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Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) on a request from the Commission related to

Flavouring Group Evaluation 5, Revision 1 (FGE.05Rev1): Esters of branched- and straight-chain aliphatic saturated primary alcohols and of one secondary alcohol, and branched- and straight-chain unsaturated carboxylic acids from chemical groups 1, 2, and 5 (Commission Regulation (EC) No 1565/2000 of 18 July 2000)[1]

(Question No EFSA-Q-2003-148B)

Adopted on 27 September 2007

PANEL MEMBERS

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SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the Panel) is asked to advise the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Scientific Panel is asked to evaluate 29 flavouring substances in the Flavouring Group Evaluation, Revision 1 (FGE.05Rev1), using the procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 29 flavouring substances belong to chemical groups 1, 2, and 5 of Annex I of the Commission Regulation (EC) No 1565/2000.

[1] For citation purposes: Flavouring Group Evaluation 5, Revision 1 (FGE.05Rev1): Esters of branched- and straight-chain aliphatic saturated primary alcohols and of one secondary alcohol, and branched- and straight-chain unsaturated carboxylic acids from chemical groups 1, 2, and 5 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). The EFSA Journal (2008) 643, 1-81.

[*] 1 member of the Panel did not participate in the discussion due to a declared interest. For details see the minutes of the 25th AFC Panel meeting : http://www.efsa.europa.eu/EFSA/ScientificPanels/AFC/efsa_locale-1178620753812_Meetings424.htm

The present Flavouring Group Evaluation deals with 29 esters of branched- and straight-chain aliphatic saturated primary alcohols, and a secondary alcohol, and branched- and straight-chain unsaturated carboxylic acids.

Twenty-four of the 29 flavouring substances can exist as stereoisomers. For seven of these substances the stereoisomeric composition has not been specified.

Twenty-six substances belong to structural class I and three substances to structural class II according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

Twenty-five of the substances in the present group have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the Maximised Survey-derived Daily Intakes (MSDIs) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Scientific Panel examined the information provided by the European Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified Theoretical Added Maximum Daily Intake (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, the 29 flavouring substances in this group have estimated intakes in Europe from 0.0012 to 12 microgram*/capita/*day, which are below the threshold of concern values for both structural class I (1800 microgram/person/day) and structural class II (540 microgram/person/day) substances.

Genotoxicity data are available only for a limited number of substances, and the genotoxicity could not be assessed adequately. However, the data available do not preclude evaluation of the candidate substances using the Procedure.

Except for the three methacrylates [FL-no: 09.375, 09.586 and 09.647], the flavouring substances are expected to be metabolised to innocuous products.

Ethyl methacrylate [FL-no: 09.375] induced neurotoxicity in a 60-day drinking water study in rats at 50 mg/kg body weight/day, the lowest dose tested. Accordingly, a No Observed Adverse Effect Level (NOAEL) could not be established. Methyl methacrylate [FL-no: 09.647] has a neurotoxic potential likewise, as shown in an EU Risk Assessment Report. However, an adequate NOAEL from an oral study is not available for methyl methacrylate. For isobutyl 2-methylprop-2-enoate [FL-no: 09.586], structurally related to ethyl methacrylate, there are neither neurotoxicity studies nor other repeated dose toxicity studies. Therefore, additional toxicity data for ethyl methacrylate [FL-no: 09.375], methyl methacrylate [FL-no: 09.647], and isobutyl 2-methylprop-2-enoate [FL-no: 09.586] are required.

It was noted, that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation.

On the basis of the default MSDI approach the Panel concluded that 26 of the 29 substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances. For the three flavouring substances, ethyl methacrylate [FL-no: 09.375], methyl methacrylate [FL-no: 09.647], and isobutyl 2-methylprop-2-enoate [FL-no: 09.586], additional toxicity data are required.

When the estimated intakes were based on the mTAMDI approach they ranged from 820 to 9500 microgram/person/day for 25 of the 26 candidate substances from structural class I. For one flavouring substance use levels are missing. Except for two of the 25 substances these intakes were above the threshold of concern for structural class I of 1800 microgram/person/day. The estimated intakes of the three candidate substances assigned to structural class II, based on the mTAMDI approach are for each 3900 microgram/person/day, which are above the threshold of concern for structural class II of 540 microgram/person/day. The substances, which have mTAMDI intake estimate below the threshold of concern for structural class I, is also expected to be metabolised to innocuous products.

Thus, for the 23 of the 26 flavouring substances allocated to structural class I and for the three substances allocated to structural class II, the intakes, estimated on the basis of the mTAMDI approach, exceed the relevant threshold for the structural class. Therefore, for 27 substances, including the flavouring substance [FL-no: 09.326] for which use levels are missing, more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Subsequently, additional data might become necessary.

In order to determine whether this evaluation could be applied to the material of commerce, it is necessary to consider the available specifications.

Adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for 28 of the 29 flavouring substances, except that information on geometrical isomerism/chirality is missing for seven of the substances ([FL-no: 09.266, 09.326, 09.329, 09.335, 09.379, 09.637 and 09.942]). For the substance [FL-no: 09.326] identity test is missing. Thus, the final evaluation of the materials of commerce cannot be performed for seven substances ([FL-no: 09.266, 09.326, 09.329, 09.335, 09.379, 09.637 and 09.942]), pending further information on specifications. For three substances [FL-no: 09.375, 09.586 and 09.647] additional toxicity data are required. The remaining 19 substances [FL-no: 09.248, 09.321, 09.324, 09.330, 09.365, 09.370, 09.372, 09.374, 09.596, 09.603, 09.624, 09.625, 09.636, 09.641, 09.652, 09.680, 09.699, 09.865 and 09.934] would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

KEYWORDS

Flavourings, safety, alcohols, carboxylic acids, esters, unsaturated.

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2006/252/EC (EC, 2006). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000), which is broadly based on the opinion of the Scientific Committee on Food (SCF, 1999). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

The Flavouring Group Evaluation (FGE) is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 (EC, 2002b) and to take into account additional information that has been made available since the previous Opinion on this FGE.

The revision also includes newly notified substances belonging to the same chemical groups evaluated in this FGE.

After the completion of the evaluation programme the positive list of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (EC, 1996).

HISTORY OF THE EVALUATION

FGE	Opinion Adopted by EFSA	Link	No. of Candidate Substances
FGE.05	23 February 2005	http://www.efsa.eu.int/science/afc/afc_opinions/1016_en.html	24
FGE.05Rev1	27 September 2007	http://www.efsa.europa.eu/EFSA/ScientificPanels/AFC/efsa_locale-1178620753812_Opinions425.htm	29

The present revision of FGE.05, FGE.05Rev1, includes the assessment of five additional flavouring substances [FL-no: 09.248, 09.266, 09.326, 09.934, 09.942]. No new toxicity or metabolism data were provided for these five substances, except for one new substance [FL-no: 09.248], for which one acute toxicity study was available. Additional information on 11 substances [FL-no: 09.324, 09.329, 09.330, 09.335, 09.372, 09.374, 09.379, 09.625, 09.637, 09.641 and 09.865] was made available since the FGE.05 was published.

TERMS OF REFERENCE

The European Food Saferty Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register prior to their authorisation and inclusion in a positive list according to Commission Regulation (EC) No 1565/2000 (EC, 2000). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

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ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 5 Revision 1

1.1. Description

The present Flavouring Group Evaluation 5, Revision 1 (FGE.05Rev1), using the procedure as referred to in the Commission Regulation (EC) No 1565/2000 (the Procedure – shown in schematic form in Annex I), deals with 28 esters of branched- and straight-chain aliphatic saturated primary alcohols and branched- and straight-chain unsaturated carboxylic acids, and one ester of a secondary aliphatic saturated alcohol and a straight-chain unsaturated carboxylic acid and a secondary alcohol. These 29 flavouring substances (candidate substances) belong to chemical groups 1, 2, and 5, Annex I of the Commission Regulation (EC) No 1565/2000 (EC, 2000).

The 29 candidate substances under consideration, with their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufactures Association- (FEMA-) numbers, structure and specifications, are listed in Table 1. This group of candidate substances includes three esters of branched-chain aliphatic saturated primary alcohols and branched-chain unsaturated carboxylic acids ([FL-no: 09.586, 09.596 and 09.942]), seven esters of straight-chain aliphatic saturated primary alcohols and branched-chain unsaturated carboxylic acids [FL-no: 09.321, 09.365, 09.375, 09.624, 09.625, 09.647, and 09.680], 18 esters of straight-chain aliphatic saturated primary alcohols and straight-chain unsaturated carboxylic acids [FL-no: 09.248, 09.266, 09.324, 09.326, 09.329, 09.330, 09.335, 09.370, 09.372, 09.374, 09.379, 09.636, 09.637, 09.641, 09.652, 09.699, 09.865 and 09.934], and one ester of a secondary alcohol and a straight-chain unsaturated carboxylic acid [FL-no: 09.603].

The hydrolysis products of candidate substances are listed in Table 2b.

The 29 candidate substances are closely related structurally to the 41 flavouring substances (supporting substances) evaluated at the 51st meeting of the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) in the group ”Linear and Branched-chain Aliphatic, Unsaturated, Unconjugated Alcohols, Aldehydes, Acids and Related Esters” (JECFA, 2000a), and to three substances evaluated at the 61st JECFA meeting in the group “Linear and Branched-chain Aliphatic, Unsaturated, Unconjugated Alcohols, Aldehydes, Acids and Related Esters” (JECFA, 2004a). The names and structures of the 44 supporting substances are listed in Table 3, together with their evaluation status (CoE, 1992; JECFA, 2000a; JECFA, 2004a; SCF, 1995).

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, they may have different chemical properties resulting in possible variation of their absorption, distribution, metabolism, elimination and toxicity. Thus information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number etc.).

Two candidate substances, methyl 2-methylpent-3-enoate [FL-no: 09.625] and 2-methylbutyl 3-methyl-2-butenoate [FL-no: 09.942], possess chiral centres. 2-methylbutyl 3-methyl-2-butenoate [FL-no: 09.942] has been presented without specification of the stereoisomeric composition.

Due to the presence and the position of a double bond, 23 of the 29 substances can exist as geometrical isomers [FL-no: 09.248, 09.266, 09.321, 09.324, 09.326, 09.329, 09.330, 09.335, 09.372, 09.374, 09.379, 09.596, 09.603, 09.624, 09.625, 09.636, 09.637, 09.641, 09.652, 09.680, 09.699, 09.865 and 09.934]. In six [FL-no: 09.266, 09.326, 09.329, 09.335, 09.379 and 09.637] of these cases, no indication has been given that one of the possible isomers has preponderance in the commercial flavouring material (see Table 1). For four of the flavouring substances [FL-no: 09.329, 09.335, 09.379 and 09.637], Industry has informed that they exist as a "mixture of isomers". However, the Panel does not consider this information sufficient and requests data on the actual ratios.

1.3. Natural Occurrence in Food

Twenty-five of the 29 candidate substances have been reported to occur in fruits, mussels, alcoholic beverages, bread, beef fat and essential oils. Quantitative data on the natural occurrence have been reported for eight of these substances.

These reports are:

- Ethyl trans-2-butenoate [FL-no: 09.248]: up to 1.25 mg/kg in guava fruit, 0.99 mg/kg in mussel
- Hexyl 2-butenoate [FL-no: 09.266]: up to 0.01 mg/kg in papaya
- Butyl but-2-enoate [FL-no: 09.324]: 0.024 mg/kg in papaya and less than 0.01 mg/kg in passion fruit
- Butyl hex-2-enoate [FL-no: 09.329]: 0.004 mg/kg in apple juice
- Butyl hex-3-enoate [FL-no: 09.330]: less than 0.01mg/kg in passion fruit
- Ethyl dec-9-enoate [FL-no: 09.370]: up to 0.05 mg/kg in parmesan cheese, up to 0.3 mg/kg in guava fruit, up to 0.9 mg/kg in various types of alcoholic beverages, up to 0.03 mg/kg in various types of wine
- Methyl dec-2-enoate [FL-no: 09.637]: trace amount in pear brandy
- Methyl oleate [FL-no: 09.652]: up to 0.15 mg/kg in cocoa

The remaining four substances (methyl 2-methylpent-3-enoate [FL-no: 09.625], hexyl 9-octadecenoate [FL-no: 09.865], methyl (5Z)-octenoate [FL-no: 09.934] and 2-methylbutyl 3-methyl-2-butenoate [FL-no: 09.942)] have not been reported to occur naturally in any food items according to TNO (TNO, 2000).

1.4. Evaluations From Other Expert Groups

Methyl methacrylate has been evaluated in the context of Council Regulation (EEC) No 793/93 on the evaluation and control of the risks of existing substances. Further there is a Risk Assessment Report (CEC, 2002) which has been peer-reviewed by the Scientific Committee for Toxicity, Ecotoxicity and the Environment (CSTEE, 2000). The following conclusions on methyl methacrylate were drawn in the EU Risk Assessment Report (CEC, 2002):

"From the two-year drinking water study in rats (Borzelleca et al., 1964) a NOAEL of 200 mg/kg body weight/day was derived."

"*In vitro* methyl methacrylate has the potential for induction of mutagenic effects, esp. clastogenicity; however, this potential seems to be limited to high doses with strong toxic effects. Furthermore, the negative *in vivo* micronucleus test and the negative dominant lethal assay indicate that this potential is probably not expressed *in viv*o."

"There is no relevant concern on carcinogenicity in humans and animals. Epidemiology data on increased tumor rates in exposed cohorts were of limited reliability and cannot be related to methyl methacrylate as the solely causal agent. Therefore there are no reasons to assume that methyl methacrylate should be considered to be carcinogenic in humans."

Five out of the 29 candidate substances have been evaluated by the Scientific Committee on Food (SCF, 2002a). Three of these substances (methyl methacrylate, ethyl methacrylate and isobutyl 2-methyl prop-2-enoate) have a temporary group-TDI of 0.1 mg/kg body weight (as methacrylic acid) derived from a study on methyl methacrylate. The remaining two (methyl crotonate, methyl oleate) of these five substances are listed on the SCF_List 7 and 8 (List 7: "Defined data still missing but no special concern expected"; List 8: "No toxicological data") as there were not enough data available.

Crotonic acid [FL-no: 08.072], which is the hydrolysis product of several of the candidate esters, has been evaluated by the SCF (SCF_list 3: Limit of migration: 50 microgram/kg food, based on the reduced core set of toxicological data according to the migration level[2]) (SCF, 2002a).

2. Specifications

Purity criteria for the 29 candidate substances have been provided by the Flavour Industry (EFFA, 2001c; Flavour Industry, 2006a).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the purity criteria for [FL-no: 09.326] are insufficient. Otherwise the specifications are adequate for all 29 candidate substances (see Table 1), except that, stereoisomeric information is needed for seven substances [FL-no: 09.266, 09.326, 09.329, 09.335, 09.379, 09.637 and 09.942] (see Section 1.2 and Table 1).

3. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the "Maximised Survey-derived Daily Intake" (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of

[2] *The Panel noted that this migration limit for crotonic acid could apparently conflict with reported use levels of the crotonates included in candidate substances (butyl but-2-enoate [FL-no: 09.324], methyl crotonate [FL-no: 09.636], propyl crotonate [FL-no: 09.699], and isopropyl crotonate [FL-no: 09.603]). However, the migration limit is set after administrative considerations (limited data submitted) rather than on toxicological data, and the Panel therefore did not find the low migration limit for crotonic acid as such in conflict with higher use levels of the crotonates, which could therefore go through the Procedure.*

consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999).

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake in most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The Maximised Survey-derived Daily Intake (MSDI (SCF, 1999)) data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the EU population[3] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

In the present Flavouring Group Evaluation (FGE.05Rev1) the total annual volume of production of the 29 candidate substances for use as flavouring substances in Europe has been reported to be approximately 214 kg (EFFA, 2001e; Flavour Industry, 2006a). For 41 of the 44 supporting substances, the total annual volume of production has been reported by JECFA to be approximately 39000 kg (hex-3(cis)-en-1-ol [FL-no: 02.056] accounts for 30000 kg and oleic acid [FL-no: 08.013] for 6800 kg) (JECFA, 2000a). For the remaining three supporting substances [FL-no: 02.110, 08.059, and 09.646] information is not available for Europe (JECFA, 2004b).

On the basis of the annual volume of production reported for the 29 candidate substances, MSDI values for each of these flavourings have been estimated (Table 2a). The estimated MSDI of ethyl trans-2-butenoate [FL-no: 09.248] from use as a flavouring substance is 12 microgram/*capita*/day

[3] *EU figure 375 millions. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.*

and that of methyl (5Z)-octenoate [FL-no: 09.934] is 3.7 microgram/*capita*/day. The daily *per capita* intakes for each of the remaining substances are all less than 2 microgram (Table 2a).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For the present evaluation of the 29 candidate substances, information on food categories and normal and maximum use levels[4,5,6] were submitted for 28 of the substances by the Flavour Industry (EFFA, 2001c; Flavour Industry, 2006a; EFFA, 2007a). Use levels for [FL-no: 09.326] are missing. The 28 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different normal use levels were reported for different food categories the highest reported normal use level was used.

