FOOD INGREDIENTS AND PACKAGING UNIT

Call for data on genotoxicity data on sweeteners

Published: 30/06/2021

Deadline for registering interest: 30/07/2021

Deadline for submission of data: 30/12/2021

New deadline for submission of data: 31/03/2022

Background

Pursuant to Article 32(1) of Regulation (EC) No 1333/2008[1], food additives which were permitted for use in the European Union before 20 January 2009 need to be re-evaluated by the European Food Safety Authority (EFSA). The programme for this re-evaluation is established by Commission Regulation (EU) No 257/2010[2].

In accordance with the above regulations EFSA started a systematic re-evaluation of authorised food additives and is issuing scientific opinions on these food additives, according to the priorities indicated in the Regulation (EU) No 257/2010, which foresees in article 3(b) that "the re-evaluation of all approved sweeteners listed in Directive 94/35/EC shall be completed by 31 December 2020".

In accordance with Article (5) of the Regulation (EU) No 257/2010, EFSA has already made open call(s) for data for the sweeteners under the re-evaluation programme.[3,4] On the basis of the information received from interested parties and those retrieved from the literature EFSA has started the assessment of these food additives.

As recorded in the minutes of the 22nd and 23rd plenary meetings of the Food Additives and Flavourings (FAF) Panel, during the course of the preliminary assessment of the data available, however, for some of these food additives, the need for additional information considered to be relevant for the re-evaluation has been identified.

Therefore, in accordance with Article 6(3) of the Regulation (EU) No 257/2010, EFSA is launching a public call for data in order to acquire documented information (published, unpublished or newly generated) on acesulfame K (E 950), salt of aspartame-acesulfame (E962), isomalt (E 953), sucralose (E 955), neohesperidine DC (E 959), neotame (E 961), lactitol (E 966), xylitol (E 967) and cyclamates (E 952 i, ii,iii).

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[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. Available here: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32008R1333

[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, OJ L 80, 26.03.2010. Available here: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010R0257&from=EN.

[3] https://www.efsa.europa.eu/en/data/call/170621

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

EFSA will consider the relevance of the information provided for the risk assessment of these food additives. The submission of the requested information is without prejudice, *inter alia*, to the final opinion of the FAF Panel.

Overall objective

The purpose of this call for data is to offer interested parties and/or stakeholders the opportunity to submit documented information (published, unpublished or newly generated) relevant to the re-evaluation of acesulfame K (E 950), salt of aspartame-acesulfame (E962), isomalt (E 953), sucralose (E 955), neohesperidine DC (E 959), neotame (E 961), lactitol (E 966), xylitol (E 967) and cyclamates (E 952 i, ii,iii).

Deadline for submission of data and disclosure of contact details

Interested parties and stakeholders should provide by **31/03/2022** the information described below.

Within **4 weeks** from the publication of this call, please communicate in writing by e-mail to: fip@efsa.europa.eu, your availability to submit the requested information by the timeline specified above or any proposal for a new deadline providing justified reasons. Depending on the replies received the final deadline will be communicated to you via e-mail and by updating the current call.

In accordance with Article 6(4) of the Regulation (EU) No 257/2010 any information not submitted within the final deadline shall not be taken into account in the re-evaluation. However, in exceptional cases, EFSA may decide with the agreement of the Commission to take into account information submitted after the deadline, if that information is significant for the re-evaluation of a food additive.

In order to facilitate the collaboration of all interested business operators and interested parties to provide the data needed, we are seeking your consent to disclose contact details to the other parties that have expressed an interest to provide the requested information. If you do not wish to make your contact details available, clearly indicate it in your first communication.

Information sought

EFSA kindly invites business operators and other interested parties (governments, interested organisations, universities, research institutions, companies) to submit information on the following food additives:

Q-Numbers	Additive	E number
EFSA-Q-2011-00721	Acesulfame K	E 950
EFSA-Q-2011-00727	Salt of aspartame-acesulfame	E 962
EFSA-Q-2011-00723	Isomalt	E 953
EFSA-Q-2011-00724	Sucralose	E 955
EFSA-Q-2011-00726	Neohesperidine DC	E 959
EFSA-Q-2011-00740	Neotame	E 961
EFSA-Q-2011-00728	Lactitol	E 966
EFSA-Q-2011-00729	Xylitol	E 967
EFSA-Q-2011-00733; EFSA-Q-2011-00734; EFSA-Q-2011-00735	Cyclamates	E 952 i, ii, iii

