

Safety assessment of flavourings in the European Union

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Abstract

In the European Union (EU) the regulatory framework for the use of flavourings in and on foods is provided by Regulation (EC) No 1334/2008. It contains as Annex the so-called Union list, i.e. a list of flavouring substances authorized for use in and on foods to the exclusion of all others. The principles underlying a group-based approach applied for the safety evaluation of flavouring substances prior to their entry into the Union list are outlined. The application of a decision-tree that takes into consideration structure-activity relationships, metabolism, intake and toxicity is described. Examples with particular emphasis on testing for genotoxic potential are given, and criteria for future safety evaluations of chemically defined substances and of flavourings other than flavouring substances are presented.

Regulatory framework

In the European Union (EU) the regulatory framework for the use of flavourings in and on foods is provided by Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 [1]. The Regulation applies to flavourings, food ingredients with flavouring properties, to food containing flavourings and/or food ingredients with flavouring properties, and to source materials for flavourings and/or source materials for food ingredients with flavouring properties. Flavourings to be used in or on food must meet the following conditions: (i) They do not, on the basis of the scientific evidence available, pose a safety risk to the health of the consumer, and (ii) their use does not mislead the consumer.

Regulation (EC) No 1331/2008 of 16 December 2008 [2] laid down a common procedure for the assessment and the authorization of so-called food improvement agents, i.e. food additives, food enzymes and food flavourings. A Union list, i.e. a list of flavourings and source materials for use in and on foods that are authorized to the exclusion of all others, is included as Annex to Regulation (EC) No 1334/2008.

Principles of the safety assessment

The procedure to establish the Union list had been laid down in Regulation (EC) No 2232/96 of the European Parliament and of the Council [3]. Member States were requested to notify to the Commission a list of flavouring substances which at that time were legally accepted on their territory. The resulting register of about 2800 substances was adopted by Commission Decision (1999/217/EC) [4]. The measures for the evaluation programme were laid down by Commission Regulation (EC) No 1565/2000 [5]. Considering the large number of substances, it was decided to make use of already existing safety assessments. Flavouring substances that had been considered as being not of safety concern at the current levels of intake either by the Scientific Committee on Food of the European Commission (SCF), the Experts on Flavouring Substances of the Council of Europe (CEFS) or by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) before 2000 did not need to be re-evaluated within the evaluation programme. Flavouring substances classified after 2000 by JECFA as to present no safety concern at the current level of intake had to be considered by the European Food Safety

Authority (EFSA), in order to decide whether no further evaluation is necessary. The remaining flavouring substances had to be evaluated by EFSA.

In order to make the evaluation process as efficient as possible, a group-based approach was followed. The flavouring substances contained in the register were divided into 34 structurally related chemical groups; substances within a group are considered to have some common metabolic and biological behaviours. An additional important feature is that data may be provided either for a candidate substance as such or for supporting representatives showing sufficient structural and metabolic similarity.

The evaluation procedure is based on a stepwise decision-tree approach that considers information on structure-activity relationships, metabolism, intake and toxicity (Figure 1). This corresponds to a procedure developed by JECFA [6] and subsequently applied in an adjusted version to the evaluation of various flavouring substances [7-9]. The only differences are that the option to accept flavouring substances with the only argument that their estimated intake is lower than the threshold of concern of 1.5 µg/person/day was not adopted and that flavouring substances should be particularly examined for structural alerts of potential genotoxicity [10].

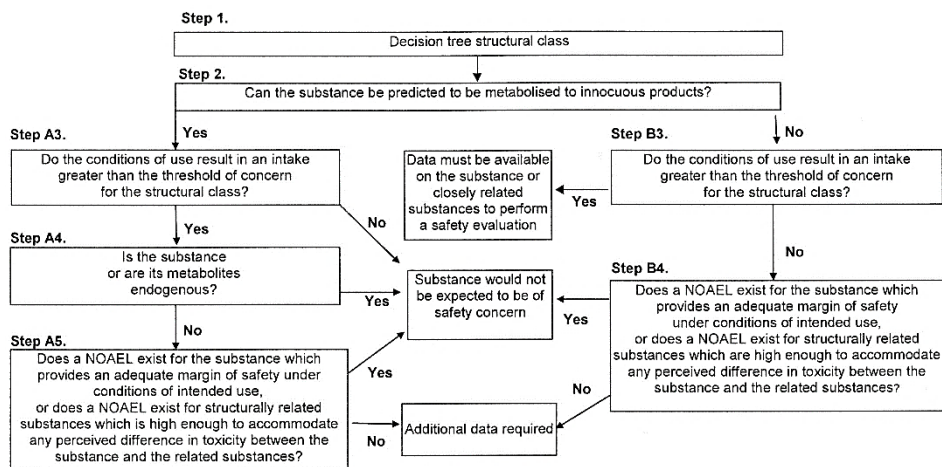


Figure 1: Procedure for the safety evaluation of chemically defined flavouring substances

The first step of the decision tree is the assignment of a flavouring substance to one of three classes for which thresholds of concern (human exposure thresholds) have been specified. Class I contains flavouring substances with simple chemical structures and efficient modes of metabolism, suggesting a low order of oral toxicity. Class II contains substances with structural features that are less innocuous, but are not suggestive of toxicity. Class III includes flavouring substances with structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity [11]. The thresholds of concern for these structural classes (1800, 540 and 90 µg/person/day, respectively) have been derived from a large dataset of subchronic and chronic animal studies [6,12].