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
Category 1	Dairy products, excluding products of category 2	All 28
Category 2	Fats and oils, and fat emulsions (type water-in-oil)	All 28 except [FL-no: 09.942]
Category 3	Edible ices, including sherbet and sorbet	All 28
Category 4.1	Processed fruits	All 28 except [FL-no: 09.248]
Category 4.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Only [FL-no: 09.934 and 09.942]
Category 5	Confectionery	All 28
Category 6	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All 28
Category 7	Bakery wares	All 28 except [FL-no: 09.942]
Category 8	Meat and meat products, including poultry and game	All 28 except [FL-no: 09.942]
Category 9	Fish and fish products, including molluscs, crustaceans and echinoderms	All 28 except [FL-no: 09.942]
Category 10	Eggs and egg products	None
Category 11	Sweeteners, including honey	None
Category 12	Salts, spices, soups, sauces, salads, protein products etc.	All 28 except [FL-no: 09.865]
Category 13	Foodstuffs intended for particular nutritional uses.	All 28 except [FL-no: 09.248, 09.865 and 09.942]
Category 14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All 28
Category 14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All 28
Category 15	Ready-to-eat savouries	All 28 except [FL-no: 09.248 and 09.942]
Category 16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All 28 except [FL-no: 09.248 and 09.942]

[4] *"Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).*

[5] *The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).*

[6] *The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007a).*

According to the Flavour Industry the normal use levels for 28 of the 29 candidate substances are in the range of 0.2 - 20 mg/kg food, and the maximum use levels are in the range of 1 - 100 mg/kg (EFFA, 2001c; Flavour Industry, 2006a).

The mTAMDI values for the 25 of the 26 candidate substances from structural class I (see Section 6) range from 820 to 9500 microgram/person/day. For the three candidate substances from structural class II the mTAMDI is for each 3900 microgram/person/day.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

4. Absorption, Distribution, Metabolism and Elimination

In vitro hydrolysis data shown in Table III.1 from studies with saturated esters and one unsaturated ester (unsaturated in both its alcohol and carboxylic acid moieties), structurally related to the candidate substances and the supporting substances, indicate that the unsaturated esters included in this Flavouring Group Evaluation are hydrolysed to yield the corresponding alcohols and carboxylic acids either prior to absorption in the gut or after distribution into the tissues, in particular the liver (Grundschober, 1977; Longland et al., 1977; Gangolli & Shilling, 1968; Leegwater & Straten, 1974a).

Short chain (<C8) straight- and branched-chain aliphatic alcohols and carboxylic acids are rapidly absorbed from the gastrointestinal tract (Dawson et al., 1964b; Gaillard & Derache, 1965). Long-chain carboxylic acids, such as linoleic acid and oleic acid, are readily absorbed from micelles in the jejunum, re-esterified with glycerol in chylomicrons and transported *via* the lymphatic system (Borgström, 1974). Radiolabeled linoleic and oleic acids have been administered by different routes to a variety of mammals and humans, demonstrating that fatty acid uptake occurs in all tissues, including the brain, by passive/facilitated diffusion and/or active transport (Dhopeshwarkar & Mead, 1973; Harris et al., 1980; Abumrad et al., 1984; Schulthess et al., 2000).

By hydrolysis of the candidate esters in the present FGE, the resulting primary alcohols and aldehydes undergo functional group oxidation to yield their corresponding carboxylic acids. The resulting carboxylic acids are metabolised *via* high capacity beta-oxidation pathways (Voet & Voet, 1990). In addition, straight long-chain carboxylic acids and branched-chain compounds may be oxidised *via* omega- and beta-oxidation to yield polar metabolites, which are excreted as the glucuronic acid or sulphate conjugates, primarily in the urine. The one resulting secondary alcohol (isopropanol) can be conjugated to glucuronic acid or can be oxidised to give acetone, which can be exhaled or further metabolised to lactic acid and ultimately carbon dioxide (Morgott, 1993).

A more detailed discussion on hydrolysis of straight- and branched-chain esters, metabolism of saturated primary alcohols, and straight- and branched-chain unsaturated carboxylic acids is given in Annex III (Section III.2.1). Evaluation of the general aspects of metabolism of these types of substances has also been performed by JECFA (JECFA, 1999a).

The hydrolysis products resulting from the hydrolysis of eight candidate substances [FL-no: 09.321, 09.375, 09.586, 09.596, 09.624, 09.625, 09.647, and 09.680] are structurally related to valproic acid (2-n-propyl pentanoic acid) in that they are branched at the *alpha* position relative to the carbonyl group. Since valproic acid is well known to be teratogenic in humans (Samren et al., 1997) the structures of these hydrolysis products might possibly be considered as structural alert for teratogenicity. However, these hydrolysis products are *alpha*-methyl-branched. *alpha*-Methyl

carboxylic acids like 2-methyl pentanoic acid, 2-methyl hexanoic acid and 2-methyl octanoic acid did not show developmental effects in rats (Narotsky et al., 1994). Further, certain alpha-methylated carboxylic acids are endogenous substances formed during the metabolism of amino acids like isoleucine and valine (Zubay, 1988). Therefore, the Panel does not consider the *alpha*-methyl branching of the eight candidate substances as structural alert for teratogenicity.

Terminal double bonds appear in four candidate substances [FL-no: 09.370, 09.375, 09.586, and 09.647] and thus also in their hydrolysis products. These double bonds may be oxidized to the corresponding epoxides. Epoxides are highly reactive molecules, due to the large strain associated with the three membered ring structure, and they react easily with nucleophilic sites of cellular macromolecules. For this reason, several aliphatic alkene-derived epoxides (e.g. ethylene, isoprene, butadiene, and glycidol) have been demonstrated to be carcinogenic (Melnick, 2002). However, epoxides can be conjugated with glutathione by glutathione S-transferases or hydrolysed to diols by epoxide hydrolases. The latter two reactions can be considered to be detoxications.

As mentioned, it has been demonstrated that terminal double bonds may be oxidised at the double bond to give the corresponding epoxide or, alternatively, at the allylic carbon to give the allylic alcohol, as was demonstrated with 1-hexene with rat and human P450s (Chiappe et al., 1998). The ratio of epoxidation over allylic oxidation, as measured with different P450 isoforms (CYP) is $\geq$1, indicating that epoxide formation is generally favoured (Chiappe et al., 1998). In the same paper (Chiappe et al., 1998), it was demonstrated that the biotransformation of 2-methyl-1-hexene proceeds exclusively via the epoxide, which was further hydrolysed by epoxide hydrolases to the diol. This pathway might apply to the hydrolysis product of [FL-no: 09.375, 09.586, and 09.647].

However, the presence of the terminal double bond in these four candidate substances is not considered of concern because of the following reasons:

- Epoxides can be detoxicated by conjugation with glutathione or by epoxide hydrolase mediated hydrolysis.
- The terminal double bonds are all present in the carboxylic acid moieties of the candidate substances. Biochemical attack of these moieties via e.g. beta-oxidation is anticipated to be more efficient and rapid than microsomal oxidation.
- Based on genotoxicity data available for seven out of 48 flavouring substances with terminal double bonds from the Register (EC, 1999a; EC, 2004) it is not indicated that a terminal double bond distal to a functional group is a structural alert for genotoxicity.

Conclusion

The candidate substances are all esters that may be expected to be hydrolysed to the corresponding aliphatic alcohols and carboxylic acids. The resulting alcohols are oxidized to carboxylic acids and together with other carboxylic acids formed during hydrolysis, oxidized in the fatty acid and tricarboxylic acid pathways. The intermediates in these pathways can be considered endogenous and based on these aspects the end products can be considered innocuous. However, due to neurotoxicity data on ethyl methacrylate [FL-no: 09.375] this substance as well as methyl methacrylate [FL-no: 09.647] and isobutyl 2-methylprop-2-enoate [FL-no: 09.586] which are structurally related to ethyl methacrylate cannot *a priori* be predicted to be metabolised to innocuous products (see Section 5).

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where, the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the safety evaluation of the 29 candidate substances from chemical groups 1, 2 and 5 the Procedure was applied. The stepwise evaluations of the 29 substances are summarised in Table 2a.

Step 1:

Twenty-six of the 29 candidate substances are classified in structural class I according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978). Three candidate substances [FL-no: 09.375, 09.586, and 09.647] are classified in structural class II, since hydrolysis of these substances results in formation of methacrylic acid.

Step 2:

Since ethyl methacrylate [FL-no: 09.375] induced neurotoxicity in a 60-day drinking water study in rats even at the lowest dose tested (0.1 % in drinking water, equivalent to approximately 50 mg/kg body weight/day) (Abou-Donia et al., 2000), this substance as well as methyl methacrylate [FL-no: 09.647] and isobutyl 2-methylprop-2-enoate [FL-no: 09.586], which are structurally related to ethyl methacrylate, cannot *a priori* be predicted to be metabolised to innocuous products. Therefore, the evaluation for these three substances proceeds via the B-side of the Procedure scheme.

The remaining 26 candidate substances would not be expected to saturate metabolic pathways, and all of them can be predicted to be metabolised to innocuous products. The evaluation of these 26 candidate substances, therefore, proceeds via the A-side of the Procedure scheme (Annex I).

Step A3:

The 26 candidate substances ([FL-no: 09.248, 09.266, 09.321, 09.324, 09.326, 09.329, 09.330, 09.335, 09.365, 09.370, 09.372, 09.374, 09.379, 09.596, 09.603, 09.624, 09.625, 09.636, 09.637, 09.641, 09.652, 09.680, 09.699, 09.865, 09.934 and 09.942]) that have been assigned to structural class I have estimated European daily *per capita* intakes (MSDI) from 0.0012 to 12 microgram (Table 2a). These intakes are below the threshold of concern of 1800 microgram/person/day for structural class I.

Based on results of the safety evaluation sequence of the Procedure, these 26 candidate substances proceeding via the A-side of the Procedure scheme do not pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

Step B3:

According to the MSDI approach, the estimated European daily *per capita* intakes for ethyl methacrylate [FL-no: 09.375], methyl methacrylate [FL-no: 09.647] and isobutyl 2-methylprop-2-enoate [FL-no: 09.586], belonging to structural class II, are 0.12, 0.061, and 0.012 microgram/*capita*/day, respectively. Thus, the intake estimated according to the MSDI approach is below the threshold of concern for structural class II substances (540 microgram/person/day). Accordingly, these three candidate substances proceed to step B4 of the Procedure.

Step B4:

Ethyl methacrylate [FL-no: 09.375] induced neurotoxicity in a 60-day drinking water study in rats even at the lowest dose tested (0.1 % in drinking water, equivalent to approximately 50 mg/kg body weight/day) (Abou-Donia et al., 2000). Accordingly, a NOAEL could not be established. Also, methyl methacrylate has a neurotoxic potential, as shown in an EU Risk Assessment Report (CEC, 2002). However, an adequate NOAEL from an oral study comparable to that of Abou-Donia et al. (2000) is not available for methyl methacrylate.

Therefore, additional toxicity data for ethyl methacrylate [FL-no: 09.375], methyl methacrylate [FL-no: 09.647] and the structurally related isobutyl 2-methylprop-2-enoate [FL-no: 09.586] are required.

6. Comparison of the Intake Estimations based on the MSDI Approach and the mTAMDI Approach

The estimated intakes for 25 of the 26 candidate substances in structural class I based on the mTAMDI range from 820 to 9500 microgram/person/day. For all of the substances except [FL-no: 09.934 and 09.942] the mTAMDI-values are above the threshold of concern for structural class I of 1800 microgram/person/day. For comparison of the intake estimate based on the MSDI approach and mTAMDI approach see Table 6.1. Use levels have not been submitted for [FL-no: 09.326].

The estimated intake for each of the three substances assigned to structural class II, based on the mTAMDI is 3900 microgram/person/day, which is above the threshold of concern for structural class II substances of 540 microgram/person/day. For comparison of the MSDI- and mTAMDI-values see Table 6.1.

For 27 of the 29 candidate substances further information is required. This would include more reliable intake data and where required additional toxicity data.

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
09.248	Ethyl trans-2-butenoate	12	9500	Class I	1800
09.266	Hexyl 2-butenoate	0.12	3900	Class I	1800
09.321	Butyl 2-methylbut-2(cis)-enoate	1.2	3900	Class I	1800
09.324	Butyl but-2-enoate	1.7	3900	Class I	1800
09.326	Butyl deca-2,4-dienoate	0.0012		Class I	1800
09.329	Butyl hex-2-enoate	1.0	3900	Class I	1800
09.330	Butyl hex-3-enoate	0.12	3900	Class I	1800
09.335	Butyl oct-2-enoate	0.66	3900	Class I	1800
09.365	Ethyl 3-methylcrotonate	0.0012	3900	Class I	1800
09.370	Ethyl dec-9-enoate	0.012	3900	Class I	1800
09.372	Ethyl dodec-2-enoate	0.34	3900	Class I	1800
09.374	Ethyl hept-2-enoate	0.61	3900	Class I	1800
09.379	Ethyl pent-2-enoate	0.037	3900	Class I	1800
09.596	Isopentyl 2-methylcrotonate	0.012	3900	Class I	1800
09.603	Isopropyl crotonate	0.24	3900	Class I	1800
09.624	Methyl 2-methylcrotonate	0.12	3900	Class I	1800
09.625	Methyl 2-methylpent-3-enoate	0.0012	3900	Class I	1800
09.636	Methyl crotonate	0.12	3900	Class I	1800
09.637	Methyl dec-2-enoate	0.37	3900	Class I	1800

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
09.641	Methyl dodec-2-enoate	0.56	3900	Class I	1800
09.652	Methyl oleate	1.2	3900	Class I	1800
09.680	Pentyl 2-methylisocrotonate	0.74	3900	Class I	1800
09.699	Propyl crotonate	0.085	3900	Class I	1800
09.865	Hexyl 9-octadecenoate	0.24	3600	Class I	1800
09.934	Methyl (5Z)-Octenoate	3.7	820	Class I	1800
09.942	2-Methylbutyl 3-methyl-2-butenoate	1.2	1630	Class I	1800
09.375	Ethyl methacrylate	0.12	3900	Class II	540
09.586	Isobutyl 2-methylprop-2-enoate	0.012	3900	Class II	540
09.647	Methyl methacrylate	0.061	3900	Class II	540

7. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this Flavouring Group Evaluation may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2001c; Flavour Industry, 2006a) the combined estimated *per capita* intake as flavouring of the 26 candidate substances assigned to structural class I is 26 microgram/day, which does not exceed the threshold of concern for the structural class of 1800 microgram/person/day. For the three candidate substances assigned to structural class II the combined intake is 0.19 microgram/day, which does not exceed the threshold of concern for structural class II of 540 microgram/person/day.

The 29 candidate substances are structurally related to 44 flavouring substances (all structural class I substances) evaluated by JECFA at its 51st session (JECFA, 1999a) and 61st session (JECFA, 2004b). The total estimated combined intake of candidate and supporting substances (in Europe) would be 4900 microgram/*capita*/day (European data were not available for three of the supporting substances), which would exceed the threshold of concern for structural class I. One substance, hex-3(cis)-en-1-ol [FL-no: 02.056] accounts for 3700 microgram/*capita*/day, but as there exists a NOAEL of 120-150 mg/kg body weight (bw)/day, this provides an adequate margin of safety under the conditions of intended use. Therefore, at the level of exposure, based on the MSDI approach, the total combined intake as flavouring substances of the candidate and supporting substances would not be expected to be of safety concern.

8. Toxicity

8.1. Acute toxicity

Data are available for seven candidate substances and for 17 structurally related supporting substances evaluated by JECFA (JECFA, 2000a; JECFA, 2004a). The oral LD50 values range from 500 to 19000 mg/kg body weight (bw) in rats and mice.

The acute toxicity data are summarised in Annex IV, Table IV.1.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Data are available for three candidate substances, ethyl methacrylate [FL-no: 09.375] methyl methacrylate [FL-no: 09.647] and methyl oleate [FL-no: 09.652], and for 11 supporting substances [FL-no: 02.001, 02.003, 02.005, 02.056, 05.074, 08.013, 08.039, 08.041, 08.044, 08.070, and 09.248].

Ethyl methacrylate [FL-no: 09.375]

Ethyl methacrylate induced neurotoxicity in a 60-day drinking water study in rats even at the lowest dose tested (0.1 % in drinking water, equivalent to approximately 50 mg/kg body weight/day) (Abou-Donia et al., 2000).

Groups of eight adult male Sprague-Dawley rats received ethyl methacrylate [FL-no: 09.375] (99 % purity) orally in drinking water at concentrations of 0.1, 0.2, or 0.5 % for 60 days (this is equivalent to approximately 50, 100, and 250 mg/kg body weight/day assuming 25 ml water consumption *per* day and 500 g body weight). Control rats were administered tap water. Alterations in clinical condition were observed at 0.2 % and 0.5 % ethyl methacrylate. Morphological alterations were observed in sections of brain, spinal cord, and sciatic nerve from rats treated with 0.1 %, 0.2 % and 0.5 % ethyl methacrylate in drinking water. Spongiform alterations were observed in fiber tracts of the forebrain, brainstem, and spinal cord. Clusters of axonal swellings were scattered throughout the dorsal, ventral, and lateral columns of the spinal cord, and typically involved internodal segments of two or three neighbouring axons. Shrunken axons with separated myelin lamellae and large axons with thinner than normal myelin sheaths were apparent in the sciatic nerve. The patterns of alterations in the white matter of the spinal cord and the sciatic nerve are consistent with myelinopathy, but the authors stressed that additional experiments are necessary to confirm whether oligodendroglia and Schwann cells are the primary sites of injury. In addition to the alterations associated with myelin, there was a decrease in the density of neurons in the ventral horn of the spinal cord (Abou-Donia et al., 2000).

