reported were still below the LOQ, at 0.005 and 0.02 mg/kg.

Another IBO provided analytical results for the analysis of arsenic, lead, cadmium, mercury and tin in 3 samples of E 955 (Documentation provided to EFSA No 5). In all samples, the elements were reported either as not detected or below their respective LOQ (arsenic, 0.01–0.04 mg/kg; lead, 0.04 mg/kg; cadmium, 0.003 mg/kg; mercury 0.01 mg/kg and tin 0.03 mg/kg).

No proposal for the lowest technologically achievable levels for impurities, including toxic elements, was provided by any IBO.

As described in the manufacturing processes provided by the IBOs (see Section 3.1.2), the food additive E 955 is a product of chemical synthesis from sucrose, involving several separation and purification steps. Taking this into account, along with the analytical data reported above, the Panel did not consider it necessary to perform the risk assessment of toxic elements, except for lead, for which a limit in the current EU specification is set.

The Panel noted that based on the data submitted by the IBOs, the reported data for lead are substantially lower than the current limit in the EU specifications and considered that the maximum limit in the EU specifications for lead should be established based on actual levels in the food additive.

The level of lead in the food additive combined with the estimated intakes of E 955 (Table 7), could result in an exposure which can be compared with the reference point of 0.5 μg/kg bw per day (BMDL01) established by the EFSA CONTAM Panel (2010) (Table F.1).

TABLE F.1 Reference points for lead potentially present in the food additive E 955.

Element/RP	Basis/reference
Lead (Pb)/0.5 mg/kg bw per day ($BMDL_{01}$)	The reference point is based on a study demonstrating perturbation of intellectual development in children with the critical response size of 1 point reduction in IQ. The EFSA CONTAM Panel mentioned that a 1-point reduction in IQ is related to a 4.5% increase in the risk of failure to graduate from high school and that a 1-point reduction in IQ in children can be associated with a decrease of later productivity of about 2%. A risk cannot be excluded if the exposure exceeds the $BMDL_{01}$ (MOE lower than 1). EFSA CONTAM Panel (2010)

The Panel assessed the risk that would result if lead was present in E 955 at: (i) the existing limit in EU specifications; (ii) considering the reported limit of 0.02 mg/kg modulated by the Panel by applying a factor of 10, to allow flexibility with respect to representativeness and homogeneity. The outcome of the risk assessment of the Panel is presented in Table F.2.

TABLE F.2 Risk assessment for lead from the use of E 955.

	MOE for Pb
Exposure to E 955 (mg/kg bw per day)	**(i) Considering a potential presence of lead at the current limit of 1 mg/kg of the EU specifications for E 955 (Commission Regulation (EU) No 231/2012)**
5[a]	100
14[b]	35
	(ii) Considering a potential presence of lead at the reported limit of 0.02 mg/kg modulated by the Panel by applying a factor of 10 to give a value of 0.2 mg/kg for the calculations
5[a]	500
14[b]	180

[a]Highest exposure level among the different population groups (refined brand-loyal scenario – toddlers – mean (Table 7).

[b]Highest exposure level among the different population groups (refined brand-loyal scenario – toddlers – 95th percentile (Table 7).

Results in Table F.2 show that the potential presence of lead in E 955 at the current exposure estimates is not of toxicological concern.

The Panel recommended to lower the EU specification limit for lead taking into account (i) the results of the calculations performed by the Panel (Table F.2) and that (ii) the maximum limits should be established based on actual levels in the commercial food additive. If the European Commission decides to revise the current limits for lead in the EU specifications, the values in Table F.2 could be considered.

F.2 | Organic impurities

Currently a limit for a maximum amount of chlorinated monosaccharides, chlorinated disaccharides and TPPO of 0.1%, 0.5% and 150 mg/kg (0.015%) respectively, are included in the EU specifications.

