Info session: (Re-)Evaluating Food Additives
DAY 2: 20 March 2024
SESSION 2 | Future work

EMERGING CHANGES IN THE EU SPECIFICATIONS OF AUTHORISED FOOD ADDITIVES

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EUROPEAN FOOD SAFETY AUTHORITY

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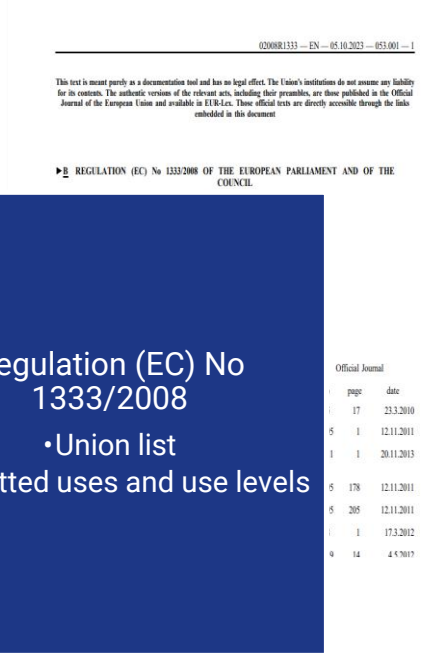
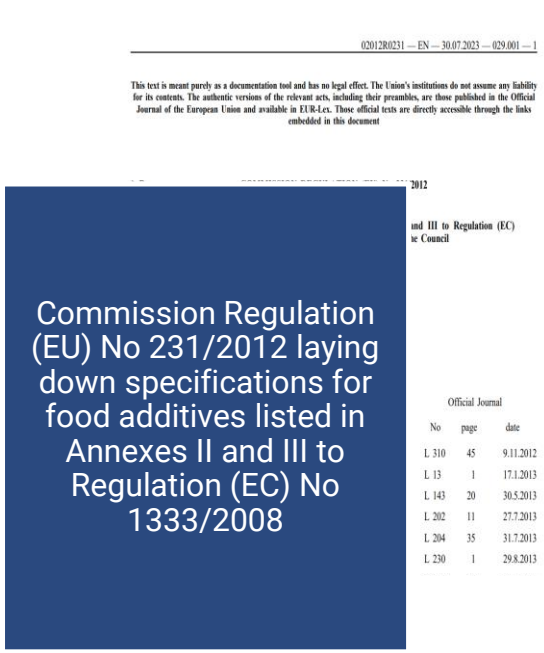
REGULATORY FRAMEWORK

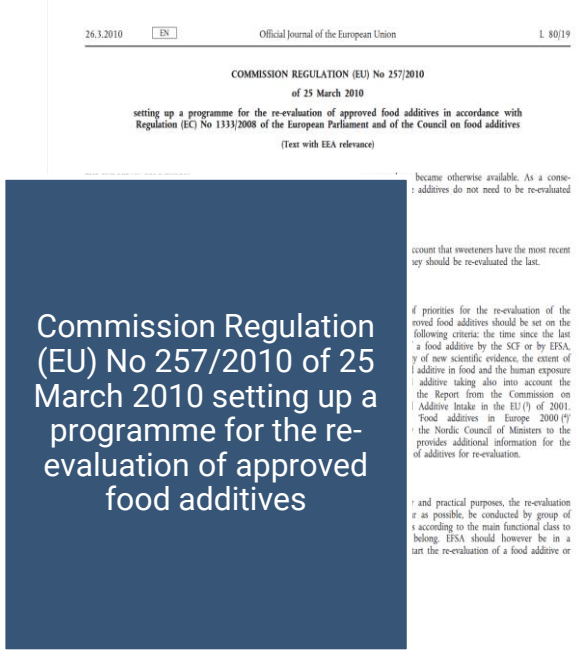


Regulation (EC) No 1333/2008

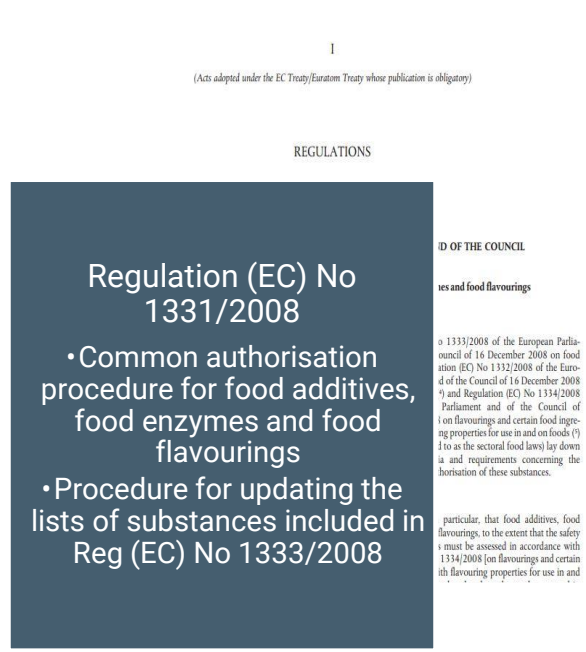
- Union list
- Permitted uses and use levels



Commission Regulation (EU) No 231/2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008



Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives

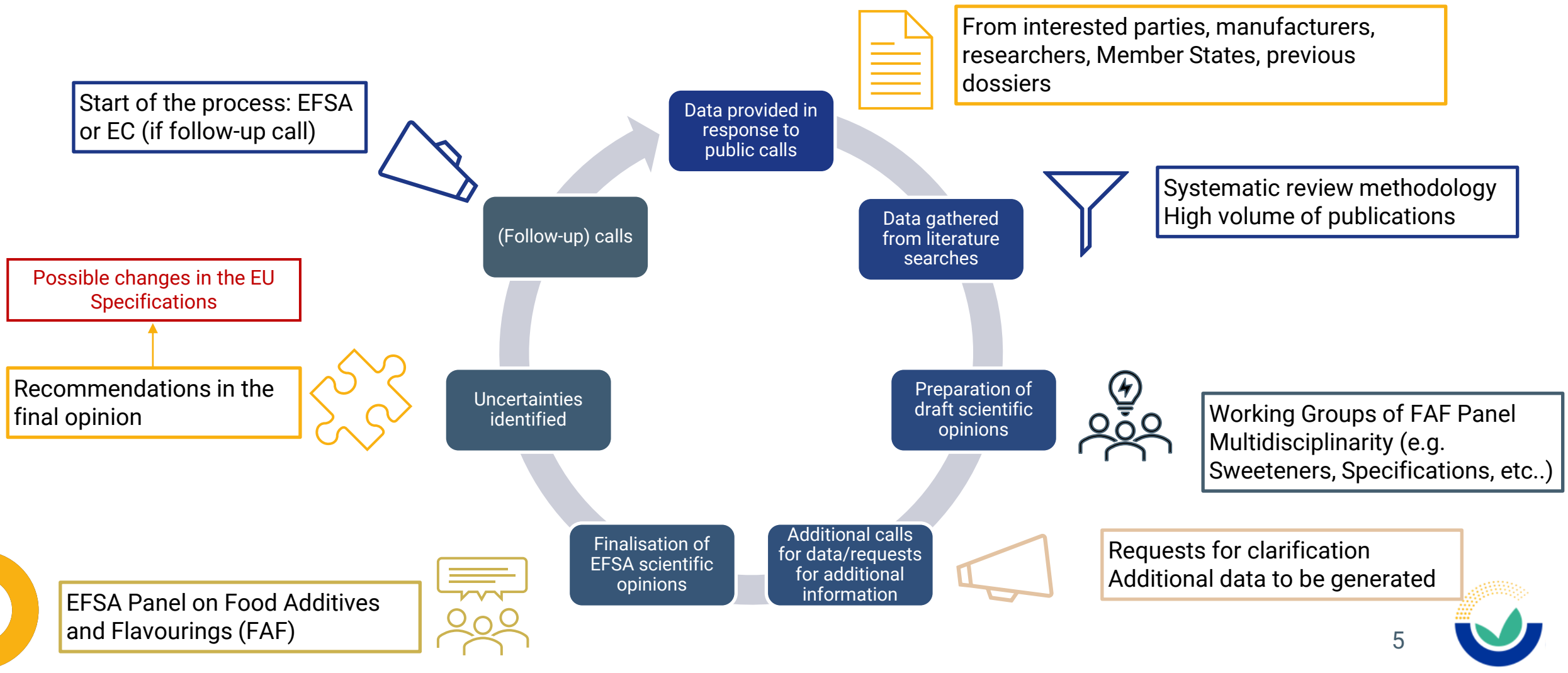


Regulation (EC) No 1331/2008

- Common authorisation procedure for food additives, food enzymes and food flavourings
- Procedure for updating the lists of substances included in Reg (EC) No 1333/2008

Guidance on submission of food additives (2012) (to be updated within 2024)

RE-EVALUATION OF FOOD ADDITIVES/FOLLOW UP PROCESS



NEW FOOD ADDITIVES: COMMON AUTHORISATION PROCEDURE

Applicants submit their dossiers to the European Commission

Through the E-Submission Food Chain Platform

Commission may ask EFSA for an opinion (mandate)

Communicates with:

FDP - suitability check

FIP - risk assessment

EFSA must give an opinion within 9 months of receipt of a valid application

Those months without including the ADRs timing, meaning when the application is under Stop the Clock

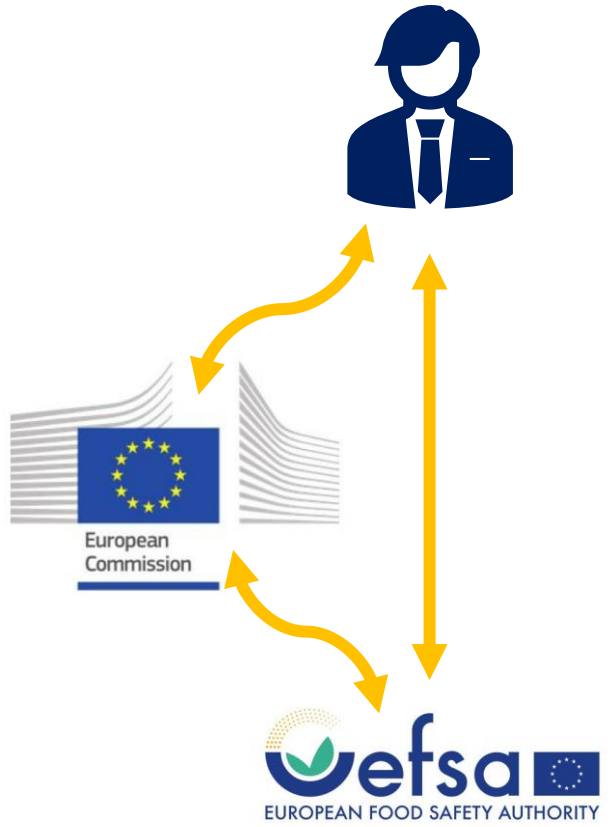
Indicative Timelines

The result may be positive, negative, or inconclusive

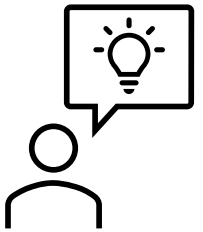
Commission acts accordingly to the outcome

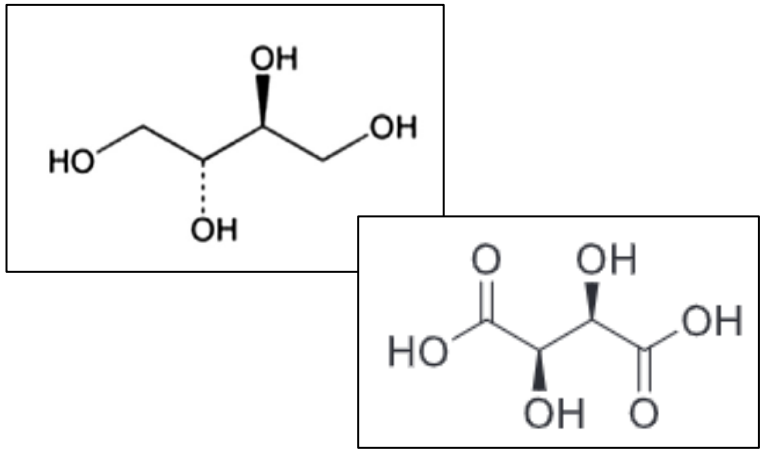
The legislation may be amended

A new food additive may be added in the Union list – Possible changes in the EU Specifications



EMERGING CHANGES: EXAMPLES





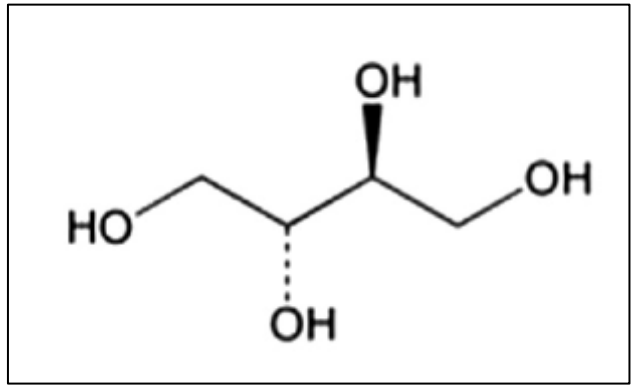
CHANGES IN THE MANUFACTURING PROCESS OR STARTING MATERIALS:

THE CASE OF ERYTHRITOL (E 968) AND TARTARIC ACID AND TARTRATES (E 334-337 AND 354)

THE CASE OF ERYTHRITOL (E 968)

- Erythritol is a polyol belonging to the **sweeteners**.