In the following step, the answer to the question whether the flavouring substance can be predicted to be metabolized to innocuous products determines whether the evaluation proceeds via the A- or the B-side of the decision tree. Another decisive

question is whether the intended conditions of use of the flavouring substance result in an intake greater than the threshold of toxicological concern for the structural class. The answer determines whether the substance is not expected to be of safety concern or whether information is required on a no-observed-adverse-effect level (NOAEL) for the flavouring substance as such or structurally related substances, which provides an adequate margin of safety under the intended conditions of use.

The intake assessment plays an important role in the application of the Procedure. As a default, the so-called “Maximised Survey-derived Daily Intakes” (MSDI) approach, which is based on annual production volumes, was used [13]. However, the MSDI approach in a number of cases grossly underestimates the intake by regular consumers of products flavoured at the use levels reported by Industry. Therefore, the intakes were also estimated using the “modified Theoretical Added Maximum Daily Intake” (mTAMDI) approach, which is based on normal use levels reported by industry and consumption data for certain food categories [13]. The mTAMDI value was not considered in the Procedure but was only used as tool to prioritise the flavouring substances according to the need for a refined intake screen and the request for more precise data. Accordingly, the following types of conclusions can be found in the scientific opinions, the so-called Flavouring Group Evaluations (FGEs): (i) Based on the default MSDI approach, the candidate substance, which was evaluated through the Procedure, would not give rise to safety concern at the estimated level of intake arising from the use as flavouring substance. (ii) Based on the mTAMDI approach, the estimated intake of a flavouring substance is above the threshold of concern for the respective structural class. In this case, more reliable exposure data are required. On the basis of such additional data, the flavouring substance should be re-evaluated using the Procedure; subsequently, additional toxicological data might become necessary.

Implementation of the Union list

The Union list of flavouring substances has been adopted by Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 [14]. It contains information on the identities and the purities (at least 95%; otherwise composition is given) of the flavouring substances. It may also contain restrictions of use, e.g. that a substance may only be added to the listed food categories and under the specified conditions of use. The scientific body that has carried out the evaluation is given and finally, footnotes indicate for which flavouring substances the evaluation is to be completed, and the time limits for applicants to comply with EFSA’s requests expressed in published opinions.

Requests for additional genotoxicity data

In the pending requests for additional information, particular attention is paid to the provision of genotoxicity data. According to the guidance expressed in the opinion of the EFSA Scientific Committee [15], genotoxicity testing should start with a basic battery of *in vitro* tests, i.e. a bacterial reverse mutation assay and an *in vitro* micronucleus test. If all *in vitro* endpoints are negative, there is no genotoxic potential. If one or two tests are positive, the following *in vivo* tests should be considered: (i) an *in vivo* mammalian erythrocyte micronucleus test, (ii) a transgenic rodent cell gene mutation assay, and (iii) an *in vivo* Comet assay. The *in vivo* test selected should relate to the genotoxic endpoint(s) identified as positive in the *in vitro* tests. If any of the *in vivo* tests is positive, there is a genotoxic potential and the flavouring substance is considered to be of safety concern.

The α,β -unsaturated aldehyde and ketone structures are considered as structural alerts for genotoxicity. FGE.19 contains 360 α,β -unsaturated aldehydes or ketones and precursors which could give rise to such carbonyl substances via hydrolysis and/or oxidation. These substances were divided into structurally related subgroups, representative substances were selected, and the Flavouring Industry had to provide additional genotoxicity data [16]. If on the basis of these data a genotoxic potential can be ruled out, the substances are merged with structurally related substances in other FGEs and evaluated using the Procedure.

An example of such a subgroup of FGE.19 are the three alicyclic aldehydes with α,β -unsaturation in the ring/side chain and the seven precursors for such aldehydes shown in Figure 2.