A limit for TPPO of 150 mg/kg is included in the specifications for E 955. This substance is not used in the manufacturing processes reported by the IBOs and therefore, no analytical data on the level of this impurity in the food additive is available (Section 3.1.2). However, the use of TPPO in other manufacturing processes of E995 cannot be excluded. Thus, the Panel assessed the risk that would result if TPPO was present in E 955 at the existing limit of 150 mg/kg in EU specifications. From a 90-day study in rat (dietary administration) reported in the REACH registration dossier,[72] the Panel identified a NOAEL of 10 mg/kg bw per day, based on several effects.[73] The Panel considered that this NOAEL identified from a 90-day study should be corrected for the time extrapolation from sub-chronic to chronic dosing using a factor of 2 (EFSA Scientific Committee, 2012) which would result in a chronic NOAEL of 5 mg/kg bw per day and used this value as a reference point. When comparing the potential exposure to TPPO from the use of E955 (Table F.3) with the reference points indicated above, the Panel noted that the MOE would be higher than 100 and no safety concern is raised.

Chlorinated monosaccharides, 4-CG and 1,6-DCF, are also degradation products resulting from the hydrolysis of sucralose under acidic conditions. Reported concentrations of 1,6-DCF in analysed samples range from 25 to 185 mg/kg, while levels of 4-CG range from 23 to 169 mg/kg (Section 3.1.1).

As presented in Section 3.1.1, chlorinated disaccharides, some of them different from the ones reported as impurities in the European Pharmacopoeia, have been quantified in E955. Sucralose-6-acetate has been reported as not detected at an LOD of 1 mg/kg in all analysed samples. The maximum amount for the sum of the reported chlorinated disaccharides was 0.42%.

Based on the available genotoxicity studies and in silico analysis of the potential organic impurities identified in E955, the Panel considered that there is no concern for their genotoxicity (Section 3.5.3.2).

Chlorinated disaccharides share the same basic structure as sucralose but contain two, three or four chlorine atoms at different positions within the molecule. The Panel noted that these substances, as potential impurities of sucralose, were likely present in the test material used in the toxicity studies available in the database. Consequently, their potential toxicity is expected to have been covered by the studies considered in the safety assessment of sucralose.

Regarding 4-CG and 1,6-DCF, the Panel has reviewed toxicological studies performed with an equimolar mixture of both substances (see Appendix E). Based on those studies, the Panel identified a NOAEL of 10 mg/kg bw per day for 1,6-DCF and of 9 mg/kg bw per day for 4-CG based on decrease in body weight observed in a 104-week chronic and carcinogenicity study. The Panel considered these NOAELs as reference points to assess the risk that would result if these impurities would be present in E 955 at the rounded up highest measured value of 200 mg/kg (Commission Regulation 231/2012). When comparing the potential exposure to 4-CG and 1,6-DCF as impurities from the use of E955 (Table F.3) with the reference points indicated above, the Panel noted that the MOE would be higher than 100 and no safety concern is raised.

[72]https://chem.echa.europa.eu/100.011.217/dossier-view/3bae81e3-b05e-4071-a81b-9e5aef1812c7/IUC5-51b36c8c-f0d2-44c7-9a7a-83d54cb1be89_539cab4f-184e-4a06-a9aa-186a792704d7

[73]From the REACH registration dossier at 50 mg/kg bw per day several effects were reported: reduced food consumption and bw gain, clinical signs, haematological and clinic chemical alterations, necropsy findings, mainly reversible; degeneration in the hepatic epithelium not completely reversible.

TABLE F.3 Risk assessment for organic impurities.

Exposure to E 955 (mg/kg bw per day)	Exposure (mg/kg bw per day) to 4-CG or 1,6-DCF at a concentration of 200 mg/kg	MOE 1,6-DCF	MOE 4-CG	Exposure to TPPO (mg/kg bw per day) at the limit of 150 mg/kg in the current EU specifications	MOE TPPO
5[a]	0.001	10,000	9000	0.00075	13,333
14[b]	0.0028	3571	3214	0.0021	4762

[a]Highest exposure level among the different population groups (refined brand-loyal scenario – toddlers – mean (Table 7).
[b]Highest exposure level among the different population groups (refined brand-loyal scenario – toddlers – 95th percentile (Table 7).