	DEFINITION
E 968 ERYTHRITOL	Obtained by fermentation of carbohydrate source by safe and suitable food grade osmophilic yeasts such as Moniliella pollinis or Moniliella megachilensis, followed by purification and drying



- Erythritol was **re-evaluated by EFSA in December 2023**, in the context of the ongoing re-evaluation of sweeteners under Regulation (EC) No 257/2010.

RE-EVALUATION OF ERYTHRITOL (E 968)

Detailed information provided by the IBOs on the **characterisation of the microorganisms and the demonstration of the absence of viable cells** in erythritol:

The manufacturing process of E 968 does not raise a safety concern

The Panel recommended **modifying the definition** to specify that E 968 is obtained by fermentation of a carbohydrate source **by non-genetically modified** M. pollinis **strain BC** or M. megachiliensis **strain KW3-6**, followed by several purification steps and drying

In addition, the Panel emphasised that the **present re-evaluation does not apply** to erythritol (E 968) produced by **other manufacturing processes (e.g. different microorganisms, strains)**

Significant changes in the production methods → Requirement of an assessment in accordance with relevant legislation

PROPOSALS OF THE PANEL

The FAF Panel proposed **amendment of the Specifications:**

- **Definition** (Introduction of the **strains**)
- **CAS No** (Introduction 149–32-6)
- **Purity** (Lowering the limit of toxic element lead)

Adopted: 25 October 2023
DOI: 10.2903/J.efsa.2023.8430

SCIENTIFIC OPINION

efsa JOURNAL

Re-evaluation of erythritol (E 968) as a food additive

EFSA Panel on Food Additives and Flavourings (FAF) | Maged Younes | Gabriele Aquilina | Laurence Castle | Gisela Degen | Karl-Heinz Engel | Paul J. Fowler | Maria José Frutos Fernandez | Peter Fürst | Ursula Gundert-Remy | Rainer Gürtler | Trine Husøy | Melania Manco | Wim Mennes | Peter Moldeus | Sabina Passamonti | Romina Shah | Ine Waalkens-Berendsen | Matthew Wright | Monika Batke | Polly Boon | Ellen Bruzell | James Chipman | Riccardo Crebelli | Rex FitzGerald | Cristina Fortes | Thorhallur Halldorsson | Jean-Charles LeBlanc | Oliver Lindtner | Alicja Mortensen | Evangelia Ntzani | Heather Wallace | Stefania Barmaz | Consuelo Civitella | Lorenzo D'Angelo | Federica Lodi | Marcello Laganaro | Ana Maria Rincon | Camilla Smeraldi | Alexandra Tard

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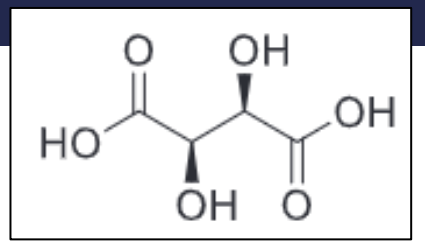
Abstract
This opinion addresses the re-evaluation of erythritol (E 968) as food additive and an application for its exemption from the laxative warning label requirement as established under Regulation (EU) No 1169/2011. Erythritol is a polyol obtained by fermentation with *Moniliella pollinis* BC or *Moniliella megachiliensis* KW3-6, followed by purifications and drying. Erythritol is readily and dose-dependently absorbed in humans and can be metabolised to erythronate to a small extent. Erythritol is then excreted unchanged in the urine. It does not raise concerns regarding genotoxicity. The dataset evaluated consisted of human interventional studies. The Panel considered that erythritol has the potential to cause diarrhoea in humans, which was considered adverse because its potential association with electrolyte and water imbalance. The lower bound of the range of no observed adverse effect levels (NOAELs) for diarrhoea of 0.5 g/kg body weight (bw) was identified as reference point. The Panel considered appropriate to set a numerical acceptable daily intake (ADI) at the level of the reference point. An ADI of 0.5 g/kg bw per day was considered by the Panel to be protective for the immediate laxative effect as well as potential chronic effects, secondary to diarrhoea. The highest mean and 95th percentile chronic exposure was in children (742 mg/kg bw per day) and adolescents (1532 mg/kg bw per day). Acute exposure was maximally 3531 mg/kg bw per meal for children at the 99th percentile. Overall, the Panel considered both dietary exposure assessments an overestimation. The Panel concluded that the exposure estimates for both acute and chronic dietary exposure to erythritol (E 968) were above the ADI, indicating that individuals with high intake may be at risk of experiencing adverse effects after single and repeated exposure. Concerning the new application, the Panel concluded that the available data do not support the proposal for exemption.