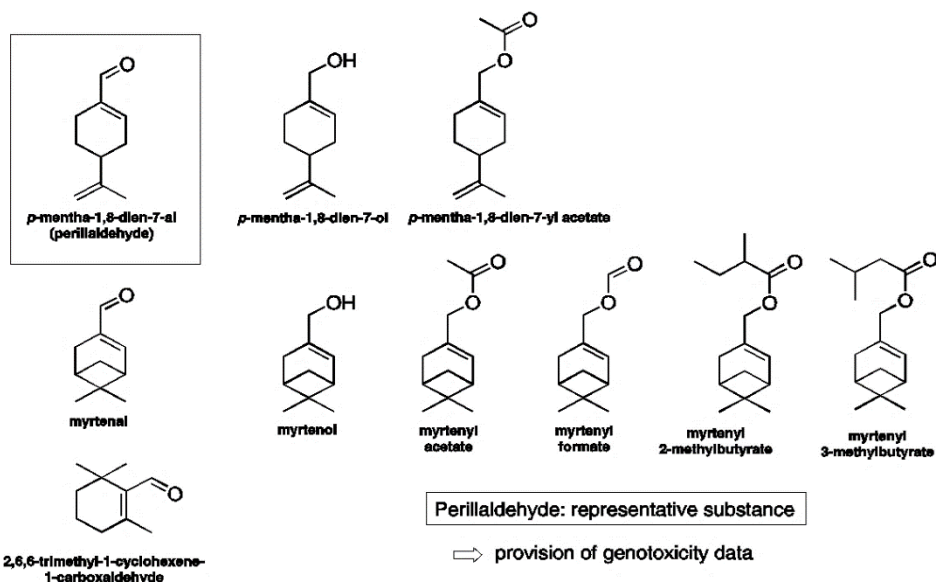


Figure 2: Examples of α,β -unsaturated carbonyls and their precursors (subgroup 2.2 of FGE.19)

p-Mentha-1,8-dien-7-al [FL-no: 05.117] was selected as representative substance for which genotoxicity data were requested. According to the data submitted, the EFSA Panel concluded that *p*-mentha-1,8-dien-7-al is genotoxic *in vivo* [17], and the flavouring substance was removed from the Union list [18]. This, however, meant that there were also concerns regarding potential genotoxicity for the other flavouring substances in this subgroup represented by *p*-mentha-1,8-dien-7-al. Subsequently, the flavor industry withdrew the support for 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde [FL-no: 05.121], myrtenyl formate [FL-no: 09.272], myrtenyl 2-methylbutyrate [FL-no: 08.899] and myrtenyl 3-methylbutyrate [FL-no: 09.900] which then were also removed from the Union list. For myrtenol [FL-no: 02.091], myrtenyl acetate [FL-no: 09.302] and *p*-mentha-1,8-dien-7-yl acetate [FL-no: 09.278] new genotoxicity data were provided; they allowed to rule out the concerns regarding genotoxicity for these substances. Only for myrtenal [FL-no: 05.106] the genotoxicity data submitted were considered equivocal and therefore this flavouring substance presently cannot be evaluated through the Procedure [19].

In contrast to this group-based approach involving a representative substance, the rather unique structure of 4,5-epoxydec-2(trans)-enal [FL-no: 16.071] resulted in an evaluation as stand-alone substance. The genotoxic effect observed *in vitro* was confirmed in an *in vivo* Comet assay in the liver of rats. Accordingly, the EFSA Panel concluded that 4,5-epoxydec-2(trans)-enal raises a safety concern with regard to genotoxicity [20], and consequentially this flavouring substance was removed from the Union list [21].

Evaluation of newly submitted flavouring substances

The established Union list is open and can be amended in the light of scientific and technical developments. EFSA has elaborated a guidance document for the risk assessment of flavourings newly submitted after the adoption of the Union list [22]. As a starting point of the assessment genotoxicity testing is required. Flavourings which can be assigned to one of the existing FGEs on the basis of structural and metabolic similarities can be evaluated according to the scientific principles and to the group-based approach underlying the former evaluation programme. For flavouring substances which cannot be assigned to one of the existing FGEs individual evaluations via the tiered approach shown in Figure 2 have to be performed. The type of data required depends on (i) whether there are experimental data available for the substance to demonstrate that the metabolites can be considered innocuous, and (ii) whether the chronic dietary exposure, based on added use levels, is below or above the threshold of concern of the structural class to which the flavouring substance belongs.

For the assessment of dietary exposure, a new approach called “Added Portions Exposure Technique” (APET) has been introduced [22]. The APET is calculated based on the occurrence levels provided by the applicant in a defined list of food categories by summing the highest potential dietary exposure within each of the two groups of “Beverages” and “Solid foods”. Such an estimate, based on daily consumption of one single standard portion of beverage and one single portion of solid food, is considered to provide a conservative assessment of long-term average dietary exposure for consumers of flavoured products. A case study on the use of the APET technique to estimate total dietary exposure to flavouring substances has been provided [23].