Taking into account the provided analytical data, and the results of the calculations performed by the Panel (Table F.3), the Panel recommended including individual limits for 4-CG and 1,6-DCF in the specifications of E955 and accordingly to delete the limit for chlorinated monosaccharides.

Based on the available information provided by the IBOs on the stability of sucralose in aqueous acidic solutions (see Section 3.1.4), the formation of 4-CG and 1,6-DCF as degradation products may occur when sucralose is used in flavoured drinks, that have a pH that tends to promote hydrolysis. Elevated temperatures further enhance hydrolysis, and the extent of degradation also increases with time. The Panel noted discrepancies between the results of different studies provided, no detectable loss of sucralose reported in a carbonated cola beverage stored at 20°C for 12 months, while in a fruit-based orange drink an approximately 10% loss after 3 months at 20°C was indicated (Documentation provided Annex F old studies).

The exposure to sucralose from its use in FC 14.1.4 'Flavoured drinks' was calculated (Annex A), with the highest mean exposure estimated at 3.8 mg/kg bw per day in toddlers. Assuming a worse-case scenario where 10% of sucralose is hydrolysed, this would correspond to an exposure of approximately 0.38 mg/kg bw per day for either 4-CG or 1,6-DCF from the consumption of flavoured drinks. Under this specific scenario, sucralose used in flavoured drinks and stored at an elevated temperature for an extended period of time, the MOE for both, 4-CG or 1,6-DCF, would be below 100 (Table F.4) which would be considered as a concern. The Panel however noted that the percentage of hydrolysis could be considerably lower depending on the specific beverage and storage conditions. This introduces uncertainty regarding the actual extent of degradation under normal conditions of use, and consequently the level of concern, as illustrated in Table F.4.

TABLE F.4 Risk assessment of degradation products under various scenarios of potential sucralose degradation by hydrolysis in Food Category 14.1.4.

Exposure to E 955 (mg/kg bw per day) from its use in FC 14.1.4	Exposure (mg/kg bw per day) to 4-CG or 1,6-DCF as degradation products	MOE 1,6-DCF	MOE 4-CG
3.8	0.38[a]	26	24
3.8	0.08[b]	125	113

[a]Exposure to the degradation products, assuming 10% degradation of sucralose by hydrolysis.
[b]Exposure to the degradation products, assuming 2% degradation of sucralose by hydrolysis.

APPENDIX G

BMD analysis

G.1 | SETTING OF THE BMR

Decreases in body weight higher than 10% were observed, particularly in the combined chronic and carcinogenicity study in rats. Therefore, the dose-effect data from this study were used to perform a Benchmark dose analysis (see Annex G). In the case of sucralose, the Panel noted that the observed response is in part driven by poor palatability and consequent reduced feed intake. The Panel estimated, from the results of the pair fed dietary restriction study (Documentation provided to EFSA No 1), that about half of the response (decrease in body weight) could be attributable to reduced palatability of the test diet and set a BMR of 15% on this basis: see explanation under Section G.2.

The BMD, BMDL and BMDU values obtained considering a BMR of 10%, 15% and 20% are reported in Section G.3.

G.2 | ANALYSIS OF THE RELATIVE CONTRIBUTION OF PALATABILITY AND TOXICITY ON THE DECREASE IN BODY WEIGHT

Palatability study

The palatability study (Documentation provided to EFSA No 1) included two parts phase I study and phase II study. The Panel considered only the results of phase II (pair fed study).

In phase II, three groups of Charles River CD(SD)BR rats (5/sex/group) were used. One group received diet with 5% sucralose ad libitum, while two other groups received either a control diet (0% sucralose) but was pair-fed with the group administered 5% sucralose in the diet or a control diet ad libitum. Clinical appearance was monitored daily, body weight and feed intake twice a week. Water intake was not quantified but noted daily. At the end of the treatment, all rats were subjected to necropsy. Gross abnormalities and weights of adrenals, kidneys, livers, spleens, brains, caeca and testes were recorded. Microscopic examination of the tissues/organs was not included in this study. Sucralose at 5% in the diet did not affect clinical condition. Feed or water consumption were similar across the three groups for males but not for females (mean total feed intake higher in the 5% sucralose group). A statistically significant lower body weight gain was recorded for males and females from 5% sucralose group relative to the pair-fed and ad libitum fed control groups. There were no gross abnormalities attributable to the sucralose administration apart from an enlargement of the caecum in 5% sucralose group. The relative organ weights were not statistically significant different in 5% sucralose group from those in the pair-fed and ad libitum fed controls except for a statistically significant increased relative caecum weight (Table G.1).