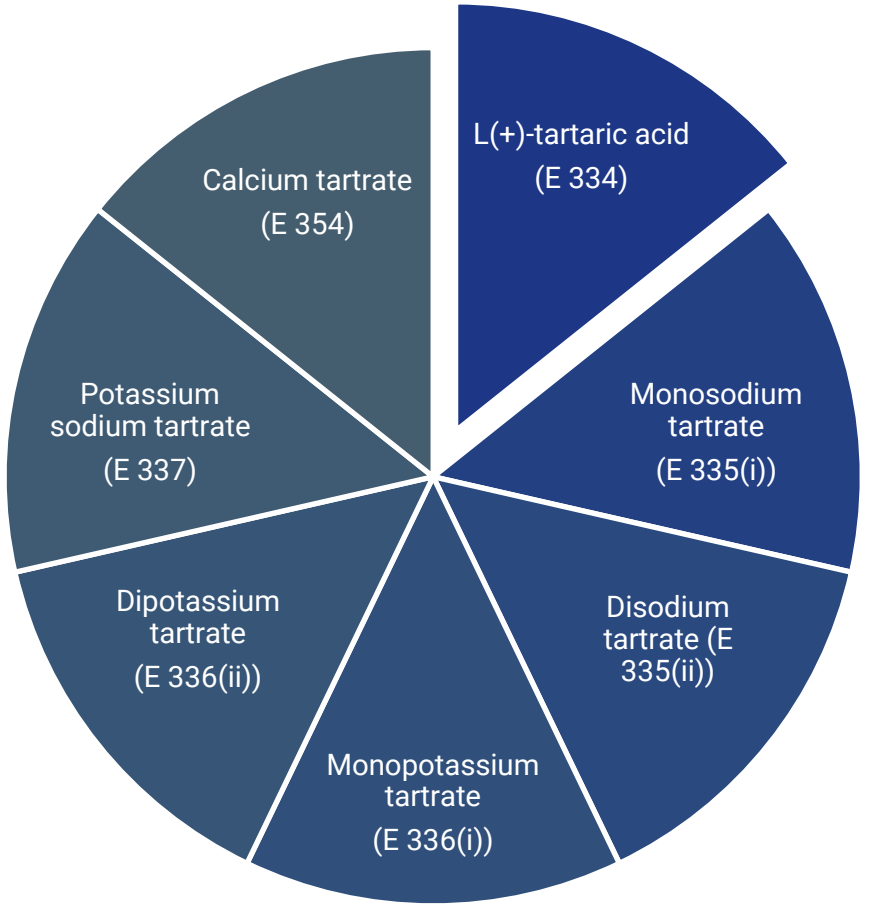
KEYWORDS
diarrhoea, E 968, erythritol, food additive, laxative, sweeteners

TARTARIC ACID AND TARTRATES (E 334-337 AND E 354)



- **Authorised food additives**

- **No definition** set in the EU Specifications, **describing the manufacturing process** used.



RE-EVALUATION: MANUFACTURING PROCESS OF TARTARIC ACID AND TARTRATES

- According to the data provided by the IBOs in the re-evaluation, tartaric acid may be **manufactured by two different methods**

- The Panel considered that the **manufacturing process** of L(+)-tartaric acid from chemical/microbiological production may result in **impurities different** from those that may be present in L(+)-tartaric acid as a by-product of wine production

As a **by-product of wine**

From **chemical/microbiological production**

Maleic anhydride is the starting material converted enzymatically to L(+)-tartaric acid

The L(+)-tartaric acid is obtained by biocatalysis using **immobilised cells of the bacteria Rhodococcus rube strain CM001 or Rhodococcus sp strain USA-AN01**

Toxic elements present such as (vanadium, molybdenum and tungsten) from the use of **catalysts** in the conversion of butane to maleic anhydride and maleic anhydride to disodium cis-epoxysuccinate solution

No viable cells, fully DNA fully degraded → not considered to pose a safety concern

PANEL CONSIDERATIONS FOR TARTARIC ACID AND TARTRATES

- The Panel, therefore, considered that **separate specifications** would be needed for L(+)-tartaric acid from **chemical/microbiological** production using Rhodococcus ruber strain CM001 or Rhodococcus sp strain USA-AN012

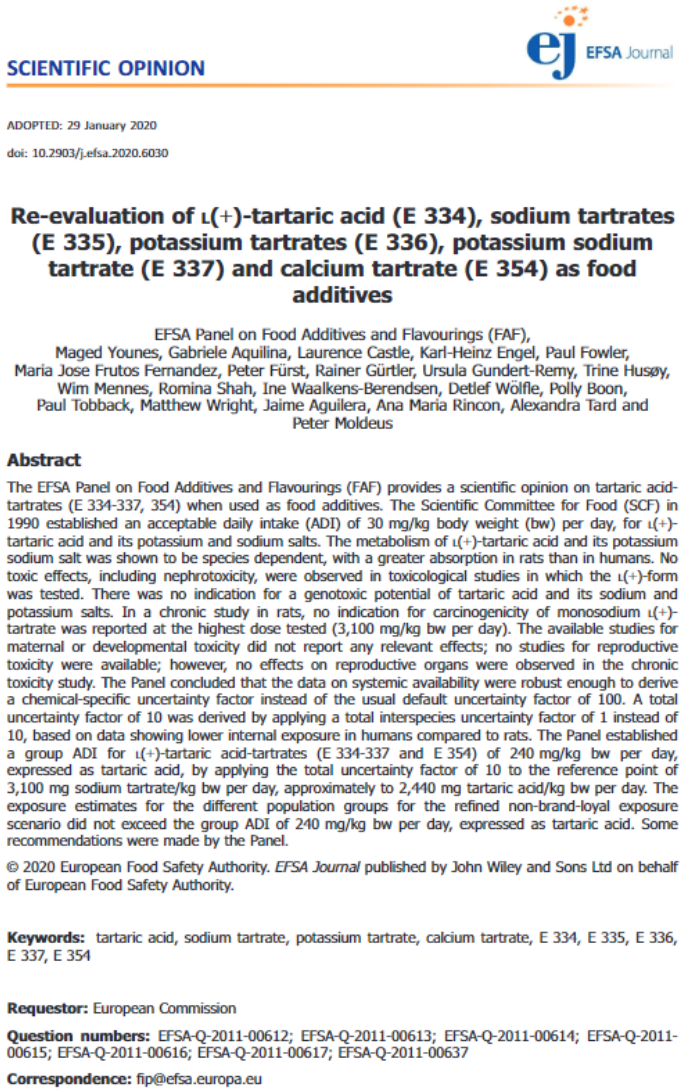
- The Panel noted that in the **peer-reviewed literature** the manufacturing process of L(+)-tartaric acid **using other microorganisms** for the bioconversion are described

- However, **no interested parties have indicated their use** and, therefore, they have **not** been **assessed** by the Panel

PROPOSALS OF THE PANEL

The FAF Panel proposed **amendment of the Specifications**:

- **Separate specifications** for L(+)-tartaric acid from chemical/microbiological production
- Specific rotation (**Introduction**)
- **Revision of wording** specific parameters (e.g., molecular weight)
- **Purity** (Lowering the limit of toxic elements lead, mercury and arsenic)



SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 29 January 2020

doi: 10.2903/j.efsa.2020.6030

Re-evaluation of L(+)-tartaric acid (E 334), sodium tartrates (E 335), potassium tartrates (E 336), potassium sodium tartrate (E 337) and calcium tartrate (E 354) as food additives