Remarks: This study was roughly in accordance with OECD guideline 424 (21 July 1997), however, some criteria were not met (e.g. no females tested, no ophthalmological examinations, number of sites examined histologically). The study was not in compliance with GLP but is published in a peer-reviewed journal and contains sufficient details about the parameters examined. The authors pointed out that the observed effects of ethyl methacrylate on the nervous system of rats are consistent with neurologic symptoms of workers exposed to ethyl methacrylate, but that additional experiments are necessary to determine if the level and route of exposures associated with occupational use produce these impairments in experimental animals. Accordingly, the study demonstrates that ethyl methacrylate can result in neurotoxicity after oral consumption. However, a NOAEL could not be established.

Methyl methacrylate [FL-no: 09.647]

Methyl methacrylate has been evaluated in the context of Council Regulation (EEC) No 793/93 on the evaluation and control of the risks of existing substances. Conclusions drawn in the EU Risk Assessment Report (CEC, 2002) and considered relevant for the current evaluation are summarized in Section 1.4 and below.

There are a number of findings which may indicate an effect of methyl methacrylate on the nervous system (CEC, 2002). In an insufficiently documented 21-day oral rat study, effects on behaviour (listlessness, locomotoric activity, learning ability, gait and rear leg function), and changes in brain chemistry and peripheral nervous system were observed at 500 mg/kg body weight (Husain et al., 1985). In an early 2-year study on Beagle dogs and Wistar rats treated orally with methyl methacrylate, histopathological findings on brains revealed no abnormalities or lesion attributable to the test material (Borzelleca et al., 1964). However, other parameters for neurotoxicity such as brain chemistry were not investigated and clinical observations on behaviour were not reported. The maximum concentrations used in the study of Borzelleca et al. (1964) were 2000 ppm in drinking water for rats (equivalent to approximately 200 mg/kg body weight/day) and 1000 ppm in corn oil administered with gelatine capsules to dogs.

The EU Risk Assessment Report on methyl methacrylate summarizes also some reports on neurotoxicity resulting from inhalative exposure of animals and humans to methyl methacrylate. These reports may additionally be used to characterise the hazard potential of methyl methacrylate, however, they are of minor relevance for assessing the risk resulting from oral exposure.

The repeated dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

Data are available on three candidate substances [FL-no: 09.375, 09.586, and 09.647] and one supporting substance [FL-no: 08.048]. However, clear conclusions could not be drawn.

Developmental/reproductive toxicity data are summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

There are *in vitro* genotoxicity data for four candidate substances [FL-no: 09.375, 09.586, 09.647, and 09.652] and for four supporting substances [FL-no: 05.074, 08.013, and a mixture of 09.646 and methyl linoleate]. *In vivo* data are available for two candidate substances [FL-no: 09.586 and 09.647] and for one supporting substance [FL-no: 05.074].

Genotoxicity data are summarised in Annex IV, Tables IV.4 and Table IV.5.

8.4.1. Studies on candidate substances

In vitro studies

Methyl oleate [FL-no: 09.652], methyl methacrylate [FL-no: 09.647], ethyl methacrylate [FL-no: 09.375] and isobutyl 2-methylprop-2-enoate [FL-no: 09.586] were reported to be nonmutagenic in standard, pre-incubation or liquid suspension protocol Ames assays including *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, TA1537, and/or TA1538 with or without metabolic activation (Table IV.4). In three instances, the results of Ames assays with methyl methacrylate were weakly positive; however, these results were accompanied by cytotoxicity.

Methyl methacrylate and ethyl methacrylate have been tested in several mammalian cell assays (Table IV.4). Positive results seen in chromosome aberrations, mouse lymphoma, Sister Chromatid Exchange (SCE), HPRT, and/or micronucleus assays in most instances were obtained at high exposure concentrations (i.e. > 10 mM or > 1000 microgram/ml) and (when reported) high levels of cytotoxicity. However, when methyl methacrylate was tested in a mouse lymphoma assay at concentrations between 5 and 10 mM in the presence of S9-mix, it revealed a positive result which was accompanied by only low cytotoxicity (about 80 % survival at 5 mM and approximately 40 % at 10 mM) (Dearfield et al., 1991).

In vivo studies

Methyl methacrylate [FL-no: 09.647] was evaluated in a mouse micronucleus study conducted by oral gavage. The result was negative, however, it is not clear whether the substance had reached the bone marrow. Two sex-linked recessive lethal mutation studies (one by inhalation and the other by injection) in *Drosophila melanogaster* were negative, as was a dominant lethal assay in mice conducted *via* inhalation exposure. Rats exposed to high inhalation concentrations of methyl methacrylate did have weak, but statistically significant, increases in chromosome aberrations in bone marrow cells at some exposure levels in comparison to the negative control values both after single and multiple exposures. However, a clear conclusion cannot be drawn from these studies. SCE and chromosome aberration studies with peripheral lymphocytes from male workers occupationally exposed to methyl methacrylate by inhalation for eight hours/day were negative (Table IV.5).

Isobutyl 2-methylprop-2-enoate [FL-no: 09.586] was evaluated in a mouse micronucleus study with oral doses as high as 5000 mg/kg. Results were reported to be negative (Table IV.5).

For methyl methacrylate, genotoxicity data were summarized the EU Risk Assessment Report (CEC, 2002) as follows:

"Methyl methacrylate was negative in bacterial gene mutation tests. From mammalian cell culture assays it may be concluded that methyl methacrylate is a high toxicity clastogen (i.e. induction of chromosomal aberrations is bound to highly toxic doses). The effect is not dependent on presence of S9-mix. These findings are in line with results from mouse lymphoma assays where positive findings seem to be due to the induction of small colonies. Marginal increases in SCE frequencies are of low significance."

"*In vivo* an oral mouse bone marrow micronucleus test was negative for doses up to 4520 mg/kg. No clear conclusion could be drawn from bone marrow chromosomal aberration assays with rats. A dominant lethal assay with male mice led to a negative result."

"*In vitro* methyl methacrylate has the potential for induction of mutagenic effects, esp. clastogenicity; however, this potential seems to be limited to high doses with strong toxic effects. Furthermore, the negative *in vivo* micronucleus test and the negative dominant lethal assay indicate that this potential is probably not expressed *in vivo*."

8.4.2. Studies on supporting substances

Mutagenicity/genotoxicity testing has been performed on four supporting substances. The results of these studies are described below and summarized in Tables IV.4 and IV.5.

In vitro studies

No evidence of mutagenicity was reported for 2,6-dimethyl-5-heptenal [FL-no: 05.074], oleic acid [FL-no: 08.013], methyl linolenate [FL-no: 09.646] or methyl linoleate in the standard or pre-

incubation protocol Ames assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 with or without the addition of metabolic activation (Shimizu et al., 1985; Mortelmans et al., 1986; Wild et al., 1983; Heck et al., 1989) (Table IV.4). The maximum doses reported for these studies ranged from 333 to 50000 microgram/plate. In further bacterial assays, such as the rec-assay utilizing *Bacillus subtili*s, incubated with oleic acid (Osawa & Namiki, 1982), the His + reversion assay utilizing *Salmonella typhimurium* incubated with methyl linoleate or methyl linolenate (MacGregor et al., 1985), and a modified Ames test utilizing *Escherichia coli WP2uvrA* incubated with oleic acid (Shimizu et al., 1985), these aliphatic unsaturated non-conjugated acids and esters were non-mutagenic.

With respect to mammalian cell assays, rat hepatocytes were tested for unscheduled DNA synthesis (UDS) after exposure to concentrations of up to 1.0 mg 2,6-dimethyl-5-heptenal/ml (Table IV.4). The results from this study showed no genotoxic effects (Heck et al., 1989).

***In vivo* studies**

A bone marrow micronucleus test was conducted *in vivo* in mice with a maximum single dose of 1540 mg/kg 2,6-dimethyl-5-heptenal. All mice survived the treatment. There were no statistically significant increases in the incidence of micronucleated polychromatic erythrocytes (PCEs) observed (Wild et al., 1983). However, the quality of the study is limited since only a single sampling time was used and the PCE/NCE ratio was not reported. Therefore, it is not clear whether the substance had reached the bone marrow.

In the *Basc* test using *Drosophila melanogaster*, 2,6-dimethyl-5-heptenal was negative when tested at a concentration of 25 mM (Wild et al., 1983).

Conclusion on genotoxicity

Genotoxicity data are available only for a limited number of substances, and the genotoxicity could not be assessed adequately. However, the data available do not preclude the evaluation of the candidate substances using the Procedure.

9. Conclusions

The 29 candidate substances are esters of branched- and straight-chain aliphatic saturated primary alcohols, and of one secondary alcohol, and branched- and straight-chain unsaturated carboxylic acids.

Twenty-three of the 29 candidate substances can exist as geometrical isomers. For six of these substances [FL-no: 09.266 09.326, 09.329, 09.335, 09.379 and 09.637] the stereoisomeric composition has not been specified. Two flavouring substances possess chiral centres. One of these [FL-no: 09.942] has been presented without any indication that the commercial flavouring substance has dominance of one or the other enantiomer.

Twenty-six candidate substances belong to structural class I while three substances were assigned to structural class II, according to the decision tree approach presented by Cramer et al., 1978.

Twenty-five of the candidate substances in the present group have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, the 29 candidate substances in this group have intakes in Europe from 0.0012 to 12 microgram/*capita*/day, which are below the threshold of concern value

for structural class I (1800 microgram/person/day) and structural class II (540 microgram/person/day) substances.

On the basis of the reported annual production volumes in Europe (MSDI approach), the combined intake of the 26 candidate substances belonging to structural class I and of the three candidate substances belonging to structural class II would result in a total intake of approximately 26 and 0.19 microgram/*capita*/day, respectively. These values are lower than the thresholds of concern for structural class I and class II substances (1800 and 540 microgram/person/day, respectively). The total combined intake of candidate and supporting substances in Europe is approximately 4900 microgram/*capita*/day, which exceeds the thresholds of concern for structural class I and II (1800 and 540 microgram/person/day, respectively). However, for one of the substances [FL-no: 02.056] (which alone accounts for 3700 microgram/*capita*/day) exists a NOAEL, which provides an adequate margin of safety. Therefore, the total combined intake would not be expected to be of safety concern.

Genotoxicity data are available only for a limited number of substances, and the genotoxicity could not be assessed adequately. However, the data available do not preclude evaluating the candidate substances using the Procedure.

Except for the three methacrylates [FL-no: 09.375, 09.647 and 09.586], the candidate substances are expected to be metabolised to innocuous products.

The three methacrylates in this group of 29 candidate substances are ethyl methacrylate [FL-no: 09.375], methyl methacrylate [FL-no: 09.647], and isobutyl 2-methylprop-2-enoate [FL-no: 09.586]. Ethyl methacrylate induced neurotoxicity in a 60-day drinking water study in rats at 50 mg/kg body weight/day, the lowest dose tested. Accordingly, a NOAEL could not be established. Methyl methacrylate has a neurotoxic potential likewise, as shown in an EU Risk Assessment Report. However, an adequate NOAEL from an oral study is not available for methyl methacrylate. For isobutyl 2-methylprop-2-enoate, there are neither neurotoxicity studies nor other repeated dose toxicity studies. Therefore, additional toxicity data for ethyl methacrylate [FL-no: 09.375], methyl methacrylate [FL-no: 09.647], and isobutyl 2-methylprop-2-enoate [FL-no: 09.586] are required.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

It is considered that on the basis of the default MSDI approach the remaining 26 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach they ranged from 820 to 9500 microgram/person/day for 25 of the 26 candidate substances from structural class I. For one flavouring substance [FL-no: 09.326] use levels are missing. Except for [FL-no: 09.934 and 09.942] these intakes were above the threshold of concern for structural class I of 1800 microgram/person/day. The estimated intakes of the three candidate substances [FL-no: 09.375, 09.586, and 09.647] assigned to structural class II, based on the mTAMDI approach are for each 3900 microgram/person/day, which are above the threshold of concern for structural class II of 540 microgram/person/day. The substances [FL-no: 09.934 and 09.942], which have mTAMDI intake estimate below the threshold of concern for structural class I, is also expected to be metabolised to innocuous products.

Thus, for the 23 of the 26 candidate substances from structural class I and for the three substances allocated to structural class II, the intakes, estimated on the basis of the mTAMDI approach, exceed

the relevant threshold for the structural class. Therefore, for 27 substances, including the flavouring substance [FL-no: 09.326] for which use levels are missing, more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Subsequently, additional data might become necessary.

In order to determine whether the conclusions for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for 28 of the 29 candidate substances, except that information on geometrical isomerism/chirality is missing for seven of the substances ([FL-no: 09.266, 09.326, 09.329, 09.335, 09.379, 09.637 and 09.942]). For the substance [FL-no: 09.326], in addition, identity test is missing.

Thus, the final evaluation of the materials of commerce cannot be performed for seven substances ([FL-no: 09.266, 09.326, 09.329, 09.335, 09.379, 09.637 and 09.942]), pending further information on specifications. For three substances [FL-no: 09.375, 09.586 and 09.647] additional toxicity data are requested. The remaining 19 substances [FL-no: 09.248, 09.321, 09.324, 09.330, 09.365, 09.370, 09.372, 09.374, 09.596, 09.603, 09.624, 09.625, 09.636, 09.641, 09.652, 09.680, 09.699, 09.865 and 09.934] would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 5 REVISION 1

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 5 Revision 1

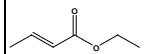
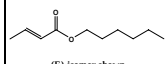
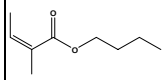
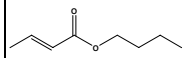
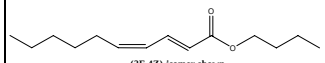
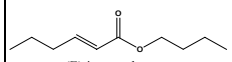
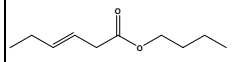
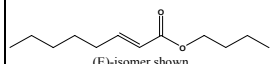
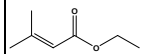
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
09.248	Ethyl trans-2-butenoate		3486 2244 623-70-1	Liquid $C_6H_{10}O_2$ 114.14	Practically insoluble or insoluble 1 ml in 1 ml	143 MS 98 %	1.423-1.427 0.914-0.918	
09.266	Hexyl 2-butenoate 6)	 (E)-isomer shown	3354 10688 19089-92-0	Liquid $C_{10}H_{18}O_2$ 170.25	Practically insoluble or insoluble 1 ml in 1 ml	97 (2 hPa) NMR 95 %	1.428-1.449 0.880-0.895	CASrn in Register refers to the (E)-isomer.
09.321	Butyl 2-methylbut-2(cis)-enoate		7785-64-0	Liquid $C_9H_{16}O_2$ 156.22	Insoluble 1 ml in 1 ml	74 (12 hPa) MS 95 %	1.432-1.438 0.906-0.912	
09.324	Butyl but-2-enoate		591-63-9	Liquid $C_8H_{14}O_2$ 142.2	Insoluble 1 ml in 1 ml	80 (56 hPa) MS 98 %	1.425-1.435 0.901-0.909	Register name to be changed to Butyl but-(2E)-enoate.
09.326	Butyl deca-2,4-dienoate 6)	 (2E,4Z)-isomer shown	10529 28369-24-6	Liquid $C_{14}H_{24}O_2$ 224.34	Practically insoluble or insoluble 1 ml in 1 ml	69 (0.001 hPa) 95 %	1.480-1.486 0.893-0.899	ID 7) CASrn in Register refers to the (2E,4Z)-isomer.
09.329	Butyl hex-2-enoate 6)	 (E)-isomer shown	13416-74-5	Liquid $C_{10}H_{18}O_2$ 170.25	Insoluble 1 ml in 1 ml	217 MS 95 %	1.439-1.445 0.890-0.895	(Z) or (E) isomer not specified by CASrn in Register.
09.330	Butyl hex-3-enoate		118869-62-8	Liquid $C_{10}H_{18}O_2$ 170.25	Insoluble 1 ml in 1 ml	217 MS 95 %	1.438-1.444 0.890-0.895	Register name to be changed to Butyl hex-(3E)-enoate.
09.335	Butyl oct-2-enoate 6)	 (E)-isomer shown	10536 57403-32-4	Liquid $C_{12}H_{22}O_2$ 198.25	Insoluble 1 ml in 1 ml	253 NMR 95 %	1.450-1.456 0.884-0.889	(Z) or (E) isomer not specified by CASrn in Register.
09.365	Ethyl 3-methylcrotonate		10610 638-10-8	Liquid $C_7H_{12}O_2$ 128.17	Insoluble 1 ml in 1 ml	150 MS 95 %	1.434-1.441 0.917-0.923	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 5 Revision 1