TABLE G.1 Mean body weight at the start, at termination, mean body weight gain during the 28 days of the treatment, mean total feed intake, mean absolute caecum weight (Documentation provided to EFSA No 1).

	Males					Females				
Group	Mean BW initial (g)	Mean BW terminal (g)	Mean BW gain (g)	Mean total feed intake (g)	Mean absolute caecum weight (g)	Mean BW initial (g)	Mean BW terminal (g)	Mean BW gain (g)	Mean total feed intake (g)	Mean absolute caecum weight (g)
5% sucralose	238	346	108	725	1.98	177	224	47	662	1.75
Pair-fed control	243	382	139	701	1.69	164	206	42	497	0.93
Control ad libitum	238	398	160	728	1.58	173	234	59	576	1.21

Note: Mean values for the groups were calculated based on individual data.

To estimate the (potential) effects of either palatability alone or sucralose alone, on body weight, the Panel compared body weight between those groups fed ad libitum and those in the pair-fed groups:

1. Calculation of body weight difference **due to sucralose consumption only** (Column D): difference between body weight of pairs of treated animals (Column B) and their respective pair-fed animals (Column C).

2. Calculation of body weight difference **due to palatability and sucralose consumption** (Column E): difference of the sucralose treated animal (Column B) and the mean value of the control animals (column A, mean).

3. Calculation of **mean percentage of body weight difference due to sucralose treatment only** (Column F): ratio of mean body weight differences of treated – pair-fed (Column D) to treated – mean control (Column E) multiplied by 100.

TABLE G.2 Data on final body weight (at 28 days) of pair-fed and control animals (Documentation provided to EFSA No 1) and respective calculations following step 1 to 3 described above.

Sex	A: Control final body weight, g	B: Treated final body weight, g	C: Control - pair-fed final body weight, g	D: Treated – Pair-fed final body weight, g	E: Treated – Mean control body weight, g	F: % red. b.w. sucralose only
Male	413	330	374	−44	−67.6	
Male	372	316	358	−42	−81.6	
Male	381	360	372	−12	−37.6	
Male	415	311	355	−44	−86.6	
Male	407	414	449	−35	16.4	
Mean	***398 +/− 18***			***−35 +/− 12***	***−51.4***	***69***
Female	240	219	163	56	−15.4	
Female	236	208	195	13	−26.4	
Female	217	233	235	−2	−1.4	
Female	235	232	210	22	−2.4	
Female	244	228	228	0	−6.4	
Mean	***234+/−9***			***18+/−21***	***−10.4***	**n.d.**

The pair-fed female animals gained not as much weight as did the treated ones, resulting in no consistence body weight difference. In fact, the data on feed consumption (Table G.2, Column D–F) show, that treated females consume about 115% of the feed consumed by the ad libitum control, whereas the pair-fed females consume 86% of the ad libitum control. For this reason, the results for females were not considered further.

69% of body weight reduction in males can be attributed to sucralose consumption only (Table G.2) and this only due to the treatment with sucralose.

Pair fed dietary restriction study

In a **pair fed dietary restriction study (**Documentation provided to EFSA No 1, please refer to Annex C for a detailed description of the study) 20 animals per sex and group were fed for 26 weeks with: (a) standard diet ad libitum (controls); (b–d) 95%, 90% and 85% restriction of controls; (e–f) 1% and 3% sucralose in the diet and ad libitum consumption; (g–h) 1% and 3% sucralose in the diet with 90% restriction.