EFSA Panel on Food Additives and Flavourings (FAF),
Maged Younes, Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Rainer Gürtler, Ursula Gundert-Remy, Trine Husøy, Wim Mennes, Romina Shah, Ine Waalkens-Berendsen, Detlef Wölfle, Polly Boon, Paul Tobback, Matthew Wright, Jaime Aguilera, Ana Maria Rincon, Alexandra Tard and Peter Moldeus

Abstract

The EFSA Panel on Food Additives and Flavourings (FAF) provides a scientific opinion on tartaric acid-tartrates (E 334-337, 354) when used as food additives. The Scientific Committee for Food (SCF) in 1990 established an acceptable daily intake (ADI) of 30 mg/kg body weight (bw) per day, for L(+)-tartaric acid and its potassium and sodium salts. The metabolism of L(+)-tartaric acid and its potassium sodium salt was shown to be species dependent, with a greater absorption in rats than in humans. No toxic effects, including nephrotoxicity, were observed in toxicological studies in which the L(+)-form was tested. There was no indication for a genotoxic potential of tartaric acid and its sodium and potassium salts. In a chronic study in rats, no indication for carcinogenicity of monosodium L(+)-tartrate was reported at the highest dose tested (3,100 mg/kg bw per day). The available studies for maternal or developmental toxicity did not report any relevant effects; no studies for reproductive toxicity were available; however, no effects on reproductive organs were observed in the chronic toxicity study. The Panel concluded that the data on systemic availability were robust enough to derive a chemical-specific uncertainty factor instead of the usual default uncertainty factor of 100. A total uncertainty factor of 10 was derived by applying a total interspecies uncertainty factor of 1 instead of 10, based on data showing lower internal exposure in humans compared to rats. The Panel established a group ADI for L(+)-tartaric acid-tartrates (E 334-337 and E 354) of 240 mg/kg bw per day, expressed as tartaric acid, by applying the total uncertainty factor of 10 to the reference point of 3,100 mg sodium tartrate/kg bw per day, approximately to 2,440 mg tartaric acid/kg bw per day. The exposure estimates for the different population groups for the refined non-brand-loyal exposure scenario did not exceed the group ADI of 240 mg/kg bw per day, expressed as tartaric acid. Some recommendations were made by the Panel.

Keywords: tartaric acid, sodium tartrate, potassium tartrate, calcium tartrate, E 334, E 335, E 336, E 337, E 354

Requestor: European Commission

Question numbers: EFSA-Q-2011-00612; EFSA-Q-2011-00613; EFSA-Q-2011-00614; EFSA-Q-2011-00615; EFSA-Q-2011-00616; EFSA-Q-2011-00617; EFSA-Q-2011-00637

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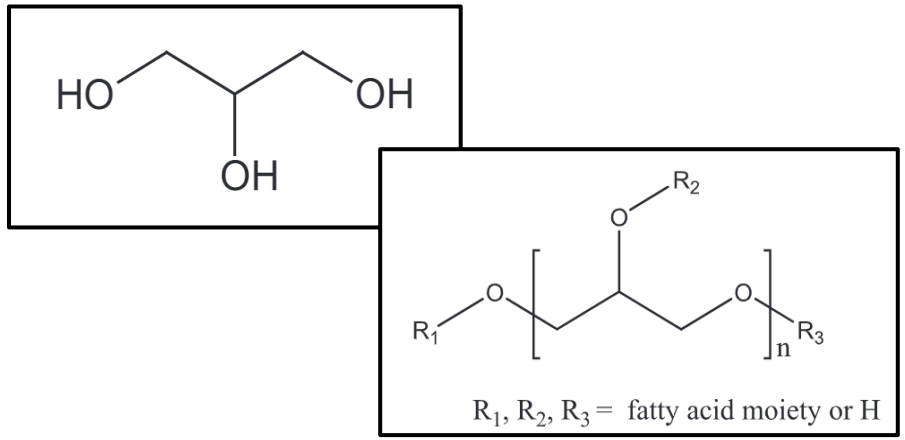


DIFFERENT IMPURITIES IDENTIFIED:

THE CASE OF GLYCEROL (E 422) AND POLYGLYCEROL ESTERS OF FATTY ACIDS (E 475)

EVALUATION OF IMPURITIES - APPROACH FAF PANEL

The potential exposure to impurities from the use of a food additive can be calculated by assuming that **the impurity is present in the food additive up to a certain value** and then by calculation pro-rata to the estimates of **exposure to the food additive itself**

The **exposure** to the impurity **is compared** with **a reference point** or a **health-based guidance value** for the undesirable impurity

This helps to determine whether there is a possible health concern if the impurity is present at a **certain level in the food additive**

Table 5: Risk assessment for toxic elements

Exposure to E 475 (mg/kg bw per day)	Based on the current limits for toxic element in the EU specifications for E 475 (Commission Regulation (EU) No 231/2012)			
	MOE for Pb at 2 mg/kg	% of the TWI for Hg at 1 mg/kg	% of the TWI for Cd at 1 mg/kg	MOE for As at 3 mg/kg
2.6[a]	96	0.46	0.73	38–1,026
6.4[b]	39	1.1	1.8	16–417
	Based on the lowest technologically achievable levels for the toxic elements in E 475 proposed by the IBO (Documentation provided to EFSA n. 1)			
Exposure to E 475 (mg/kg bw per day)	MOE for Pb at 2 mg/kg	% of the TWI for Hg at 0.1 mg/kg	% of the TWI for Cd at 0.2 mg/kg	MOE for As at 1 mg/kg
2.6[a]	96	0.05	0.15	115–3,077
6.4[b]	39	0.11	0.36	47–1,250

GLYCEROL E 422

Re-evaluation
EFSA ANS Panel, 2017

EFSA ANS Panel recommended limits to be included in the EU specification for E422 regarding:

- ✓**genotoxic impurities e.g., glycidol, epichlorohydrin,** which could be formed during manufacturing process
- ✓**potential impurities of toxicological concern** (e.g. dichlorohydrin) which could be formed during manufacturing process
- ✓a numerical **limit for acrolein**
- ✓**the maximum limits for the impurities of toxic elements** (arsenic, lead, mercury and cadmium)

More data should be generated to decrease uncertainty arising from the presence in the food additive (E 422) of compounds of toxicological concern (**e.g. acrolein, 3-MCPD or 3-MCPD ester**), which can be produced under some food processing conditions (e.g. use of glycerol(E 422) in parallel with lactic acid bacteria; use of glycerol (E 422) in food containing significant amounts of sodium chloride (more than 5%) and treated at temperatures above160°C...).