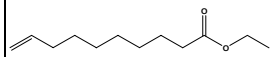
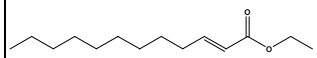
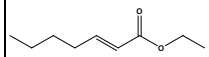
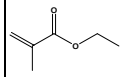
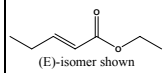
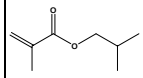
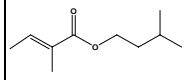
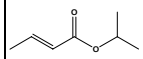
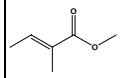
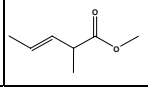
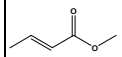
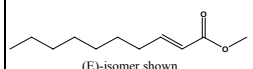
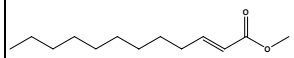
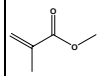
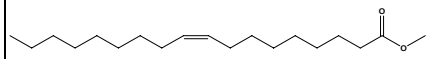
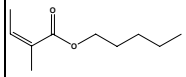
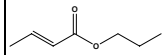
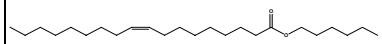
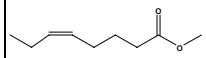
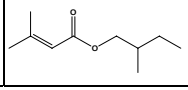
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
09.370	Ethyl dec-9-enoate		10579 67233-91-4	Liquid $C_{12}H_{22}O_2$ 198.31	Insoluble 1 ml in 1 ml	135 (37 hPa) MS 95 %	1.434-1.440 0.874-0.879	
09.372	Ethyl dodec-2-enoate		10584 28290-90-6	Liquid $C_{14}H_{26}O_2$ 226.36	Insoluble 1 ml in 1 ml	144 (20 hPa) MS 95 %	1.436-1.442 0.864-0.870	Register name to be changed to Ethyl dodec-(2E)-enoate.
09.374	Ethyl hept-2-enoate		54340-72-6	Liquid $C_9H_{16}O_2$ 156.22	Insoluble 1 ml in 1 ml	80 (27 hPa) MS 95 %	1.435-1.441 0.885-0.891	Register name to be changed to Ethyl hept-(2E)-enoate.
09.375	Ethyl methacrylate		97-63-2	Liquid $C_6H_{10}O_2$ 114.14	Insoluble 1 ml in 1 ml	117 MS 95 %	1.410-1.416 0.910-0.916	
09.379	Ethyl pent-2-enoate 6)	 (E)-isomer shown	10623 2445-93-4	Liquid $C_7H_{12}O_2$ 128.17	Insoluble 1 ml in 1 ml	157 MS 95 %	1.428-1.434 0.904-0.910	(Z) or (E) isomer not specified by CASrn in Register.
09.586	Isobutyl 2-methylprop-2-enoate		97-86-9	Liquid $C_8H_{14}O_2$ 142.20	Insoluble 1 ml in 1 ml	155 MS 95 %	1.409-1.415 0.882-0.888	
09.596	Isopentyl 2-methylcrotonate		10482-55-0	Liquid $C_{10}H_{18}O_2$ 170.25	Insoluble 1 ml in 1 ml	202 MS 95 %	1.437-1.442 0.889-0.894	CASrn in Register refers to the (Z)-isomer. CASrn to be changed.
09.603	Isopropyl crotonate		10729 6284-46-4	Liquid $C_7H_{12}O_2$ 128.17	Practically insoluble or insoluble 1 ml in 1 ml	146 MS 95 %	1.419-1.425 0.889-0.895	
09.624	Methyl 2-methylcrotonate		6622-76-0	Liquid $C_6H_{10}O_2$ 114.14	Insoluble 1 ml in 1 ml	137 MS 95 %	1.430-1.436 0.938-0.944	
09.625	Methyl 2-methylpent-3-enoate		33603-30-4	Liquid $C_7H_{12}O_2$ 128.17	Insoluble 1 ml in 1 ml	142 NMR 95 %	1.415-1.421 0.902-0.907	Racemate. Register name to be changed to Methyl 2-methylpent-3(E)-enoate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 5 Revision 1

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
09.636	Methyl crotonate		623-43-8	Liquid $C_5H_8O_2$ 100.12	Slightly soluble 1 ml in 1 ml	119 MS 98 %	1.424-1.427 0.977-0.983	
09.637	Methyl dec-2-enoate 6)	 (E)-isomer shown	11799 2482-39-5	Liquid $C_{11}H_{20}O_2$ 184.28	Insoluble 1 ml in 1 ml	123 (21 hPa) MS 95 %	1.442-1.448 0.887-0.892	(Z) or (E) isomer not specified by CASrn in Register.
09.641	Methyl dodec-2-enoate		10792 6208-91-9	Liquid $C_{13}H_{24}O_2$ 212.33	Insoluble 1 ml in 1 ml	151 (20 hPa) MS 95 %	1.445-1.451 0.881-0.886	Register name to be changed to Methyl dodec-(2E)-enoate.
09.647	Methyl methacrylate		4002 80-62-6	Liquid $C_5H_8O_2$ 100.12	Insoluble 1 ml in 1 ml	100 MS 95 %	1.409-1.415 0.933-0.939	
09.652	Methyl oleate		10836 112-62-9	Liquid $C_{19}H_{36}O_2$ 296.54	Insoluble 1 ml in 1 ml	160 (4 hPa) MS 95 %	1.448-1.454 0.876-0.882	
09.680	Pentyl 2-methylisocrotonate		7785-63-9	Liquid $C_{10}H_{18}O_2$ 170.25	Insoluble 1 ml in 1 ml	213 MS 95 %	1.439-1.445 0.891-0.896	
09.699	Propyl crotonate		10352-87-1	Liquid $C_7H_{12}O_2$ 128.17	Insoluble 1 ml in 1 ml	158 MS 98 %	1.425-1.431 0.903-0.909	(Z)- or (E)- isomer not specified by CASrn in Register. CASrn to be changed.
09.865	Hexyl 9-octadecenoate		20290-84-0	Liquid $C_{24}H_{46}O_2$ 366.63	Insoluble 1 ml in 1 ml	207 (7 hPa) MS 95 %	1.454-1.460 0.866-0.872	Register name to be changed to Hexyl (9Z)-octadecenoate.
09.934 1630	Methyl (5Z)-Octenoate		4165 41654-15-3	Liquid $C_9H_{16}O_2$ 156.20	Very slightly soluble Freely soluble	187 (97.5 hPa) IR NMR MS 95.1 %	1.438-1.432 0.921-0.925	
09.942	2-Methylbutyl 3-methyl-2-butenoate 6)		97890-13-6	Liquid $C_{10}H_{18}O_2$ 170.25	Pratically insoluble or soluble 1 ml in 1 ml	58 (4.7 hPa) NMR MS 98%	1.451-1.461 0.881-0.891	CASrn reported refers to racemate.

1) Solubility in water, if not otherwise stated.

2) Solubility in 95% ethanol, if not otherwise stated.

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

6) Stereoisomeric composition not specified.

7) ID: Missing identification test.

TABLE 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI Approach)

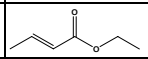
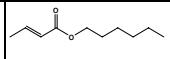
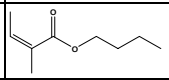
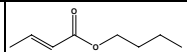
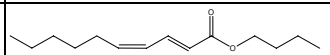
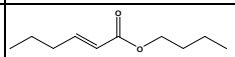
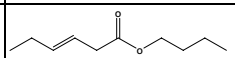
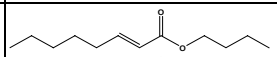
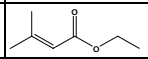
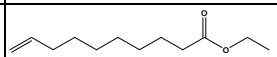
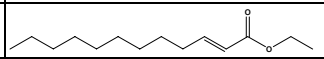
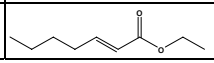
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.248	Ethyl trans-2-butenoate		12	Class I A3: Intake below threshold	4)	6)	
09.266	Hexyl 2-butenoate	 (E)-isomer shown	0.12	Class I A3: Intake below threshold	4)	7)	
09.321	Butyl 2-methylbut-2(cis)-enoate		1.2	Class I A3: Intake below threshold	4)	6)	
09.324	Butyl but-2-enoate		1.7	Class I A3: Intake below threshold	4)	6)	
09.326	Butyl deca-2,4-dienoate	 (2E,4Z)-isomer shown	0.0012	Class I A3: Intake below threshold	4)	7)	
09.329	Butyl hex-2-enoate	 (E)-isomer shown	1.0	Class I A3: Intake below threshold	4)	7)	
09.330	Butyl hex-3-enoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.335	Butyl oct-2-enoate	 (E)-isomer shown	0.66	Class I A3: Intake below threshold	4)	7)	
09.365	Ethyl 3-methylcrotonate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.370	Ethyl dec-9-enoate		0.012	Class I A3: Intake below threshold	4)	6)	
09.372	Ethyl dodec-2-enoate		0.34	Class I A3: Intake below threshold	4)	6)	
09.374	Ethyl hept-2-enoate		0.61	Class I A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI Approach)

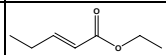
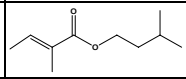
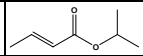
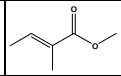
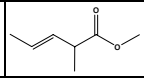
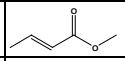
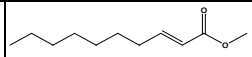
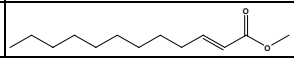
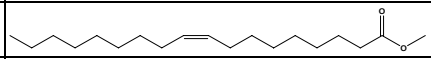
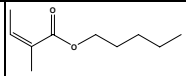
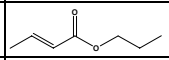
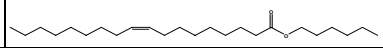
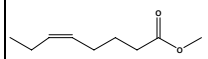
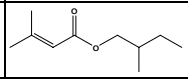
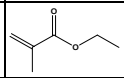
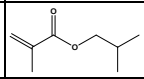
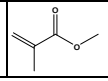
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.379	Ethyl pent-2-enoate	 (E)-isomer shown	0.037	Class I A3: Intake below threshold	4)	7)	
09.596	Isopentyl 2-methylcrotonate		0.012	Class I A3: Intake below threshold	4)	6)	
09.603	Isopropyl crotonate		0.24	Class I A3: Intake below threshold	4)	6)	
09.624	Methyl 2-methylcrotonate		0.12	Class I A3: Intake below threshold	4)	6)	
09.625	Methyl 2-methylpent-3-enoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.636	Methyl crotonate		0.12	Class I A3: Intake below threshold	4)	6)	
09.637	Methyl dec-2-enoate	 (E)-isomer shown	0.37	Class I A3: Intake below threshold	4)	7)	
09.641	Methyl dodec-2-enoate		0.56	Class I A3: Intake below threshold	4)	6)	
09.652	Methyl oleate		1.2	Class I A3: Intake below threshold	4)	6)	
09.680	Pentyl 2-methylisocrotonate		0.74	Class I A3: Intake below threshold	4)	6)	
09.699	Propyl crotonate		0.085	Class I A3: Intake below threshold	4)	6)	
09.865	Hexyl 9-octadecenoate		0.24	Class I A3: Intake below threshold	4)	6)	
09.934 1630	Methyl (5Z)-Octenoate		3.7	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
09.942	2-Methylbutyl-3-methyl-2-butenoate		1.2	Class I A3: Intake below threshold	4)	7)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI Approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.375	Ethyl methacrylate		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
09.586	Isobutyl 2-methylprop-2-enoate		0.012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
09.647	Methyl methacrylate		0.061	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

a) See sections 5 and 9.

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

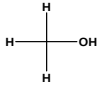
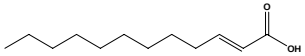
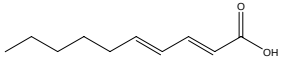
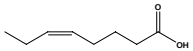
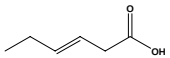
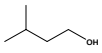
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	Methanol		Not evaluated as flavour Not evaluated as flavour Not evaluated as flavour		Not in EU-Register.
	Methacrylic acid		Not evaluated as flavour Not evaluated as flavour Not evaluated as flavour		Not in EU-Register.
	2-Dodecenoic acid		Not evaluated as flavour Not evaluated as flavour Not evaluated as flavour		Not in EU-Register.
	Deca-2,4-dienoic acid	 (2E, 4Z)-isomer shown	Not evaluated as flavour Not evaluated as flavour Not evaluated as flavour		Not in EU-Register.
	(5Z)-Octenoic acid		Not evaluated as flavour Not evaluated as flavour Not evaluated as flavour		Not in EU-Register.
	trans-Hex-3-enoic acid		Not evaluated as flavour Not evaluated as flavour Not evaluated as flavour		Not in EU-Register.
02.001	2-Methylpropan-1-ol 251		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.002	Propan-1-ol 82		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.003	Isopentanol 52		Category 1 a) No safety concern d) Category A c)	Class I A3: Intake below threshold	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

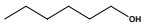
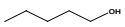
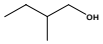
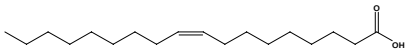
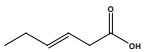
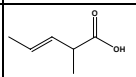
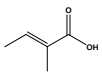
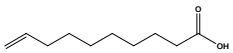
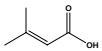
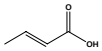
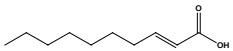
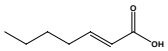
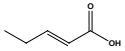
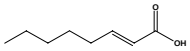
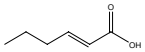
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
02.004	Butan-1-ol 85		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.005	Hexan-1-ol 91		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.040	Pentan-1-ol 88		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.076	2-Methylbutan-1-ol 1199		Category 1 a) No safety concern e) Category B c)	Class I A3: Intake below threshold	
02.078	Ethanol 41		Category 1 a) No safety concern d)	No evaluation	At the forty-sixth JECFA meeting (JECFA, 1997a), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents.
02.079	Isopropanol 277		Category 1 a) No safety concern f)	Class I A3: Intake above threshold, A4: Endogenous	
08.013	Oleic acid 333		Category 1 a) No safety concern f) Deleted c)	Class I A3: Intake below threshold	
08.050	Hex-3-enoic acid 317		Category 1 a) No safety concern f) Category B c)	Class I A3: Intake below threshold	
08.058	2-Methylpent-3-enoic acid 347	 (E)-isomer shown	Category 1 a) No safety concern f)	Class I A3: Intake below threshold	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.064	2-Methylcrotonic acid 1205		No safety concern e)	Class I A3: Intake below threshold	
08.065	Dec-9-enoic acid 328		Category 1 a) No safety concern f)	Class I A3: Intake below threshold	
08.070	3-Methylcrotonic acid 1204		No safety concern e)	Class I A3: Intake below threshold	
08.072	But-2-enoic acid			No evaluation	
08.073	Dec-2-enoic acid 1372		No safety concern g)	Class I A3: Intake below threshold	
08.083	Hept-2-enoic acid			No evaluation	
08.107	Pent-2-enoic acid		FGE.28	No evaluation	
08.114	2-Octenoic acid		FGE.28	No evaluation	
08.119	2-Hexenoic acid			No evaluation	
08.128	2-Methylbut-2(cis)-enoic acid			No evaluation	

1) Category 1: Considered safe in use Category 2: Temporarily considered safe in use Category 3: Insufficient data to provide assurance of safety in use Category 4): Not acceptable due to evidence of toxicity.

2) No safety concern at estimated levels of intake.

3) Category A: Flavouring substance, which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.

4) Threshold of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.

5) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

a) (SCF, 1995).

b) (JECFA, 1999b).

c) (CoE, 1992).

d) (JECFA, 1997a).

e) (JECFA, 2004a).

f) (JECFA, 2000a).

g) (JECFA, 2005c).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

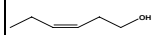
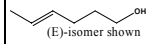
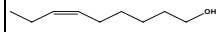
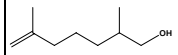
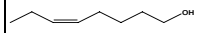
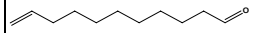
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
02.056	Hex-3(cis)-en-1-ol		2563 750c 928-96-1	315 JECFA specification (JECFA, 1998b)	3700	Category 1 a) No safety concern b) Category A c)	
02.074	Hex-4-en-1-ol	 (E)-isomer shown	3430 2295 6126-50-7	318 JECFA specification (JECFA, 1998b)	2.4	Category 2 a) No safety concern b) Category B c)	JECFA evaluated 4-hexen-1-ol (CASrn as in Register). (Z)- or (E)-isomer not specified by CASrn in Register.
02.093	Non-6-en-1-ol		3465 10294 35854-86-5	324 JECFA specification (JECFA, 2000d)	2.2	No safety concern b)	JECFA evaluated cis-6-nonen-1-ol (CASrn as in Register). CASrn in Register refers to (Z)-isomer. Register name to be changed to Non-6Z-en-1-ol.
02.094	Oct-3-en-1-ol		3467 10296 20125-84-2	321 JECFA specification (JECFA, 1998b)	4.7	Category 2 a) No safety concern b)	JECFA evaluated cis-3-octen-1-ol (CASrn as in Register). CASrn in Register refers to the (Z)-isomer. Register name to be changed to Oct-3Z-en-1-ol.
02.110	2,6-Dimethylhept-6-en-1-ol		3663 36806-46-9	348 JECFA specification (JECFA, 2003b)	ND	Category 3 a) No safety concern b)	JECFA evaluated 2,6-dimethyl-6-hepten-1-ol (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
02.113	Oct-5(cis)-en-1-ol		3722 64275-73-6	322 JECFA specification (JECFA, 2003b)	0.4	Category 2 a) No safety concern b)	
05.035	Undec-10-enal		3095 122 112-45-8	330 JECFA specification (JECFA, 2001c)	0.32	No safety concern b) Category B c)	