1. Data on toxicology: genotoxicity

Current guidance on the genotoxicity assessment of substances (EFSA SC, 2011[5]; EFSA SC, 2017[6]), applicable to the safety evaluation of food additives, recommends a set of core tests for the detection of three important genetic endpoints: gene mutation, structural chromosomal aberrations (i.e. clastogenicity) and numerical chromosome aberrations (i.e. aneugenicity). Moreover, a substantial proportion of the genotoxicity studies available for the food additives under re-evaluation were completed prior to the provision of the current OECD test guidelines, thus resulting in limitations for several of the current assessments.

A preliminary assessment conducted by the WG Sweeteners of the FAF Panel was to establish whether the available genotoxicity data for each substance would be considered adequate with respect to the current standards or whether the need for additional information considered relevant for the genotoxicity assessment had been identified in order to progress with the overall safety assessment and to reach a conclusion. This assessment has highlighted the need for the following additional data to be generated for each of the following food additives:

Acesulfame K (E 950): in the first instance, data from the basic battery of *in vitro* tests, i.e. bacterial reverse mutation assay (OECD TG 471) and an *in vitro* micronucleus assay (OECD TG 487).

Salt of aspartame-acesulfame (E 962): data submitted for the food additive acesulfame K (E 950) will be used also for the re-evaluation of the food additive salt of aspartame-acesulfame (E 962).

Isomalt (E 953): in the first instance, data from the basic battery of *in vitro* tests, i.e. bacterial reverse mutation assay (OECD TG 471) and an *in vitro* micronucleus assay (OECD TG 487).

Sucralose (E 955): Additional data would be needed to further assess the positive effects observed in vitro (gene mutations for 1,6-DCF and DNA strand breaks for sucralose, both without exogenous metabolic activation), and in accordance with the recommendation of the EFSA SC on the follow-up of *in vitro* positive results (EFSA, 2011). The data required would be an *in vivo* Comet assay by the oral route both for the food additive sucralose (E 955) and for its degradation/hydrolysis product 1,6-dichloro-1,6-dideoxyfructose (1,6-DCF). Based on the *in vitro* evidence for a direct genotoxic mechanism for both substances, and the inconclusive results provided by a previous *in vivo* comet assay with sucralose, the recommended tissues to be assessed in the new Comet assay are stomach, duodenum, colon, liver, lung and blood cells.

Neohesperidine DC (E 959): data from a new *in vitro* micronucleus assay using the cytokinesis block protocol (OECD TG 487).

In the event of positive results in the *in vitro* micronucleus assay, a staining of the micronuclei with fluorescent in situ hybridisation (FISH) or antikinetochore antibodies (CREST) analysis will be needed to determine the appropriate follow-up for an *in vivo* study.

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[5] EFSA Scientific Committee; Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011; 9(9):2379. [69 pp.] https://doi:10.2903/j.efsa.2011.2379

[6] EFSA Scientific Committee, Hardy, A, Benford, D, Halldorsson, T, Jeger, M, Knutsen, HK, More, S, Naegeli, H, Noteborn, H, Ockleford, C, Ricci, A, Rychen, G, Silano, V, Solecki, R, Turck, D, Younes, M, Aquilina, G, Crebelli, R, Gürtler, R, Hirsch-Ernst, KI, Mosesso, P, Nielsen, E, van Benthem, J, Carfì, M, Georgiadis, N, Maurici, D, Parra Morte, J and Schlatter, J, 2017. Scientific Opinion on the clarification of some aspects related to genotoxicity assessment. EFSA Journal 2017;15(12):5113, 25 pp. https://doi.org/10.2903/j.efsa.2017.5113

Neotame (E 961): Additional data would be needed to investigate the potential for neotame to induce the formation of micronuclei in mammalian cells *in vivo*, as no exposure of the target tissue (bone marrow) to the test item was demonstrated under identical conditions to the mouse *in vivo* micronucleus test.

Alternatively, in the absence of evidence of systemic or bone marrow exposure under identical experimental conditions to the *in vivo* micronucleus assay in the mouse, a FISH/CREST analysis of the *in vitro* micronucleus assay will be needed to determine the appropriate follow-up for an *in vivo* study.