EC Call for data
November 2018-June 2019

- Call for data aiming at gathering information needed to address the recommendation of the EFSA ANS Panel

GLYCEROL E 422

Follow up

Mandate received December 2019

- **Assessment of received data began and necessary ADR requests are sent.**

✓The IBOs stated that glycerol for use as **E 422 is manufactured only** from vegetable oils and fats, either directly or from the crude glycerol obtained as a by-product of biodiesel production. Therefore, analytical data on those impurities potentially arising from the alternative manufacturing process involving chemical synthesis or microbiological fermentation have not been submitted. **Consequently, the Panel did not assess safety of glycerol obtained through these processes**.

F'up published May 2022

- **Publication of the EFSA FAF Panel Opinion recommending changes in the EU specifications of E422**:

✓**Definition of E422**: to indicate that the **food additive is obtained only from vegetable oils and fats** and undergoes purification processes that involve distillation, and other clean up steps to obtain refined glycerol

✓**Inclusion** of the numerical limit **for acrolein** based on Panel considerations

✓**Lowering** the maximum limits of **toxic elements** based on Panel considerations

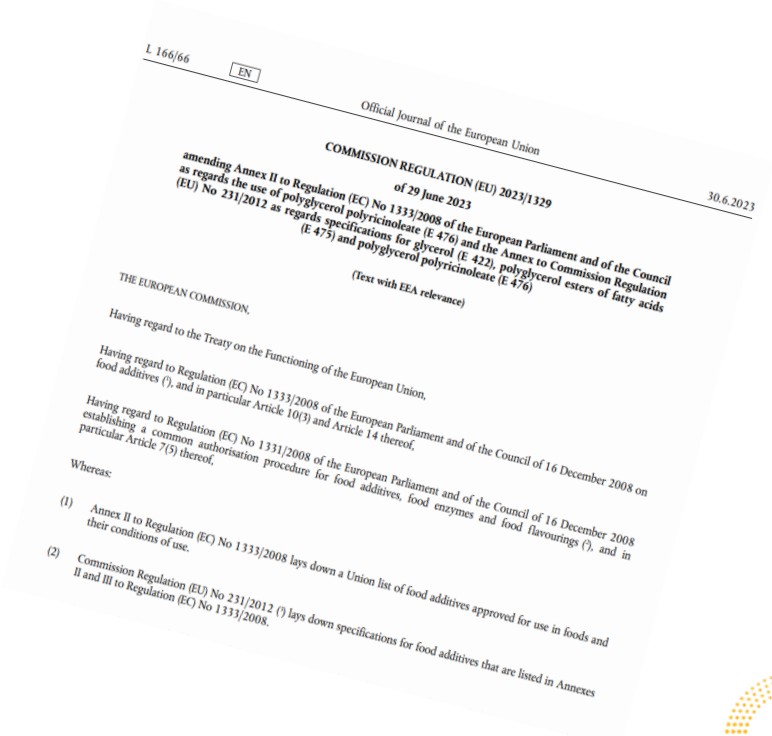
GLYCEROL E 422

Changes in the Eu Specifications

June 2023

- **COMMISSION REGULATION (EU) 2023/1329** of 29 June 2023 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the use of polyglycerol polyricinoleate (E 476) and the Annex to Commission Regulation (EU) No 231/2012 as regards **specifications for glycerol (E 422)**, polyglycerol esters of fatty acids (E 475) and polyglycerol polyricinoleate (E 476)

	Previous E422 EU Specifications	Amendments in E422 Specifications in Commission Regulation (EU) No 231/2012
Definition	-	**Glycerol is obtained only from vegetable oils and fats, either directly or from the crude glycerol obtained as a by-product of biodiesel production and undergoes purification processes that involve distillation, and other clean up steps to obtain refined glycerol.**
Purity		
Acrolein, glucose and ammonium compounds	Heat a mixture of 5 mL of glycerol and 5 mL of potassium hydroxide solution (1 in 10) at 60 °C for five minutes. It neither becomes yellow nor emits an odour of ammonia	**Acrolein: Not more than 3 mg/kg**
Arsenic	Not more than 3 mg/kg	**Not more than 0.1 mg/kg**
Lead	Not more than 2 mg/kg	**Not more than 0.1 mg/kg**
Mercury	Not more than 1 mg/kg	**Not more than 0.1 mg/kg**
Cadmium	**Not more than 1 mg/kg**	**Not more than 0.1 mg/kg**


L 166/66 EN Official Journal of the European Union 30.6.2023

COMMISSION REGULATION (EU) 2023/1329

of 29 June 2023

amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the use of polyglycerol polyricinoleate (E 476) and the Annex to Commission Regulation (EU) No 231/2012 as regards specifications for glycerol (E 422), polyglycerol esters of fatty acids (E 475) and polyglycerol polyricinoleate (E 476)

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (1), and in particular Article 10(3) and Article 14 thereof,

Having regard to 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 (2), and in particular Article 7(5) thereof,

Whereas:

(1) Annex II to Regulation (EC) No 1333/2008 lays down a Union list of food additives approved for use in foods and their conditions of use.

(2) Commission Regulation (EU) No 231/2012 (3) lays down specifications for food additives that are listed in Annexes II and III to Regulation (EC) No 1333/2008.

POLYGLYCEROL ESTER OF FATTY ACIDS E 475

Re-evaluation

EFSA ANS Panel, 2017

EFSA ANS Panel recommended to revise the EU specification for E475 considering:

- ✓ **lowering the current limits** for toxic elements (arsenic, lead, mercury and cadmium)
- ✓ including **maximum limits for epichlorohydrin and glycidol**, given that during the **manufacturing processes** of polyglycerols these genotoxic impurities could be present.
- ✓ including **maximum limits for trans fatty acids** because PEFA (E 475) can be manufactured by glycerolysis of hydrogenated fats and/or oils, which contain significant amounts of trans fatty acids.
- ✓Including **maximum limits for glycidyl esters/glycidol and 3-MCPD esters**, because it is likely that **residues of those substances occur** in the food additive PEFA (E 475), **if** they were present in the **raw materials** used in the manufacturing of the food additive by trans-esterification.
- ✓Including **maximum limits for erucic acid** since erucic acid can be present among the fatty acids in edible oils, which can be used for manufacturing of PEFA (E 475).
- ✓Including **maximum limits for impurities currently included** in the EU specifications for **glycerol** (E 422) **or recommended** by the Panel in the re-evaluation of glycerol (E 422)

EC Call for data

June 2018-December 2020

- Call for data aiming at gathering information needed to address the recommendation of the EFSA ANS Panel

POLYGLYCEROL ESTER OF FATTY ACIDS E 475

Follow up

Mandate received June 2021

- **Assessment of received data began and necessary ADR requests are sent.**

✓An IBO stated that **epichlorohydrin is not used in the manufacturing process of polyglycerols** to be used for the production of E 475 and, therefore, it is not expected to be present in E 475, consequently no data were submitted.