Table 3: Supporting Substances Summary

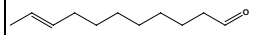
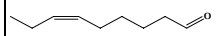
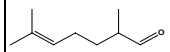
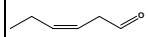
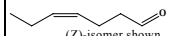
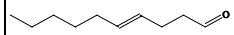
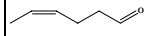
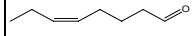
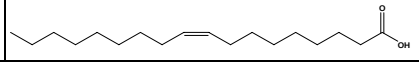
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
05.036	Undec-9-enal	 (E)-isomer shown	3094 123 143-14-6	329 JECFA specification (JECFA, 2003b)	0.97	No safety concern b) Category A c)	JECFA evaluated 9-undecenal (CASrn as in Register). (Z)- or (E)-isomer not specified by CASrn in Register.
05.059	Non-6(cis)-enal		3580 661 2277-19-2	325 JECFA specification (JECFA, 2003b)	1.7	No safety concern b) Category B c)	
05.074	2,6-Dimethylhept-5-enal		2389 2006 106-72-9	349 JECFA specification (JECFA, 2003b)	27	Category 1 a) No safety concern b) Category B c)	JECFA evaluated 2,6-dimethyl-5-heptenal (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
05.075	Hex-3(cis)-enal		2561 2008 6789-80-6	316 JECFA specification (JECFA, 2000d)	4.1	No safety concern b) Category B c)	
05.085	Hept-4-enal	 (Z)-isomer shown	3289 2124 6728-31-0	320 JECFA specification (JECFA, 2000d)	1.6	No safety concern b) Category B c)	JECFA evaluated 4-heptenal (CASrn as in Register). CASrn in Register refers to the (Z)-isomer.
05.096	4-Decenal	 (E)-isomer shown	3264 2297 30390-50-2	326 JECFA specification (JECFA, 2001c)	0.97	No safety concern b) Category B c)	JECFA evaluated 4-decenal (CASrn as in Register). (Z)- or (E)-isomer not specified by CASrn in Register.
05.113	Hex-4-enal		3496 10337 4634-89-3	319 JECFA specification (JECFA, 2000d)	0.024	No safety concern b)	JECFA evaluated cis-4-hexenal (CASrn as in Register). CASrn in Register refers to the (Z)-isomer. Register name to be changed to Hex-4Z-enal.
05.128	Oct-5(cis)-enal		3749 41547-22-2	323 JECFA specification (JECFA, 2003b)	0.0012	No safety concern b)	
08.013	Oleic acid		2815 13 112-80-1	333 JECFA specification (JECFA, 2000d)	830	Category 1 a) No safety concern b) Deleted c)	

Table 3: Supporting Substances Summary

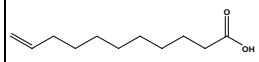
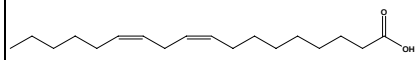
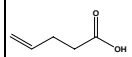
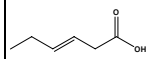
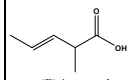
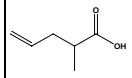
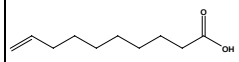
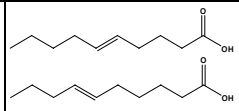
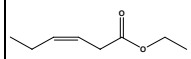
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
08.039	Undec-10-enoic acid		3247 689 112-38-9	331 JECFA specification (JECFA, 1998b)	26	Category 1 a) No safety concern b) Category A c)	
08.041	Octadeca-9,12-dienoic acid		3380 694 60-33-3	332 JECFA specification (JECFA, 2003b)	110	Category 1 a) No safety concern b) Deleted c)	Register name to be changed to Linoleic acid.
08.048	Pent-4-enoic acid		2843 2004 591-80-0	314 JECFA specification (JECFA, 1998b)	3.9	No safety concern b) Category B c)	
08.050	Hex-3-enoic acid	 (E)-isomer shown	3170 2256 4219-24-3	317 JECFA specification (JECFA, 2000d)	9.4	Category 1 a) No safety concern b) Category B c)	JECFA evaluated 3-hexenoic acid (CASrn as in Register). (Z)- or (E)-isomer not specified by CASrn in Register.
08.058	2-Methylpent-3-enoic acid	 (E)-isomer shown	3464 10147 37674-63-8	347 JECFA specification (JECFA, 2001c)	1.2	Category 1 a) No safety concern b)	JECFA evaluated 2-methyl-3-pentenoic-acid (CASrn as in Register). (Z)- or (E)-isomer not specified by CASrn in Register.
08.059	2-Methylpent-4-enoic acid		3511 10148 1575-74-2	355 JECFA specification (JECFA, 1998b)	ND	Category N a) No safety concern b)	JECFA evaluated 2-methyl-4-pentenoic-acid (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
08.065	Dec-9-enoic acid		3660 10090 14436-32-9	328 JECFA specification (JECFA, 2001c)	0.097	Category 1 a) No safety concern b)	
08.068	Dec-(5- and 6)-enoic acid	 (E)-isomers shown	3742 72881-27-7	327 JECFA specification (JECFA, 2000d)	3.4	Category N a) No safety concern b)	JECFA evaluated 5 & 6-decenoic acid (mixture) (CASrn as in Register). CASrn in Register refers to incompletely defined substance.
09.191	Ethyl hex-3-enoate	 (Z)-isomer shown	3342 2396-83-0	335 JECFA specification (JECFA, 1998b)	3.2	No safety concern b)	JECFA evaluated ethyl-3-hexenoate (CASrn as in Register). (Z)- or (E)-isomer not specified by CASrn in Register.

Table 3: Supporting Substances Summary

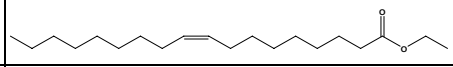
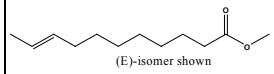
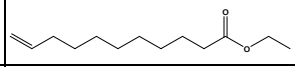
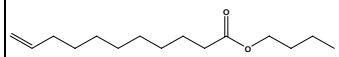
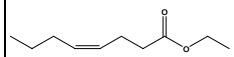
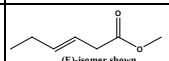
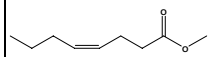
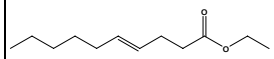
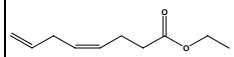
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.192	Ethyl oleate		2450 633 111-62-6	345 JECFA specification (JECFA, 1998b)	60	No safety concern b) Category A c)	
09.236	Methyl undec-9-enoate	 (E)-isomer shown	2750 2101 5760-50-9	342 JECFA specification (JECFA, 2000d)	34	No safety concern b) Deleted c)	JECFA evaluated methyl 9-undecanoate (CASrn as in Register). (Z)- or (E)-isomer not specified by CASrn in Register.
09.237	Ethyl undec-10-enoate		2461 10634 692-86-4	343 JECFA specification (JECFA, 1998b)	1.5	No safety concern b) Deleted c)	
09.238	Butyl undec-10-enoate		2216 2103 109-42-2	344 JECFA specification (JECFA, 2001c)	0.037	No safety concern b) Category B c)	
09.265	Ethyl oct-4-enoate		3344 10619 34495-71-1	338 JECFA specification (JECFA, 2003b)	1.2	No safety concern b)	JECFA evaluated ethyl cis-4-octenoate (CASrn as in Register). CASrn in Register refers to (Z)-isomer. Register name to be changed to Ethyl oct-4Z-enoate.
09.267	Methyl hex-3-enoate	 (E)-isomer shown	3364 10801 2396-78-3	334 JECFA specification (JECFA, 2001c)	0.56	No safety concern b)	Z- or E-isomer not specified by name and CASrn in Register.
09.268	Methyl oct-4(cis)-enoate		3367 10834 21063-71-8	337 JECFA specification (JECFA, 2003b)	0.37	No safety concern b)	
09.284	Ethyl dec-4-enoate		3642 10578 76649-16-6	341 JECFA specification (JECFA, 2000d)	1.8	No safety concern b)	JECFA evaluated ethyl trans-4-decenoate (CASrn as in Register). CASrn refers to (E)-isomer. Register name to be changed to Ethyl dec-4E-enoate.
09.290	Ethyl octa-4,7-dienoate		3682 69925-33-3	339 JECFA specification (JECFA, 2000d)	1.8	No safety concern b)	JECFA evaluated ethyl cis-4,7-octadienoate (CASrn as in Register). CASrn in Register refers to the (Z)-isomer. Register name to be changed to Ethyl octa-4Z,7-dienoate.

Table 3: Supporting Substances Summary

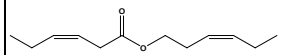
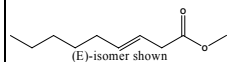
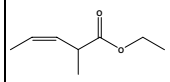
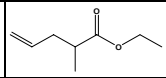
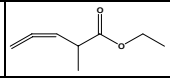
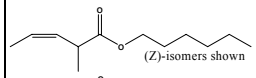
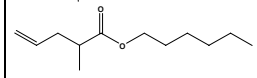
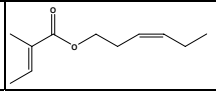
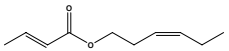
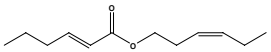
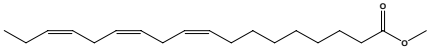
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.291	Hex-3-enyl hex-3-enoate		3689 61444-38-0	336 JECFA specification (JECFA, 1998b)	3.2	No safety concern b)	JECFA evaluated cis-3-hexenyl cis-3-hexenoate (CASrn as in Register). CASrn in Register refers to the (Z)/(Z)-isomer. Register name to be changed to Hex-3Z-enyl hex-3Z-enoate.
09.298	Methyl non-3-enoate	 (E)-isomer shown	3710 13481-87-3	340 JECFA specification (JECFA, 2000d)	1.6	No safety concern b)	JECFA evaluated methyl 3-nonenoate (CASrn as in Register). (Z)- or (E)-isomer not specified by CASrn in Register.
09.524	Ethyl 2-methylpent-3-enoate	 (Z)-isomer shown	3456 10612 1617-23-8	350 JECFA specification (JECFA, 2001c)	4.9	No safety concern b)	JECFA evaluated ethyl 2-methyl-3-pentenoate (CASrn as in Register). (Z)- or (E)-isomer nor (R) or (S) enantiomer not specified by Register CASrn.
09.527	Ethyl 2-methylpent-4-enoate		3489 10613 53399-81-8	351 JECFA specification (JECFA, 1998b)	0.024	No safety concern b)	(R) or (S) enantiomer not specified by Register CASrn.
09.540	Ethyl 2-methylpenta-3,4-dienoate		3678 60523-21-9	353 JECFA specification (JECFA, 2000d)	0.012	No safety concern d)	(R) or (S) enantiomer not specified by Register CASrn.
09.546	Hexyl-2-methylpent-(3 and 4)-enoate	 (Z)-isomers shown 	3693 58625-95-9	352 JECFA specification (JECFA, 2001c)	0.024	No safety concern b)	JECFA evaluated hexyl 2-methyl-3&4-pentenoate (mixture) (CASrn as in Register). Register CASrn refers to the (E)-isomer. (R) or (S) enantiomer not specified by Register CASrn.
09.559	Hex-3(cis)-enyl 2-methylcrotonate		3931 67883-79-8	1277 JECFA specification (JECFA, 2003b)	0.024	No safety concern e)	

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.566	Hex-3-enyl but-2-enoate	 (Z)-isomer in alcohol moiety and (E)-isomer in carboxylic acid moiety shown	3982 65405-80-3	1276 JECFA specification (JECFA, 2003b)	0.24	No safety concern e)	Register name to be changed to Hex-3Z-enyl but-2E-enoate.
09.568	Hex-3-enyl hex-2-enoate	 (Z)-isomer in alcohol moiety and (E)-isomer in carboxylic acid moiety shown	3928 53398-87-1	1279 JECFA specification (JECFA, 2003b)	0.12	No safety concern e)	JECFA evaluated 3-hexenyl 2-hexenoate (CASrn as in Register). Register CASrn refers to the (3Z,2E)-isomer.
09.646	Methyl linolenate		3411 714 301-00-8	346 JECFA specification (JECFA, 2003b)	ND	No safety concern b) Category A c)	JECFA evaluated a mixture of methyl linoleate and methyl linolenate (CASrn as in Register). Register CASrn refers to the (Z)/(Z)/(Z)-isomer (i.e. methyl linolenate).

1) EU MSDI: Amount added to food as flavouring substance in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.

3) No safety concern at estimated levels of intake.

4) Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

a) (SCF, 1995).

b) (JECFA, 2000a).

c) (CoE, 1992).

d) (JECFA, 2007c).

e) (JECFA, 2004a).

ND: not detected

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products[7] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[8] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

[7] *"Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).*

[8] *"Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).*

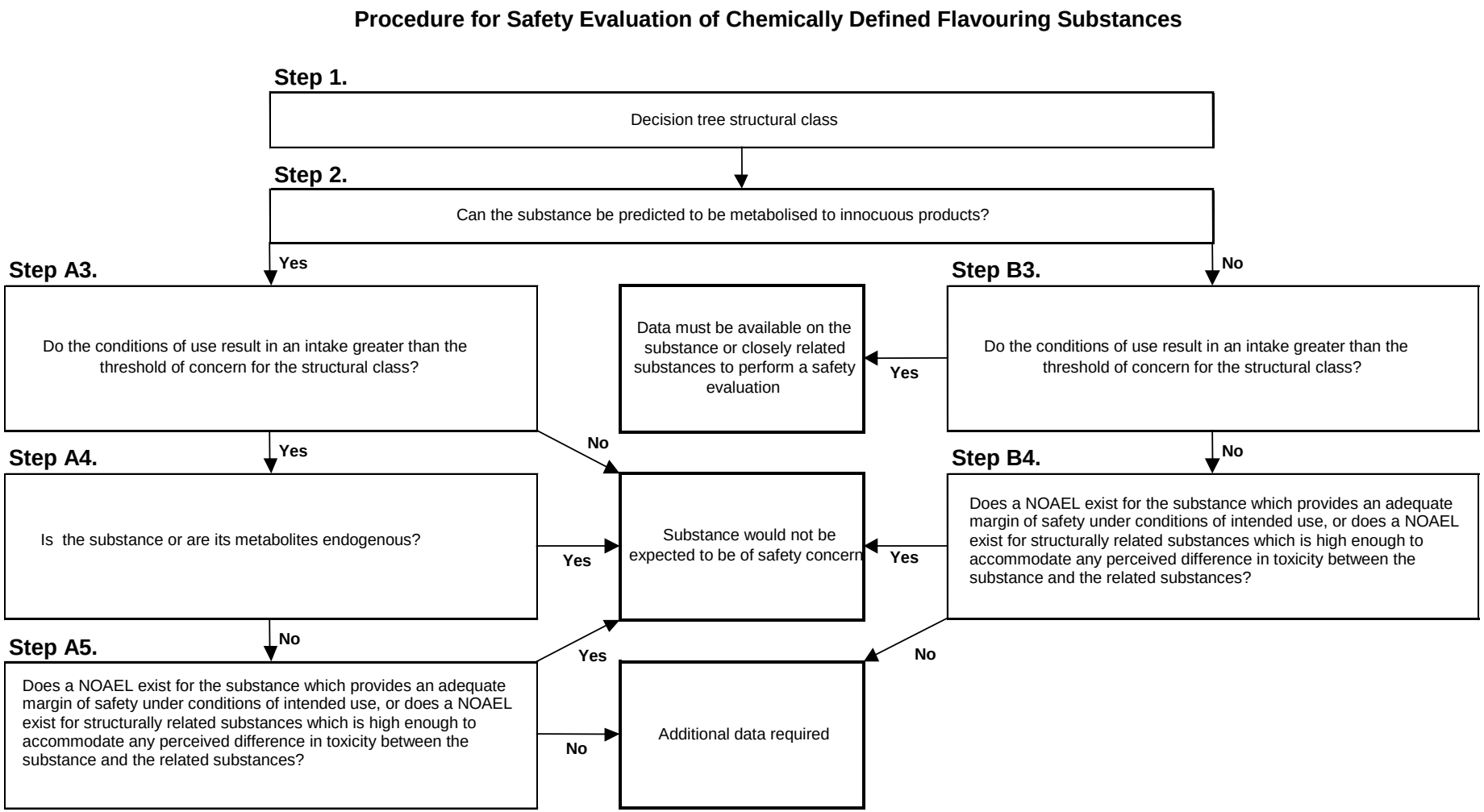


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and maximum use levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a "normal use level" and a "maximum use level" (EC, 2000). According to the Industry the "normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

Table II.1.1 Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000)

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ("soft") beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0

The "normal and maximum use levels" are provided by Industry for 28 of the 29 candidate substances in the present flavouring group (Table II.1.2). For one flavouring substance [FL-no: 09.326] use levels are missing.

Table II.1.2. Normal and Maximum use levels (mg/kg) for candidate substances in FGE.05rev1 (EFFA, 2001c; Flavour Industry, 2006a)

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
09.248	6 8	0 1	3 8	- -	- -	60 100	1,2 7	2,4 6	1 2	1 5	- -	- -	1 2	- -	20 50	30 100	1 10	- -
09.266	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.321	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.324	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.329	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.330	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.335	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.365	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.370	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.372	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25

09.374	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.375	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.379	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.586	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.596	7 35	5 25	10 50	7 35	- -	10 50	5 25	1 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.603	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.624	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.625	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.636	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.637	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.641	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.647	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	5 25	5 25	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.652	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	5 25	5 25	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.680	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.699	7 35	5 25	1 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.865	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	- -	- -	5 25	10 50	10 50	5 25
09.934	1 3	2 5	1 2	0,5 1	0,5 1	1 3	2 5	5 10	5 10	5 10	- -	- -	1 3	1 3	0,2 2	0,2 2	2 5	1 5
09.942	2 5	- -	5 10	4 10	4 10	5 10	5 10	- -	- -	- -	- -	- -	5 10	- -	2 8	4 10	- -	- -

II.2 mTAMDI calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000) and reported by the Flavour Industry in the following way (see Table II.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000)

- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No. 1565/2000 (EC, 2000) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation 1565/2000	**Distribution of the seven SCF food categories**		
Key	Food category	Food	Beverages	Exceptions
01	Dairy products, excluding products of category 02.0	Food		
02	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05	Confectionery			Exception a
06	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07	Bakery wares	Food		
08	Meat and meat products, including poultry and game	Food		
09	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10	Eggs and egg products	Food		
11	Sweeteners, including honey			Exception a
12	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15	Ready-to-eat savouries			Exception b
16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values (see Table II.2.3) are presented for 28 of the 29 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2001c; Flavour Industry, 2006a). Use levels have not been submitted for [FL-no: 09.326]. The mTAMDI values are only given for highest reported normal use level (see Table II.2.3).