Lactitol (E 966): in the first instance, data from the basic battery of *in vitro* tests, i.e. bacterial reverse mutation assay (OECD TG 471) and an *in vitro* micronucleus assay (OECD TG 487).

Xylitol (E 967): in the first instance, data from the basic battery of *in vitro* tests, i.e. bacterial reverse mutation assay (OECD TG 471) and an *in vitro* micronucleus assay (OECD TG 487).

Cyclamates (E 952 i, ii,iii): Genotoxicity studies on cyclamic acid (E 952i), sodium cyclamate (E 952 ii) and calcium cyclamate (E 952 iii) and on their metabolite cyclohexylamine (CHA) published after the IARC evaluation (1999) are of limited relevance and/or reliability. The positive findings reported in some *in vitro* and *in vivo* studies on cyclamate salts are considered of insufficient reliability due to methodological shortcomings, while the *in vitro* UDS (negative) is now considered obsolete. Similarly, the two bacterial tests performed with CHA (negative) used a test method not validated for regulatory use. For cyclohexylamine, inadequate data are available to assess whether the clastogenic activity reported in some of the *in vitro* studies evaluated in the IARC Monograph is also expressed *in vivo*. As a consequence, the additional data required would be an *in vivo* Comet assay by the oral route for the food additive cyclamates (E 952 i, ii, iii) and for its metabolite cyclohexylamine (CHA). Based on limited data indicating effects at the sites of contact, the recommended tissues to be assessed in the *in vivo* Comet assay are: stomach, duodenum, colon, liver and blood cells.

For all the *in vitro* assays, in the event of positive results obtained in the test, *in vivo* follow-up would be needed in accordance with the 2011 EFSA SC Guidance on genotoxicity and the draft Scientific Committee Guidance on aneugenicity[7].

Confidentiality

In accordance with Article 8 of Regulation (EU) No 257/2010 setting up a re-evaluation programme of approved food additives, confidential treatment may be given to information the disclosure of which might significantly harm the competitive position of business operators or other interested parties.
Therefore, data providers should indicate any information they wish to be treated as confidential and provide verifiable justification supporting this request. Please also note that the information described in Article 8(2) of Regulation (EU) No 257/2010 cannot be regarded as confidential in any circumstances.

In application of Article 8(4) of Regulation (EU) 257/2010, following a proposal from EFSA, the Commission will decide after consulting the interested business operator and/or the other interested parties, which information may remain confidential.

[7] https://open.efsa.europa.eu/questions/EFSA-Q-2019-00262 - Draft undergoing finalisation by 31.08.2021

Submission of information

Interested business operators and/or interested parties should submit the information to EFSA through their chosen internet-based software (submission by email is not allowed) with a

- cover letter that should contain:
  - Reference to the specific call Reference to the substance(s) concerned and its E numbers and its EFSA question number;
  - The contact details[8] (name of contact person, name of company/organisation, e-mail address and telephone number) of the person responsible for the data submission and, if applicable, the list of interested business operators and/or interested parties represented and their contact details;

- statement of the submitter that they hold all the necessary rights to grant EFSA permission to use and, where appropriate, to disclose the submitted information, data, document, paper or study for the purposes better defined in this call. In case the submitter does not enjoy such rights for the submitted subject matter, they should share the contact details of the respective owner(s) of data and/or the holder(s) of any relevant intellectual property rights, so that EFSA may seek their approval directly.
- separate folders with the confidential and with the non-confidential parts.

Possibility for EFSA to use the data for the safety assessment of the same or other substance under the same or other legal or regulatory frameworks.

In case future mutual interests arise in exchanging any relevant information (i.e. technical or toxicological data) with the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for the re-evaluation of food additives or with other EU agencies (such as the European Medicines Agency (EMA)), we would appreciate your written consent for data sharing between EFSA and other EU agencies or JECFA on this additive.

Please note that EFSA may, where legally possible, use or re-use relevant information or data (i.e. technical, toxicological data) for the evaluation of the same or another substance under the same or a different legal or regulatory framework from the one mentioned above.

Correspondence

Once internet-based software chosen please kindly send the link and login to . As the password must be provided by phone only you are kindly asked to call the following phone nr +39 0521 036 246 as soon as email sent.

[8] The interested parties shall notify EFSA of any change in the contact details by sending an e-mail to the FIP mailbox (fip@efsa.europa.eu).