✓However, the **Panel noted that the present definition** of E 475 in the EU specifications does not define how the polyglycerol is manufactured and therefore **the use of epichlorohydrin as a raw material cannot be excluded**.

F'up published May 2022

- **Publication of the EFSA FAF Panel Opinion recommending changes in the EU specifications of E475**:
- **The definition of E 475 to be included** indicating that polyglycerol used for the manufacturing of E 475 should be **produced from glycerol meeting the specifications for E 422** in Commission Regulation (EU) No 231/2012. In this case, respective specification limits for epichlorohydrin, acrolein and butanetriol would not be needed for E 475.
- **Maximum limits to be included** for **Sum of 3MCPDs and 3MCPDs fatty esters** (expressed as 3-MCPDs), **glycidyl fatty acid esters** (expressed as glycidol) **and erucic acid**. As an alternative to introducing individual specifications for the impurities/constituents, the definition of E 475 could include a requirement that the fats and oils used in the manufacturing of E 475 comply with the respective EU legislation regarding suitability for human consumption.
- **Maximum limit of toxic elements (Pb, Hg, Cd and As) to be lowered** on the basis of the information provided and on the considerations of the Panel

POLYGLYCEROL ESTER OF FATTY ACIDS E 475

Changes in the Eu Specifications June 2023

- **COMMISSION REGULATION (EU) 2023/1329** of 29 June 2023 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the use of polyglycerol polyricinoleate (E 476) and the Annex to Commission Regulation (EU) No 231/2012 as regards specifications for glycerol (E 422), **polyglycerol esters of fatty acids (E 475)** and polyglycerol polyricinoleate (E 476)

	Previous E455 EU Specifications	Amendments in E475 Specifications in Commission Regulation (EU) No 231/2012
Definition	Polyglycerol esters of fatty acids are produced by the esterification of polyglycerol with food fats and oils or with fatty acids occurring in foods fats and oils. The polyglycerol moiety is predominantly di-, tri- and tetraglycerol and contains notmore than 10% of polyglycerols equal to or higher than heptaglycerol	Polyglycerol esters of fatty acids are produced by the esterification of polyglycerol with food fats and oils or with fatty acids occurring in foods fats and oils. The polyglycerol moiety is predominantly di-, tri- and tetraglycerol and contains not more than 10 % of polyglycerols equal to or higher than heptaglycerol. **The polyglycerol is produced from glycerol complying with the specifications for E 422.**
Purity		
Sum-of 3-monochloroèropanediol (3MCPD) and 3MCPD fatty acid esters, expressed as 3-MCPD	-	**Not more than 2,5 mg/kg** .
Glycidyl fatty acid esters, expressed as glycidol	-	**Not more than 5 mg/kg. This applies from 20 January 2024**
Erucic acid	-	**Not more than 2%**
Arsenic	Not more than 3 mg/kg	**Not more than 0.1 mg/kg**
Lead	Not more than 2 mg/kg	**Not more than 0.3 mg/kg**
Mercury	Not more than 1 mg/kg	**Not more than 0.1 mg/kg**
Cadmium	Not more than 1 mg/kg	**Not more than 0.1 mg/kg**

CHANGES IN THE MANUFACTURING PROCESS OR STARTING MATERIALS:

THE CASE OF STEVIOL GLYCOSIDES (E 960) – NEW APPLICATIONS

REGULATORY FRAMEWORK

- Originally, both Regulations (EC) No 1333/2008 and (EU) No 231/2012 referred to the food additive **'Steviol glycosides (E 960)'**
- Over time, these two **Regulations were amended**
- The entry 'Steviol glycosides (E 960)' has been replaced by the three entries:
  - Steviol glycosides from Stevia **(E 960a)**
  - Enzymatically produced steviol glycosides **(E 960c)**
  - Glucosylated steviol glycosides **(E 960d)**

INDIVIDUAL SPECIFICATION(s)

	DEFINITION
E 960a STEVIOL GLYCOSIDES	The manufacturing process comprises two main phases: the first involving water extraction of **the leaves of the *Stevia rebaudiana Bertoni* plant** and preliminary purification of the extract by employing ion exchange chromatography to yield a steviol glycoside primary extract, and the second involving recrystallisation of the steviol glycosides from methanol or aqueous ethanol resulting in a final product consisting mainly (at least 75 %) of stevioside and/or rebaudioside A. The additive may contain residues of ion-exchange resins used in the manufacturing process. Several other related steviol glycosides that may be generated as a result of the production process, but do not occur naturally in the *Stevia rebaudiana* plant have been identified in small amounts (0,10 to 0,37 % w/w).

INDIVIDUAL SPECIFICATION(s)