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	**EU Register name**	**mTAMDI (µg/person/day)**	**Structural class**	**Threshold of concern (µg/person/day)**
09.248	Ethyl trans-2-butenoate	9500	Class I	1800
09.266	Hexyl 2-butenoate	3900	Class I	1800
09.321	Butyl 2-methylbut-2(cis)-enoate	3900	Class I	1800
09.324	Butyl but-2-enoate	3900	Class I	1800
09.326	Butyl deca-2,4-dienoate		Class I	1800
09.329	Butyl hex-2-enoate	3900	Class I	1800
09.330	Butyl hex-3-enoate	3900	Class I	1800
09.335	Butyl oct-2-enoate	3900	Class I	1800
09.365	Ethyl 3-methylcrotonate	3900	Class I	1800
09.370	Ethyl dec-9-enoate	3900	Class I	1800
09.372	Ethyl dodec-2-enoate	3900	Class I	1800
09.374	Ethyl hept-2-enoate	3900	Class I	1800
09.379	Ethyl pent-2-enoate	3900	Class I	1800
09.596	Isopentyl 2-methylcrotonate	3900	Class I	1800
09.603	Isopropyl crotonate	3900	Class I	1800
09.624	Methyl 2-methylcrotonate	3900	Class I	1800
09.625	Methyl 2-methylpent-3-enoate	3900	Class I	1800
09.636	Methyl crotonate	3900	Class I	1800
09.637	Methyl dec-2-enoate	3900	Class I	1800
09.641	Methyl dodec-2-enoate	3900	Class I	1800

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
09.652	Methyl oleate	3900	Class I	1800
09.680	Pentyl 2-methylisocrotonate	3900	Class I	1800
09.699	Propyl crotonate	3900	Class I	1800
09.865	Hexyl 9-octadecenoate	3600	Class I	1800
09.934	Methyl (5Z)-Octenoate	820	Class I	1800
09.942	2-Methylbutyl 3-methyl-2-butenoate (CN91)	1630	Class I	1800
09.375	Ethyl methacrylate	3900	Class II	540
09.586	Isobutyl 2-methylprop-2-enoate	3900	Class II	540
09.647	Methyl methacrylate	3900	Class II	540

ANNEX III: METABOLISM

III.1. Introduction

The group of substances in this flavouring group evaluation consists of 29 substances, which are all esters. They differ by chain length and chain type: branched/linear and saturated/unsaturated. The alcohol moieties of all esters are saturated and differ in chain length from C1 to C6. Branching of the carbon chain in the alcohol moieties occurs only in three of the candidate esters and in one of the esters a secondary alcohol moiety is present. The carboxylic acid moieties of the candidate esters are all unsaturated, and the double bond occurs at even or at odd positions from the carboxylic acid group. As a result of the presence of double bonds (geometric isomerism), both *cis* and *trans* configurations occur. In addition, the carboxylic acid moieties of 19 candidate esters are linear, while those of the remaining 10 candidates are branched.

In the text below the consequences of these structural features for the metabolism of these candidate esters are discussed.

III.2. Absorption, Distribution, Metabolism and Excretion studies

III.2.1. Hydrolysis and Metabolism of Esters

Ester Hydrolysis

Aliphatic esters are hydrolysed to their corresponding alcohols and carboxylic acids as shown in Figure III.1. Ester hydrolysis is catalysed by carboxylesterases or esterases, the most important of which are the B-carboxylesterase isoenzymes (Heymann, 1980). In mammals, these enzymes occur throughout the body in most tissues, but they predominate in the hepatocytes (Heymann, 1980). The substrate specificity of B-carboxylesterases has been correlated with the structure of the alcohol and carboxylic acid moieties (i.e. R and R_1; see Figure III.2; Heymann, 1980).

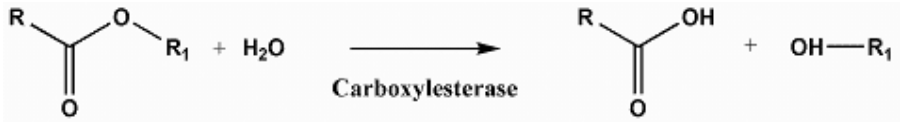


Figure III.1 *Ester hydrolysis*

In vitro hydrolysis studies of various esters have been performed with specific carboxylesterase isoenzymes isolated from pig and rat livers (Junge & Heymann, 1979; Arndt & Krisch, 1973).

Arndt & Krisch (1973) have studied the influence of the chain length (C1-C7) of the alcohol and the carboxylic acid moieties of saturated aliphatic esters on kinetic parameters of rat liver carboxylesterase. It was found that an elongation of the alcohol chain up to C4 leads to a linear increase of the K_m values approaching a plateau from C4 to C7. A variation in chain length of the carboxylic acid moiety had no significant influence on K_m (Arndt & Krisch, 1973). In a later study with similar substrates, other authors found that short chain unbranched aliphatic esters are good substrates for pig-liver carboxyl esterase with respect to reaction rates and affinity. However, different isoenzymes showed striking differences in the hydrolysis rates. When varying the chain

length of the carboxylic acid moiety, isoenzyme V had an optimum for the C5 compound (methyl pentanoate), while with acetate esters of alcohols with varying chain length, this isoenzyme exhibited a minimum activity with butyl and pentyl acetate. In contrast, the activity of isoenzyme I increased with increasing chain length of both the carboxylic acid and the alcohol moiety. The authors concluded that it appears reasonable to assume a cooperative and complementary function of the different carboxylesterase enzymes in the hydrolysis of the various esters (Junge & Heymann, 1979).

No hydrolysis data have been provided for the candidate esters of the present group of chemically defined flavouring substances. However, there are *in vitro* hydrolysis data for some short-chain aliphatic saturated esters (see Table III.1) indicating that these can be hydrolysed *in vivo* in the gut to yield the corresponding alcohols and carboxylic acids of the esters prior to absorption (Grundschober, 1977; Longland et al., 1977; Gangolli & Shilling, 1968; Leegwater & Straten, 1974a).

These esters were shown to be hydrolysed in artificial gastric juice with half-lives ($T_{1/2}$) of 4-770 min (Longland et al., 1977; Gangolli & Shilling, 1968). Hydrolysis in artificial pancreatic juice/pancreatin or in pig jejunum homogenate was found to be faster than in artificial gastric juice (Gangolli & Shilling, 1968; Longland et al., 1977; Leegwater & Straten, 1974a; Grundschober, 1977).

There is a variation in the degree of hydrolysis between different structurally related esters. Hydrolysis by artificial pancreatic juice was rather fast for some esters ($T_{1/2}$ of ethyl butyrate, isoamyl butyrate, ethyl hexanoate, ethyl heptanoate, ethyl nonanoate, and ethyl laurate were 6, 11, 3, 10, 6, and 6 min, respectively) and relatively slow for other esters ($T_{1/2}$ of butyl acetate and isoamyl hexanoate were 66 and 38 min., respectively). Rat liver homogenate and small intestinal mucosa preparation were found to be much more efficient in hydrolysing esters. While half-life of butyl acetate was 491 and 108 sec. for hydrolysis by liver homogenate and intestinal mucosa preparation, respectively, half-lives of isoamyl butyrate, ethyl hexanoate, and ethyl heptanoate were less than 1 second in liver homogenate or small intestinal mucosa preparation (Longland et al., 1977).

It should be noted that in contrast to the esters mentioned in Table III.1, all of the candidate esters have unsaturated carboxylic acid moieties and some of these are branched in the alcohol and/or carboxylic acid moiety.

McCarthy and Witz (1997) have studied the structure-activity relationship for the *in vitro* hydrolysis of several esters of acrylic acid (2-propenoic acid) and methacrylic acid (2-methyl-2-propenoic acid) using purified pig liver carboxylesterase. The esters studied were ethyl acrylate, butyl acrylate, ethyl methacrylate, butyl methacrylate, ethyleneglycol dimethacrylate and tetraethyleneglycol dimethacrylate. None of these substances are included in the current submission. It was shown, that the substrate affinity decreased with increasing length of the alcohol chain length. Also, the maximum rate of hydrolysis diminished with increasing alcohol chain. No effect on ester hydrolysis was observed that could be related to the presence of the alpha-methyl group. Based on Vmax and K_m *in vitro* data provided in the paper, it can be calculated that for the esters mentioned, this esterase at a concentration of approximately 11 microgram/ml can hydrolyse over 85% of an initial substrate concentration of 100 microM within 30 to 120 minutes (McCarthy & Witz, 1997).

An indication of rapid hydrolysis of methyl methacrylate [FL-no: 09.647] can be derived from Bratt and Hathway who observed that after administration of this substance to rats, 65% of the dose could be retrieved in the exhaled air in the form of CO_2 after 2 h (Bratt & Hathway, 1977).

In a study of Basak et al. (1997) in which the hydrolysis of an ester of an alpha, beta-unsaturated carboxylic acid (methylcinnamate) and the corresponding saturated ester was studied *in vitro* using pig liver esterase, the rate of hydrolysis was lower for unsaturated methylcinnamate compared to the corresponding saturated ester. The rates of hydrolysis differ by a factor of 70. When a mixture containing both the saturated and the unsaturated ester was studied, an exclusive hydrolysis of the saturated ester was observed. Accordingly, the authors suggest that steric constraints, e.g. double bounds or small rings near to the ester functionality can be expected to slow down the rate of hydrolysis (Basak et al., 1997). However, it should be noted that in this study the alpha, beta-unsaturated part was in conjunction with a benzene ring and this may have altered the conditions of hydrolysis compared to the aliphatic alpha, beta-unsaturated candidate esters.

Based on the data above and on general knowledge of esterase activity (e.g. (Heymann, 1980)), it may be expected that the 29 candidate esters will be hydrolysed to their corresponding acids and alcohols, either before or after absorption, but this has not been demonstrated unambiguously. In addition, there is some evidence from published and on-going research that the hydrolysis of alpha,beta-unsaturated esters is slower than that of saturated esters (Basak et al., 1997), Engel, personal communication). On the other hand, in the evaluation of 42 esters of straight-chain unsaturated carboxylic acids JECFA (JECFA, 1999a) assumed rapid hydrolysis. In addition, JECFA has accepted rapid hydrolysis of 26 esters from 4 terpenoid alcohols and 9 aliphatic saturated carboxylic acids. Also, the *in vivo* study by Bratt and Hathway indicates that hydrolysis and metabolism of the methyl ester of the alpha,beta-unsaturated methacrylic acid is rapid (Bratt & Hathway, 1977). Therfore, it is concluded that the candidated esters of this flavouring group evaluation are also hydrolysed. The expected hydrolysis products for the 29 candidate esters are shown in Table 2b to the main text of this group evaluation.

Ester Conjugation with Glutathione

It has been demonstrated that methyl esters of crotonic acid and methacrylic acid ([FL-no: 09.636 and 09.647], respectively) may undergo direct conjugation with glutathione via a direct Michael-type reaction at the double bond position. This reaction may lead to glutathione depletion after intraperitoneal injection (i.p) administration. When ester hydrolysis is inhibited by pre-treatment with TOTP[9], the urinary excretion of these conjugates is strongly increased, indicating that only the ester, but not the free unsaturated acid, is subject to this conjugation reaction (Delbressine et al., 1981a). This conjugation has been observed after i.p. of the esters, and it is questionable whether it is very relevant after oral exposure, due to presystemic ester hydrolysis.

III.3. Absorption, Distribution, Metabolism and Excretion of Hydrolysis Products

III.2.2. Absorption

In general, short (<C8) straight- and branched-chain aliphatic alcohols and carboxylic acids are rapidly absorbed from the gastrointestinal tract (Dawson et al., 1964b; Gaillard & Derache, 1965). Long-chain carboxylic acids, such as linoleic acid and oleic acid, are readily absorbed from micelles in the jejunum, re-esterified with glycerol in chylomicrons and transported *via* the lymphatic system (Blomstrand & Rumpf, 1954; Baxter et al., 1967; Borgström, 1974). Studies with radiolabeled linoleic and oleic acids, in mammalian tissues *in vitro* and in mammals *in vivo*, demonstrated that fatty acid uptake occurs in brain, heart, gut and adipose tissues by passive/facilitated diffusion

[9] *tri-ortho-tolyl phosphate*

and/or active transport (Dhopeshwarkar & Mead, 1973; Harris et al., 1980; Abumrad et al., 1984; Schulthess et al., 2000). From these data uptake in other tissues can also be expected to occur.

III.2.3. Metabolism

Upon hydrolysis, all candidate esters of FGE.05Rev1 produce short-chain (≤ C6) saturated alcohols. Three of the candidate substances ([FL-no: 09.586, 09.596 and 09.942]) will produce branched alcohols and one candidate ester [FL-no. 09.603] will produce a secondary alcohol. All acid moieties of the candidate flavouring substances are unsaturated. The acids of substances [FL-no: 09.248, 09.266, 09.324, 09.326, 09.329, 09.330, 09.335, 09.370, 09.372, 09.374, 09.379, 09.603, 09.636, 09.637, 09.641, 09.652, 09.699, 09.865 and 09.934] are not branched; the acids of substances [FL-no: 09.321, 09.365, 09.375, 09.586, 09.596, 09.624, 09.625, 09.647, 09.680, 09.942] are branched.

A relevant discussion of the general aspects of metabolism of esters and the corresponding alcohols and carboxylic acids may be found in FAO/WHO JECFA 42/51 (JECFA, 1999a). The text below is a more specific discussion on the metabolism of straight- and branched chain saturated primary alcohols, and straight- and branched-chain unsaturated carboxylic acids.

Oxidation of Straight-chain Alcohols to Carboxylic Acids

The primary alcohols formed after ester hydrolysis are subsequently oxidized to the corresponding aldehydes, which are efficiently oxidized to the corresponding carboxylic acids by high capacity enzyme pathways. Isoenzyme mixtures of NAD^+/NADH-dependent alcohol dehydrogenase (ADH) obtained from human liver microsomes have been reported to catalyse oxidation of straight-chain primary aliphatic saturated and unsaturated alcohols (Pietruszko et al., 1973). Comparison of the enzyme binding affinities of ADH for the various alcohols indicated that increased binding (lower K_m) occurs with increasing chain length (i.e. C1 to C6) of the substrate and the presence of unsaturation. However, maximum reaction rates of oxidation are essentially constant regardless of the alcohol structure suggesting that alcohol-enzyme binding is not the rate limiting step for oxidation (Pietruszko et al., 1973). Rather, the lipophilic character of the alcohol substrate seems to determine the activity of this enzyme at pre-steady state enzyme kinetics (Klesov et al., 1977).

Similarly, aldehyde dehydrogenase (ALDH), which converts aldehydes into the corresponding carboxylic acids and which is present predominantly in hepatic cytosol, exhibits broad specificity for oxidation of aldehydes (Feldman & Weiner, 1972; Blair & Bodley, 1969). Rat liver cytosolic ALDH has measurable activities with formaldehyde and acetaldehyde, while it has maximum activity with hexanal. In rat liver a microsomal ALDH has also been found and this ALDH is more active for higher molecular weight aldehydes (tested up to C12), while it has hardly any activity with formaldehyde and acetaldehyde (Nakayasu et al., 1978). Xanthine oxidase and aldehyde oxidase also catalyse oxidation of a wide range of aldehydes (Beedham, 1988).

At elevated levels of exposure and prior to oxidation to the corresponding acid, aldehydes may conjugate with sulfhydryl groups such as glutathione to yield thiohemiacetals. In addition, oxidation of low molecular weight aldehydes requires glutathione, which implies that the substrate for ALDH-mediated oxidation may be the thiohemiacetal (Brabec, 1993).

Oxidation of Branched-chain Alcohols to Carboxylic Acids

Generally, branched-chain aliphatic alcohols are oxidized to the corresponding aldehydes (Bosron & Li, 1980), which in turn are oxidized to the corresponding carboxylic acids (Bosron & Li, 1980; Levi & Hodgson, 1989). Branched-chain aliphatic alcohols and aldehydes have been reported to be

substrates for ADH (Hedlund & Kiessling, 1969a; Albro, 1975) and ALDH (Hedlund & Kiessling, 1969a), respectively.

Alcohol Glucuronidation

In addition it has also been demonstrated that alcohols can be converted into glucuronide conjugates, which will be excreted via the urine. Conjugation with glucuronide is not very important for primary alcohols, but gains in importance for branched alcohols and especially for tertiary alcohols (Bosron & Li, 1980; Kamil et al., 1953a). It has been demonstrated that isopropanol can be conjugated with glucuronic acid and subsequently excreted via the urine (Kamil et al., 1953a).

Metabolism of Fatty Acids

All of the straight-chain carboxylic acids, which are produced after oxidation of the component alcohols of the parent esters, can be expected to be metabolised via common physiological pathways, including beta-oxidation and citric acid cycle, which finally lead to the total oxidation of these substances. For example, a single oral dose of 4, 40 or 400 mg/kg [^{14}C] n-butanol (component alcohol of [FL-no: 09.324, 09.326, 09.329, 09.330, 09.335, and 09.321]) given to male Charles River CD rats by gavage and 400 mg/kg given to female rats is rapidly eliminated in the breath as expired $^{14}CO_2$. Within four hours of dosing, 75, 83 or 67% of the 4, 40 or 400 mg/kg dose, respectively, was eliminated as CO_2 (DiVincenzo & Hamilton, 1979).