The authors calculated the impact of sucralose on terminal body weight by regression analysis and concluded that 4%–6% lower total body weight was achieved due to 3% sucralose in diet, whereas 1% sucralose had no significant impact (Table G.3).

In order to assess the impact of sucralose alone on difference in terminal body weight the following approach was chosen:

1. Selection of mean terminal group body weight of controls, 95% feed restriction and 1% and 3% sucralose in the diet with ad libitum consumption. The Panel considered the data from the 95% feed restriction group since this group showed similar feed consumption data versus the treated groups, whereas the other restricted groups differed in feed consumption. The Panel further noted that the results using the 90% feed restriction group would be similar.

Calculation of the impact of feed restriction only on body weight (Column A)

2. Calculation of the impact of **sucralose treatment and reduced feed consumption** due to palatability on body weight (Column B (1% sucralose), Column C (3% sucralose))
3. Calculation of the effect of **sucralose treatment only** on body weight by subtraction of the impact of feed restriction (Column D (1% sucralose), Column E (3% sucralose))
4. Calculation of the percentage of the effect on body weight by sucralose treatment only related to the respective effect of sucralose and palatability (Column F (1% sucralose), Column G (3% sucralose))

TABLE G.3 Terminal body weight of control, 95% feed restriction and treated groups (1% and 3% ad libitum) and calculation of the effect size attributable to sucralose treatment only (Documentation provided to EFSA No 1).

	Control	95%	1% sucr	3% sucr	A:95% - control	B:1%sucr - control	C:3% sucr - control	D: Effect only 1%, (B-A)	E: Effect only 3%, (C-A)	F: % effect only 1%	G: % effect only 3%
Males											
Term. b.w.	796.6	720.4	703	681.7	−76.2	−93.6	−114.9	−17.4	−38.7	19	34
%SD	10	6	12	8							
Females											
Term.b.w	407.2	353.4	360.1	340.9	−53.8	−47.1	−66.3	*6.7*	−12.5	n.d.	19
%SD	15	7	12	13							

3% sucralose in the diet has an impact on terminal body weight. Effects are more pronounced in males than females, with 34% reduced terminal body weight in males and 19% in females.

G.3 | BMR SETTING AND BMD RESULTS

The Panel estimated, from the analysis of data from the pair fed animals study that about half of the response (decrease in body weight) could be attributable to reduced palatability of the test diet and the other half to toxicity of sucralose. This is supported by the analysis of the data from the palatability study (males only). The Panel considered this in setting the BMR to be considered in the BMD analysis.

The BMD, BMDL and BMDU obtained considering a BMR of 10%, 15% and 20% are reported in Section G.3. The Panel noted that the range in the BMDL and BMDU for males only when a BMR of 20% is used is large. The Panel additionally noted that the variation in the body weight of the control group in the combined chronic and carcinogenicity study in rats (expressed as standard deviation from the mean) at the end of the experiment was 15% for males and 20% for females. Taking a conservative approach and considering the outcome of the analysis in Section G.2, the results of the BMD with a BMR of 15% were further considered in the assessment (see Section 3.5.5, Table G.4).

TABLE G.4 BMD, BMDL and BMDU obtained considering a BMR of 10%, 15% and 20% for the endpoint body weight using the data from Documentation provided to EFSA No 1.

BMR		BMDL	BMD	BMDU
10%	M	34.643	69.144	123.988
	F	28.344	79.465	195.249
15%	M	60.651	109.716	221.389
	F	54.906	117.148	239.927
20%	M	72.604	261.539	2376.525
	F	79.69	177.317	403.956

ANNEXES

Annexes A–F can be found in the online version of this output ('Supporting information' section).

Annex A – Exposure data and estimates

Annex B – ADME studies

Annex C – Data extraction: toxicological studies

Annex D – Data extraction: genotoxicity studies

Annex E – Outcome of the risk of bias assessment

Annex F1 – Weight of Evidence (WoE) tables: animal studies

Annex F2 – Weight of Evidence (WoE) tables: human studies

Annex G – BMD report

Annex H – Environmental data