The applicant needs to provide: (i) Normal and maximum occurrence levels as added flavouring substance; (ii) normal and maximum occurrence levels of the substance from other sources, e.g. as natural constituent, as substance developed through the processing of foods, as carry-over originating from the use in animal feed or as residues of packaging; (iii) normal and maximum combined occurrence levels of the substance, taking into account all sources. In addition, the applicant needs to indicate the non-food uses of the flavouring substance.

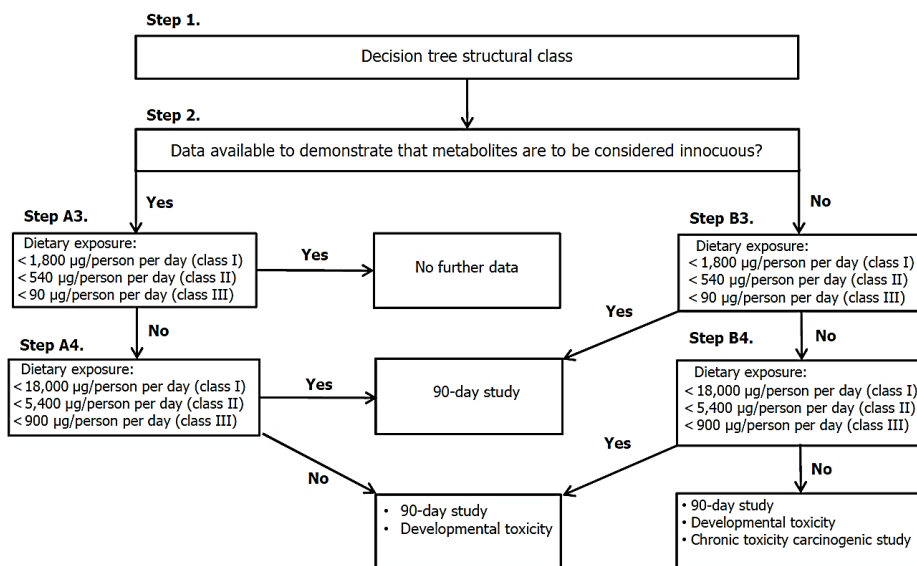


Figure 3: Individual evaluation of flavouring substances

A recent example for the application of the approach outlined in Figure 2 is the assessment of 3-(1-((3,5-dimethylisoxazol-4-yl)methyl)-1H-pyrazol-4-yl)-1-(3-hydroxybenzyl)-imidazolidine-2,4-dione [FL-no: 16.127], a substance intended to be used as flavour modifier [24]. Data provided for the substance demonstrated that there is no concern regarding genotoxicity. It was assigned to Cramer class III; potential metabolites could not be considered to be innocuous. The cumulative dietary exposure using the APET technique was 850 µg/person/day for an adult (60 kg) and 536 µg/person/day for a 3-year-old child (15 kg). Considering that this intake is higher than the threshold of concern of substances belonging to Cramer class III, i.e. 90 µg/person/day, but lower than 10 times this threshold, i.e. 900 µg/person/day, a 90-day feeding study and a developmental study were required. In a developmental toxicity study with rats no differences between treated and control groups up to 100 mg/kg bw/day were observed. In a 90-day feeding study with rats an NOAEL of 100 mg/kg bw/day could be derived. The comparison of this NOAEL with the estimated intakes resulted in margins of safety of > 7,000 for adults and > 2,000 for a 3-year-old child.

Evaluation of flavourings other than flavouring substances

In addition to flavouring substances, Article 9 of Regulation (EC) No 1334/2008 of the European Parliament specifies the following categories of flavourings for which an evaluation and approval is required: (i) Flavouring preparations obtained from material of vegetable, animal or microbiological origin, other than food. (ii) Thermal process flavourings for which ingredients for their production are source materials other than food and/or for which the conditions of their production and/or the maximum levels of undesirable substances set out in Annex V of Regulation 1334/2008 (EU, 2008) are not met. (iii) Flavour precursors obtained from source material other than food. (iv) Other flavourings. The information requested for a safety evaluation of these categories of flavourings is described in a guidance document [22].

For the categories (i) – (iii) no applications have been submitted so far. Examples of recently assessed “Other flavourings” are two “grill flavours”, i.e. high oleic sunflower oils subjected to short-time heating at high temperatures [24, 25], and “rum ether”, a complex mixture of volatiles obtained by pyrolysis of wood (oak, beech, hickory) and esterification of the resulting pyroligneous acid with ethanol, under oxidative conditions in the presence of sulfuric acid and manganese oxide [26].

Conclusion

The establishment of the Union list of flavourings substances constitutes a basis change in paradigm in the regulatory oversight on flavourings in the EU. On the one hand, this creates economically relevant hurdles for applicants, on the other hand such a list increases transparency, it can serve as reliable platform for involved stakeholders, and it may finally help to increase the acceptance of flavourings by consumers.

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