For the three branched-chain component alcohols of candidate esters [FL-no: 09.586, 09.596 and CN91] these pathways include an additional step to eliminate the side chain. This additional step will be described below in the section on fatty acid metabolism.

Metabolism of Straight-chain Saturated and Unsaturated Carboxylic Acids

The straight-chain unsaturated carboxylic acids resulting from component alcohol oxidation after ester hydrolysis (see above), participate in normal fatty acid metabolism (see Figure III.2). In this pathway, the acid is condensed with coenzyme A (CoA) followed by catalytic dehydrogenation mediated by acyl-CoA dehydrogenase (Voet & Voet, 1990). The resulting *trans*-2,3-unsaturated ester (*trans*-delta^2-enoyl-CoA) is converted to the 3-ketothioester, which undergoes beta-cleavage to yield an acetyl-CoA fragment and a new thioester reduced by two carbons.

For the unsaturated carboxylic acids, splitting-off of acetyl-CoA units will continue along the carbon chain until the position of unsaturation is reached. If the unsaturation begins at an odd-numbered carbon, acetyl-CoA fragmentation will eventually yield a delta^3-enoyl-CoA, which cannot enter the fatty acid cycle until it is isomerised to the *trans*-delta^2-enoyl -CoA by enoyl-CoA isomerase. If unsaturation begins at an even-numbered carbon, acetyl-CoA fragmentation yields a delta^2-enoyl-CoA product, which is a substrate for further fatty acid oxidation. If the stereochemistry of the double bond is *cis*, it is isomerised to the *trans* double bond by the action of 3-hydroxyacyl-CoA-epimerase prior to resuming cycling further down the fatty acid oxidation pathway. Even-numbered carbon acids continue to be cleaved to acetyl-CoA, while odd-numbered carbon acids yield acetyl-CoA and propionyl-CoA. Acetyl-CoA enters the citric acid cycle directly while propionyl-CoA is transformed into succinyl-CoA that then enters the citric acid cycle (Stryer, 1988).

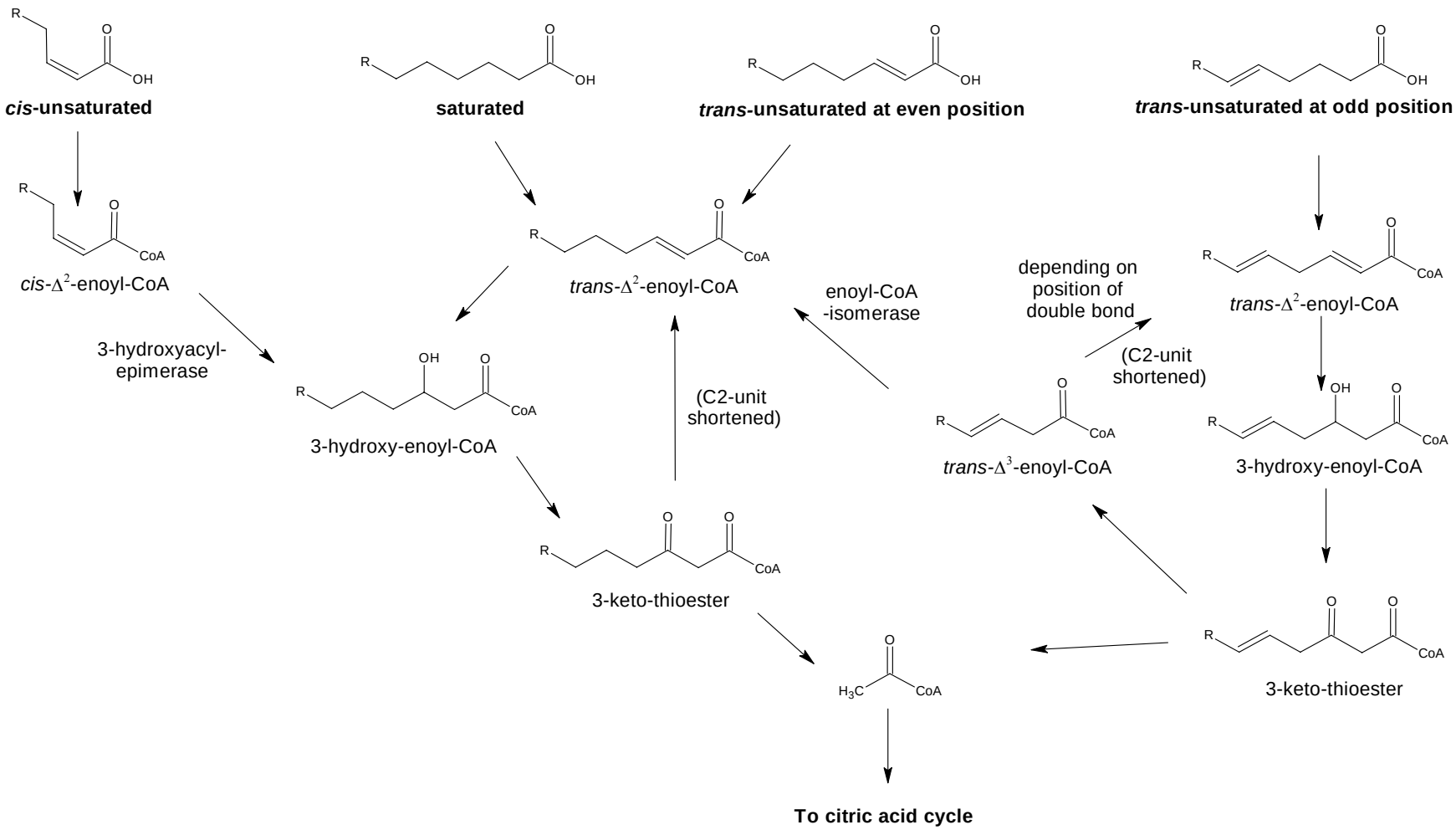


Figure III.2 *Metabolism of straight-chain carboxylic acids*

Alternative minor metabolic pathways have been characterized for straight long-chain fatty acids and short-chain acids containing unsaturation. While linoleic and oleic acids participate in beta-oxidation and normal fatty acid metabolism in most tissues (Masoro, 1977), they may undergo omega-oxidation in the liver and alpha-oxidation in the brain (Wakil & Barnes, 1971; Gibson et al., 1982; Bell et al., 1976).

Unsaturated short-chain acids may also be metabolised *via* saturation to yield a substrate that may participate in the fatty acid pathway. For example, the mechanism for oxidative metabolism of 4-pentenoic acid has been studied in rat heart mitochondria. *In vitro* 4-pentenoic acid is converted to the CoA-thioester, which is dehydrogenated to yield the *trans*-2,4-pentadienoyl -CoA (see Figure 3). Two enzyme-catalysed processes then compete for this conjugated thioester. In the first pathway, NADPH-dependent enzyme-catalysed reduction (saturation) of the delta^4-alkene leads to *trans*-2-pentenoic acid. The second pathway involves beta-oxidation to yield 3-keto-4-pentenoyl-CoA. In *vitro* hydrogenation predominates to yield *trans*-2-pentenoic acid, which then participates in normal fatty acid oxidation (Schulz, 1983).

Metabolism of Branched-chain Saturated and Unsaturated Carboxylic Acids

Short-chain (<C6) branched aliphatic acids undergo beta-oxidation, preferentially in the longer chain. Beta-cleavage of the branched aliphatic acids yields straight-chain acid fragments, which are sources of carbon in the fatty acid metabolism pathway or tricarboxylic acid cycle (Voet & Voet, 1990).

Methacrylic acid (2-methylpropenoic acid) given to rats by gavage is rapidly eliminated almost exclusively as CO_2. The CO_2 is presumed to arise from beta- oxidation and decarboxylation of methacrylic acid to yield methyl-malonyl-CoA, which, after conversion to succinyl -CoA, can participate in the tricarboxylic acid cycle (Bratt & Hathway, 1977).

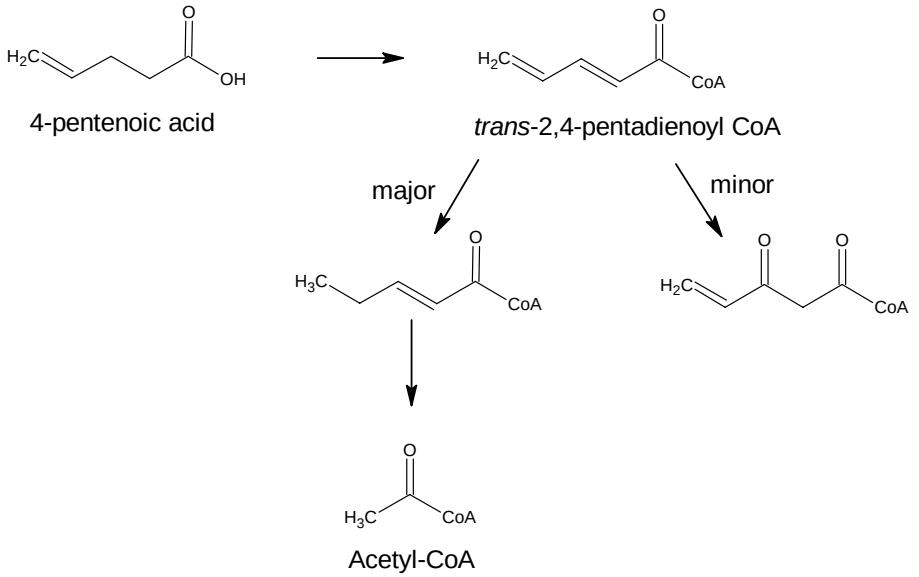


Figure III.3 *Metabolism of 4-Pentenoic acid showing hydrogenation at the delta^4 position*

In the metabolism of branched-chain aliphatic acids, the position of the substituent also play a role. Acids with a methyl substituent located at an even-numbered carbon (e.g. 2- methylpentanoic acid

or 4-methyldecanoic acid) are extensively metabolised to CO_2 *via* beta- oxidative cleavage in the fatty acid pathway. If the methyl group is located at the 3-position, beta- oxidation is inhibited and omega-oxidation predominates, primarily leading to polar, acidic metabolites capable of being excreted in the urine (Williams, 1959).

The component alcohols of [FL-no: 09.586 and 09.596] give rise to branched-chain carboxylic acids: 2-methyl propanoic acid and 3-methyl butanoic acid, respectively. 2-Methyl propionic acid (= isobuteric acid) is broken down via the same metabolic pathway along which the carbon skeleton of the amino acid valine is broken down. An intermediate in this pathway is 2 methyl-prop-2-enoic acid (= methacrylic acid, the acid component of [FL-no: 09.647, 09.375, and 09.586]). This pathway is described below and leads to the formation of succinyl -CoA, which is easily metabolised in the citric acid cycle. 3-Methyl butanoic acid (= isovalereric acid, the carboxylic acid formed from the component alcohol of [FL-no: 09.596]) is broken down via the same pathway as the amino acid leucine. An intermediate in this pathway is beta-methyl crotonic acid (= 3-methyl-but-2-enoic acid, the acid component of [FL-no: 09.365]). This pathway leads to the formation of acetoacetic acid and acetyl-CoA, which can be used as substrates for energy production. Alternatively, acetoacetate can be excreted in the urine (Bell et al., 1976; Lehninger, 1982; Stryer, 1988).

As chain length and lipophilicity increase, omega-oxidation competes favourably with beta-oxidative cleavage (Bell et al., 1976). This is in particular important for candidate substances [FL-no: 09.652 and 09.865], which have an (unsaturated) C18 component acid. The resulting omega-hydroxy carboxylic acid can be further metabolised via dicarboxylic acids through the beta-oxidation.

Oxidation of Terminal Double Bonds

Terminal double bonds appear in 4 candidate substances [FL no: 09.370, 09.375, 09.586, and 09.647] and thus also in their hydrolysis products. These double bonds may be oxidized to the corresponding epoxides. Epoxides are highly reactive molecules, due to the large strain associated with the three membered ring structure, and they react easily with nucleophilic sites of cellular macromolecules. For this reason, several aliphatic alkene-derived epoxides have been demonstrated to be carcinogenic (e.g. ethylene, isoprene, butadiene, glycidol) (Melnick, 2002). Alternatively, epoxides can be conjugated with glutathione (GSH) by glutathione S-transferases (GSTs) or hydrolysed to diols by epoxide hydrolases (EHs). The latter two reactions can be considered to be detoxications.

It has been demonstrated that terminal double bonds may be oxidatised at the double bond to give the corresponding epoxide or, alternatively, at the allylic carbon to give the allylic alcohol, as was demonstrated with 1-hexene with rat and human P450s (Chiappe et al., 1998). The ratio of epoxidation over allylic oxidation, as measured with different P450 isoforms (CYP) is ≥1, indicating that epoxide formation is generally favoured (Chiappe et al., 1998). Theoretically these pathways could occur with the acid moiety of one candidate substance [FL no: 09.370].

In the same paper (Chiappe et al., 1998) it was demonstrated that the biotransformation of 2-methyl-1-hexene proceeds exclusively via the epoxide, which was further hydrolysed by EH to the diol. This pathway might apply to the acid moiety of [FL no: 09.375, 09.375, and 09.647].

However, the risk associated with the epoxidation of the terminal double bond of these candidate substances is expected to be low for these reasons:

- Epoxides can be detoxicated by conjugation with glutathione or by epoxide-hydrolase mediated hydrolysis.
- The terminal double bonds are all present in carboxylic acid moieties of the candidate chemical substances. Biochemical attack of these moieties via e.g. beta-oxidation is much more efficient and rapid than microsomal oxidation.

Studies on Individual Candidate Substances

Methyl crotonate [FL-no: 09.636]

The mercapturic acid derivative formed from reaction of glutathione with the 3-position of the crotonic acid moiety of methyl crotonate (*N*-acetyl-S-(1-methyl-2-carboxyethyl)cysteine-methyl ester) was identified as a metabolite in the urine of adult female Wistar rats given a single intraperitoneal injection of methyl crotonate (0.14 mmol/kg dissolved in arachis oil). After administration of the esterase inhibitor, TOTP[7] (0.34 mmol/kg in arachis oil 18 hours before injection with methyl crotonate), there was a significant increase in urinary thioether excretion as compared to the controls (increasing from 2 to 16 % of the dose), indicating that hydrolysis by carboxylesterase influences conjugation with glutathione in that the ester (methyl crotonate) reacts more readily in a Michael-type reaction with glutathione than the unesterified crotonic acid (Delbressine et al., 1981a).

Methyl methacrylate [FL-no: 09.647]

In an *in vitro* hydrolysis study, 0.184 microl/ml of methyl methacrylate was added to fresh human whole blood. Using GC analysis it was found that concentrations in blood cells were twice as high as concentrations measured in plasma, and methyl methacrylate disappeared exponentially with time from plasma at a rate that was 10 times faster than the rate for blood cells. The half-life ($T_{1/2}$) of methyl methacrylate in fresh human whole blood at 20^{o}C was 3 h (Rijke et al., 1977).

In another *in vitro* hydrolysis study, twice diluted human serum samples from 10 subjects were incubated with methyl methacrylate. The disappearance of methyl methacrylate in the incubates followed pseudo-first order kinetics. For the 10 subjects, $T_{1/2}$s of 18 to 40 minutes were determined. On average, after 90 minutes of incubation, 40 % of the amount of methyl methacrylate was recovered as free methacrylic acid. The authors suggested that the disappearance of methyl methacrylate was attributable to enzymatic hydrolysis to yield methyl methacrylic acid and methanol (Corkill et al., 1976). These studies indicate that unhydrolysed esters absorbed from the gastrointestinal tract, will continue to be hydrolysed in the blood.

Twenty male Wistar rats were administered a single dose of methyl methacrylate by oral gavage at 8 mmol/kg (801 mg/kg). Methacrylic acid was found in rat serum after five minutes, with the maximum concentration seen between 10 and 15 minutes after dose administration indicating rapid absorption and hydrolysis. Methacrylic acid concentration declined rapidly essentially reaching zero by 60 minutes after dose administration. It also was reported that in both human and rat serum methyl methacrylate is hydrolysed due to a nonspecific serum carboxylesterase. In rat serum the substrate affinity of the enzyme is 3 times as high as in human serum and the Vmax in rat serum is twice as high as in human serum (Bereznowski, 1995).

The mercapturic acid derivative formed from reaction of glutathione with the 3-position of the methacrylic acid moiety of methyl methacrylate (*N*-acetyl-S-(2-carboxypropyl)cysteine-methyl ester) was not identified as a metabolite in the urine of adult female Wistar rats given a single

intraperitoneal injection of methyl methacrylate (0.14 mmol/kg dissolved in arachis oil). However, after administration of the esterase inhibitor, TOTP[7] (0.34 mmol/kg in arachis oil 18 hours before injection with methyl methacrylate), there was a significant increase in urinary thioether excretion as compared to the controls (increasing from "not significant" to 11 % of the dose), demonstrating that hydrolysis by carboxylesterase influences conjugation with glutathione in that the ester (methyl methacrylate) reacts more readily in a Micheal-type reaction with glutathione than the unesterified methacrylic acid (Delbressine et al., 1981a).