	DEFINITION
E 960c(i) REBAUDIOSIDE M PRODUCED VIA ENZYME MODIFICATION OF STEVIOL GLYCOSIDES FROM STEVIA	**Rebaudioside M** is a steviol glycoside composed predominantly of rebaudioside M with minor amounts of other steviol glycosides such as rebaudioside A, rebaudioside B, rebaudioside D, rebaudioside I, and stevioside. Rebaudioside M is obtained **via enzymatic bioconversion** of purified steviol glycoside leaf extracts (95% steviol glycosides) of the *Stevia rebaudiana* Bertoni plant **using** UDP- glucosyltransferase and sucrose synthase **enzymes produced by** the genetically modified **yeasts *K. phaffii*** (formerly known as *Pichia pastoris*) **UGT-a** and ***K. phaffii* UGT-b** that facilitate the transfer of glucose from sucrose and UDP-glucose to steviol glycosides via glycosidic bonds. [……]
E 960c(ii) REBAUDIOSIDE M PRODUCED VIA ENZYMATIC CONVERSION OF HIGHLY PURIFIED REBAUDIOSIDE A STEVIA LEAF EXTRACTS	**Rebaudioside M** produced via enzymatic conversion of highly purified rebaudioside A stevia leaf extracts is a steviol glycoside composed predominantly of rebaudioside M with minor amounts of other steviol glycosides such as rebaudioside A and rebaudioside D. Rebaudioside M is produced via enzymatic conversion of highly purified steviol glycoside rebaudioside A extracts (95 % steviol glycosides) obtained from *Stevia rebaudiana* Bertoni plant using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified **strains of *E. coli*** (pPM294, pFAF170 and pSK401) that facilitate the transfer of glucose from sucrose and UDP-glucose to steviol glycosides via glycosidic bonds. [……]
E 960c(iii) REBAUDIOSIDE D PRODUCED VIA ENZYMATIC CONVERSION OF HIGHLY PURIFIED REBAUDIOSIDE A STEVIA LEAF EXTRACTS	**Rebaudioside D** produced via enzymatic conversion of highly purified rebaudioside A stevia leaf extracts is a steviol glycoside composed predominantly of rebaudioside D with minor amounts of other steviol glycosides such as rebaudioside A and rebaudioside M. **Rebaudioside D** is produced via enzymatic conversion of highly purified steviol glycoside rebaudioside A extracts (95 % steviol glycosides) obtained from *Stevia rebaudiana Bertoni* plant using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified **strains of *E. coli*** (pPM294, pFAF170 and pSK401) that facilitate the transfer of glucose from sucrose and UDP-glucose to steviol glycosides via glycosidic bonds. [……]
E 960c(iv) REBAUDIOSIDE AM PRODUCED VIA ENZYMATIC CONVERSION OF HIGHLY PURIFIED STEVIOSIDE STEVIA LEAF EXTRACTS	**Rebaudioside AM** produced via enzymatic conversion of highly purified stevioside stevia leaf extracts is a steviol glycoside composed predominantly of rebaudioside AM with minor amounts of other steviol glycosides such as stevioside and rebaudioside E. **Rebaudioside AM** is produced via enzymatic conversion of highly purified steviol glycoside stevioside extracts (95 % steviol glycosides) obtained from *Stevia rebaudiana* Bertoni plant using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified **strains of *E. coli*** (pPM294, pFAF170 and pSK401) that facilitate the transfer of glucose from sucrose and UDP-glucose to steviol glycosides via glycosidic bonds. [……]

INDIVIDUAL SPECIFICATION(s)

	DEFINITION
E 960d GLUCOSYLATED STEVIOL GLYCOSIDES (GSG)	Mixture of larger **glycosides of steviol derived by glucosylation of steviol glycosides** extracted from leaves of *Stevia rebaudiana* Bertoni plant. The mixture is composed of glucosylated steviol glycosides and residual parent steviol glycosides from Stevia leaf. Glucosylated steviol glycosides are produced by treating the steviol glycosides, extracted from Stevia leaf, and starch suitable for human consumption with **Cyclomaltodextrin glucanotransferase** (EC 2.4.1.19) derived from a non-GMO strain of ***Anoxybacillus caldiproteolyticus*** St-88. The enzyme transfers glucose units from the starch to the steviol glycosides. The resulting material is heated and treated with activated carbon to remove the enzyme, then passed through adsorption/desorption resin to remove residual hydrolysed starch (dextrin), followed by purification and preparation of the final product using processes that may include decolourisation, concentration and spray drying.

CASE STUDY (1) – *EFSA JOURNAL* 2023; 21: e8387

Safety evaluation of the food additive steviol glycosides, predominantly Rebaudioside M, produced by fermentation using *Yarrowia lipolytica* VRM.

- New manufacturing process → *Yarrowia lipolytica* VRM (i.e., yeast).
- The new manufacturing process may result in impurities different from those present in the other Steviol glycosides. This would require new specifications.
- The FAF Panel proposed **amendment of the Specifications**:
  - Definition,
  - Solubility, changes in the qualitative description;
  - Purity,
    - introduction of entries on toxic elements cadmium (Cd) and mercury (Hg)
    - introduction of entry on kaurenoic acid. This is formed by fermentation with *Y. lipolytica* VRM. Although its absence was demonstrated (through mass spectrometry analysis) by the applicant, the Panel recommended its inclusion in the Specifications.

CASE STUDY (2) - *EFSA JOURNAL* 2022; 20(5): 7291

Safety of the proposed amendment of the specifications for enzymatically produced steviol glycosides (E 960c): Rebaudioside D produced via enzymatic bioconversion of purified stevia leaf extract

- New manufacturing process for production of Reb D → yeast *K. phaffii* UGT [(as in E960c(i)] – but different from *E. coli as E960c(iii)*
- FAF Panel considered that **separate specifications** would be needed for this food additive [(distinct from E960c(i)]
- The FAF Panel noted that the *K. phaffii* strain contains a gene conferring resistance to kanamycin due to the genetic modification introduced.
  - The applicant confirmed the absence of viable cells in the food additive; however, some positive signals were detected when looking for the presence of DNA in the food additive.
  - The Panel concluded that the safety of Reb D produced via this enzymatic bioconversion was not sufficiently demonstrated with the available data, as the absence of recombinant DNA was not shown.

SUMMARY

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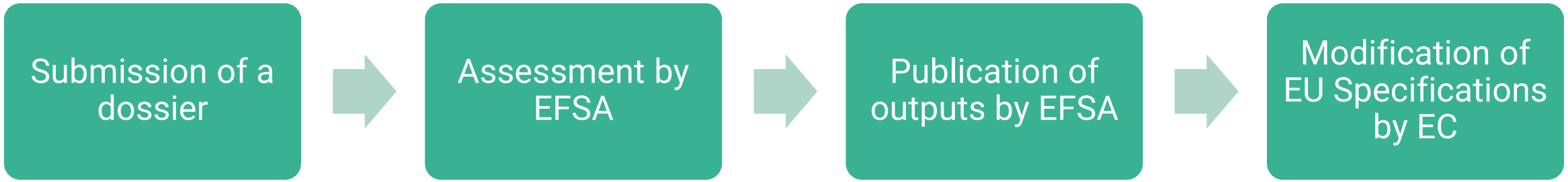
- Food additives obtained with **new manufacturing process(es)** may be considered food additives **different** from the currently authorised ones.

- **New specifications** for the new food additive(s) may be recommended.
  - New manufacturing process(es) might result in **impurities different** from those present in the currently authorised ones.
  - The **updated specifications** shall include the (potentially) different impurities and, in general, **all the differences** from the authorised products (e.g., definition, qualitative descriptors of parameters).

PROCESS TO THE AMENDMENT OF SPECIFICATIONS



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