A high proportion of methyl methacrylate [FL-no: 09.647] is completely oxidized in rats, irrespective of whether administration was oral or intravenous. Up to 65 % of a single 5.7 mg/kg dose of M-[1,3-^{14}C]-M was expired as $^{14}CO_2$ within two hours and 84 to 88 % within 10 days. The same pattern of excretion was seen after oral or intravenous administration of M-[2-^{14}C]-M. Less than 2 % of the administered dose was exhaled as parent compound and 4 to 7 % remained in the carcass 10 days after dosing. The initial rate of $^{14}CO_2$ expiration was the same for both types of M-[^{14}C]-M, suggesting that all three propylene carbons are sources for the evolved CO_2. The authors proposed that following hydrolysis of methyl methacrylate, methacrylic acid complexes with coenzyme A (CoA) to enter the pathway shown in Figure 4. In this metabolic pathway methylmalonyl -CoA, which also is formed in valine catabolism (Crout et al., 1982), is converted to succinyl -CoA. Therefore, all four carbons would enter the citric acid cycle (Bratt & Hathway, 1977) (See Figure 4).

III.4. Conclusion

The 29 candidate substances in FGE.05Rev1 can be assumed to be hydrolysed either, in the lumen of the gastro-intestinal tract or after absorption in the blood or the liver. The resulting hydrolysis products, alcohols and saturated and unsaturated carboxylic acids will be metabolised to carbon dioxide and water, or in one case to an innocuous glucuronide conjugate. As many of the intermediates in these metabolic conversions are endogenous substances, it can also be assumed that after ingestion, these candidate flavouring substances or their break-down products may be incorporated in other body constituents.

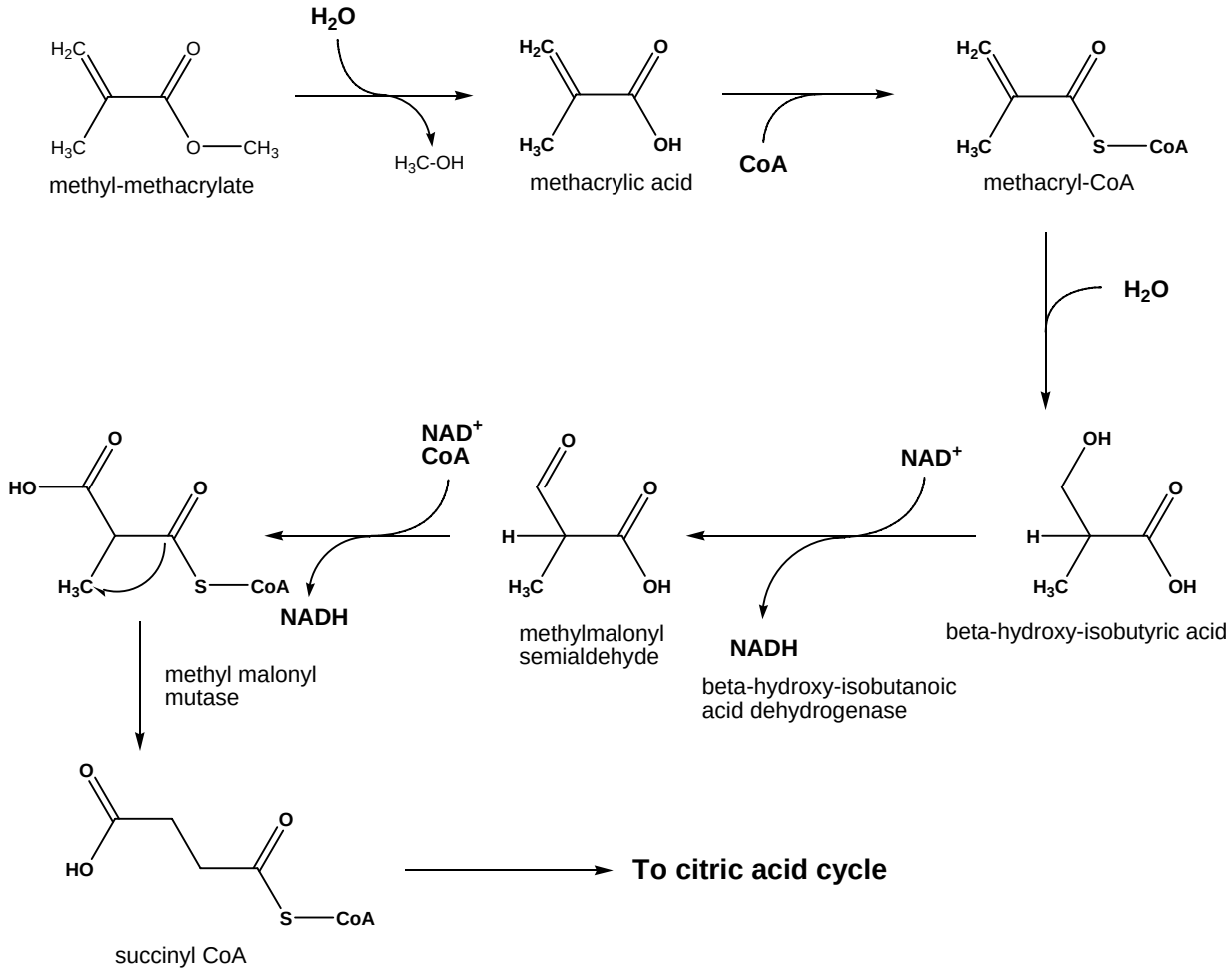


Figure III.4 *Metabolism of Methyl methacrylate and methacrylic acid (according to Bratt & Hathway, 1977)*

TABLE III.1: *IN VITRO* HYDROLYSIS DATA FOR ESTERS RELATED TO THE CANDIDATE CHEMICALS IN FGE.05REV1

The tabulated substances have been discussed in FAO/WHO JECFA 49/52. Lack of entry in a block indicates no data available for that endpoint.

Table III.1: *In vitro* hydrolysis data for esters related to the candidate chemicals in FGE.05rev1

Chemical Name	Test System	Results			Reference
		T1/2	% Hydrolysis After 2 Hours	% Hydrolysis After 4 Hours	
SATURATED					
Ethyl acetate	Whole homogenate of pig jejunum		100		(Grundschober, 1977).
Butyl acetate	Artificial gastric juice	318 min.			(Longland et al., 1977).
	Artificial pancreatic juice	66.0 min.			(Longland et al., 1977).
	Rat liver preparation	491 sec.			(Longland et al., 1977).
	Rat small intestinal mucosa	108 sec.			(Longland et al., 1977).
	Artificial gastric juice		23	41	(Gangolli & Shilling, 1968).
	Artificial pancreatic juice		72	92	(Gangolli & Shilling, 1968).
Isoamyl acetate	Pancreatin		20		(Leegwater & Straten, 1974a).
	Whole homogenate of pig jejunum		100		(Grundschober, 1977).
Ethyl butyrate	Artificial gastric juice	490 min.			(Longland et al., 1977).
	Artificial pancreatic juice	5.67 min.			(Longland et al., 1977).
	Artificial gastric juice		15		(Gangolli & Shilling, 1968).
	Artificial pancreatic juice		100 (after 1 hour)		(Gangolli & Shilling, 1968).
Isopropyl butyrate	Whole homogenate of pig jejunum		40		(Grundschober, 1977).
	Pancreatin		40		(Leegwater & Straten, 1974a).
Isoamyl butyrate	Artificial gastric juice	660 min.			(Longland et al., 1977).
	Artificial pancreatic juice	11.3 min.			(Longland et al., 1977).
	Rat liver preparation	0.492 sec.			(Longland et al., 1977).
	Rat small intestinal mucosa	0.0713 sec.			(Longland et al., 1977).
	Artificial gastric juice		12	22	(Gangolli & Shilling, 1968).
	Artificial pancreatic juice		100		(Gangolli & Shilling, 1968).
Ethyl hexanoate	Artificial gastric juice	293 min.			(Longland et al., 1977).
	Artificial pancreatic juice	3.47 min.			(Longland et al., 1977).
	Rat liver preparation	0.145 sec.			(Longland et al., 1977).
	Rat small intestinal mucosa	0.501 sec.			(Longland et al., 1977).
Isoamyl hexanoate	Artificial gastric juice	146 min.			(Longland et al., 1977).
	Artificial pancreatic juice	37.8 min.			(Longland et al., 1977).
Ethyl heptanoate	Artificial gastric juice	770 min.			(Longland et al., 1977).
	Artificial pancreatic juice	9.78 min.			(Longland et al., 1977).

Table III.1: *In vitro* hydrolysis data for esters related to the candidate chemicals in FGE.05rev1

Chemical Name	Test System	Results			Reference
		T1/2	% Hydrolysis After 2 Hours	% Hydrolysis After 4 Hours	
	Rat liver preparation	0.164 sec.			(Longland et al., 1977).
	Rat small intestinal mucosa	0.550 sec.			(Longland et al., 1977).
	Artificial gastric juice		10	19	(Gangolli & Shilling, 1968).
	Artificial pancreatic juice		100		(Gangolli & Shilling, 1968).
Ethyl nonanoate	Artificial gastric juice	177 min.			(Longland et al., 1977).
	Artificial pancreatic juice	5.92 min.			(Longland et al., 1977).
	Artificial gastric juice		37	61	(Gangolli & Shilling, 1968).
	Artificial pancreatic juice		100 (after 1 hour)		(Gangolli & Shilling, 1968).
Ethyl decanoate	Pancreatin		80		(Leegwater & Straten, 1974a).
	Pancreatin		80		(Grundschober, 1977).
Ethyl laurate	Artificial gastric juice	640 min.			(Longland et al., 1977).
	Artificial pancreatic juice	6.10 min.			(Longland et al., 1977).
	Artificial gastric juice		12	23	(Gangolli & Shilling, 1968).
	Artificial pancreatic juice		100 (after 1 hour)		(Gangolli & Shilling, 1968).
UNSATURATED					
Propenyl (2E) 2-methylbut-2-enoate	Whole homogenate of pig jejunum		100		(Grundschober, 1977).

ANNEX IV: TOXICITY

Acute toxicity data available for seven candidate substances of the present flavouring group of substances from chemical groups 1 and 2 and for 17 supporting substances evaluated at the 51st JECFA meeting (JECFA, 2000a). The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY STUDIES

Table IV.1: Acute Toxicity Studies

Chemical Name [FL-no]	Species	Sex	LD50 (mg/kg bw)	Reference	Comments
(4-Pentenoic acid [08.048])	Mouse	M, F	610	(Jenner et al., 1964)	
	Rat	M, F	470	(Jenner et al., 1964)	
(cis-3-Hexen-1-ol [02.056])	Mouse	M, F	7000	(Gaunt et al., 1969)	
	Mouse	F	7200	(Gaunt et al., 1969)	
	Rat	M, F	4700	(Moreno, 1973b)	
	Rat	M, F	10100	(Gaunt et al., 1969)	
	Rat	F	7300	(Gaunt et al., 1969)	
(cis-3-Hexenal [05.075])	Rat	M, F	<5000	(Palanker & Lewis, 1979)	
	Rat	M, F	1560	(Palanker & Lewis, 1979)	
(3-Hexenoic acid [08.050])	Mouse	NR	1840	(Senior & Sherratt, 1969)	
(cis-6-Nonenal [05.059])	Mouse	NR	>5000	(Moreno, 1978b)	
(10-Undecenal [05.035])	Rat	NR	>5001	(Hart & Wong, 1971)	
(10-Undecenoic acid [08.039])	Mouse	NR	8150	(Newell et al., 1949)	
	Mouse	NR	2300-6600	(Tislow et al., 1950)	
	Rat	NR	2500	(Tislow et al., 1950)	
(Oleic acid [08.013])	Rat	NR	>5000	(Moreno, 1977b)	
	Rat	NR	>19000	(Briggs et al., 1976)	
Methyl crotonate [09.636]	Rat	NR	>3200	(MFRM, 1979)	
	Mouse	NR	1600	(MFRM, 1979)	
(Ethyl cis-4,7-octadienoate [09.290])	Rat	M	>10000	(Mondino, 1979)	
(Methyl 9-undecenoate [09.236])	Rat	M	3000	(Moreno, 1977b)	
(Ethyl 10-undecenoate [09.237])	Rat	NR	>5000	(Moreno, 1977b)	
(Butyl 10-undecenoate [09.238])	Rat	NR	5000	(Moreno, 1977b)	
(Ethyl oleate [09.192])	Rat	NR	>5000	(Bailey, 1976d)	
(2,6-Dimethyl-5-heptenal [05.074])	Rat	NR	>5000	(Levenstein, 1974a)	
	Rat	M, F	4550	(Mayyasi et al., 1981)	
Methyl methacrylate [09.647]	Rat	NR	8400	(Deichmann, 1941)	
	Rat	NR	7872	(Deichmann, 1941)	
	Rat	NR	9400	(Spealman et al., 1945)	

Table IV.1: Acute Toxicity Studies

Chemical Name [FL-no]	Species	Sex	LD50 (mg/kg bw)	Reference	Comments
	Rat	NR	7900	(Deichmann, 1941)	
	Rat	NR	8000	(ACGIH, 1986)	
	Rat	NR	8500	(Ouyang et al., 1988)	
	Rat	NR	7800	(ACGIH, 1991)	
	Rat	NR	>2000	(Dow Chemical Company, 1957)	
	Rat	NR	8460 - 9400	(DuPont, 1989)	
	Rat	NR	>3200	(Eastman Kodak Co., 1965)	
	Rat	M	>5000	(Rohm & Haas Co., 1982)	
	Rat	NR	3625	(Gigiena, 1976)	
	Rat	NR	5300	(Lawrence et al., 1974)	
	Rat	NR	5200	(Tanii & Hashimoto, 1982)	
	Rat	NR	5200	(Schwach & Hofer, 1978)	
	Rat	NR	6550	(Deichmann, 1941)	
	Rat	NR	6000	(ACGIH, 1986)	
	Rat	NR	8700	(Gigiena, 1976)	
	Rat	NR	5900	(Spealman et al., 1945)	
	Rat	NR	5954	(DFG, 1984)	
	Rat	NR	4725	(Spealman et al., 1945)	
Ethyl methacrylate [09.375]	Rat	NR	14800	(Deichmann, 1941)	
	Rat	NR	12700 - 14510	(Deichmann, 1941)	
	Rat	NR	12700 - 18140	(Pang, 1995)	
	Rabbit	NR	3630	(Deichmann, 1941)	
	Rabbit	NR	3630 - 5440	(Deichmann, 1941)	
	Mouse	NR	7834	(Tanii & Hashimoto, 1982)	
	Mouse	NR	7800	(Pang, 1995)	
Isobutyl 2-methylprop-2-enoate [09.586]	Rat	NR	6400	(Krasavage & Terhaar, 1981)	
	Rat	NR	9600	(Roehm GmbH., 1977)	
	Rat	NR	6400 - 12800	(Autian, 1975)	
	Mouse	NR	11824	(Tanii & Hashimoto, 1982)	LD50-estimation based on mmol/kg dose provided in the article.
	Mouse	NR	11990	(Lewis, 1996a)	
Methyl 2-methylcrotonate [09.624]	Rat	NR	>5000	(Moreno, 1980a)	
(Hex-3(cis)-enyl 2-methylcrotonate [09.559])	Rat	NR	>5000	(Moreno, 1976g)	
(Hexyl 2-methyl-3&4-pentenoate [09.546])	Rat	M, F	>5000	(Elleman, 1979)	
(Ethyl 2-methyl-3,4-pentadienoate [09.540])	Mouse	M	1316	(Babish, 1978c)	
	Mouse	F	892	(Babish, 1978c)	
	Mouse	M, F	770	(Moran et al., 1980)	
Ethyl trans-2-butenoate [09.248]	Rat	NR	3000	(Smyth & Carpenter, 1944)	
Hexyl 2-butenoate [09.266]	Rat	NR	5000	(Moreno, 1978o)	

Subacute/subchronic/chronic/carcinogenicity data are available for three candidate substance of the present flavouring group of substances from chemical group 1 and 2, and for 11 supporting substances of the present evaluation group evaluated at the 51st JECFA meeting (JECFA, 2000a). The supporting substances are listed in brackets.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species/Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg/day)	Reference	Comments
(cis-3-Hexen-1-ol [02.056])	Rat; M, F 30	Drinking water	0, 310, 1250, 5000 ppm equal to M: 0, 30, 127, 410 mg/kg bw/day, F: 0, 42, 168, 721 mg/kg bw/day	98 days	120-150	(Gaunt et al., 1969)	NOAEL corresponds to 1250 mg/kg feed.
(10-Undecenoic acid [08.039])	Rat; M, F 152	Gavage	0, 100, 200, and 400 mg/kg bw/day	6 months	400	(Tislow et al., 1950)	Total number of animals studied was 152. Endpoints included body weight and changes in autopsy (only poorly reported abstract available). Footnote 3.
	Rat; M, F NR	Gavage	0, 100, 200, and 400 mg/kg bw/day	up to 9 months	400	(Tislow et al., 1950)	Footnote 3.
	Rat; M 7	Diet	0, 0.5, 1.0, 2.5 % in feed equivalent to 0, 500, 1000, 2500 mg/kg mw7day	8 weeks	500	(Newell et al., 1949)	Footnote 3.
(Linoleic acid [08.041])	Rat; M 20	Diet	0, 1,5 % in feed equivalent to to 0, 1500 mg/kg bw/day	36 weeks	1500	(Scimeca, 1998)	This study was conducted using conjugated linoleic acid.
(Oleic acid [08.013])	Mouse; Control 36, Experimental 55	Diet	0, 75 mg/day in feed equivalent to 0, 3750 mg/kg bw/day.	24 months	3750	(El-Khatib & Cora, 1981)	
(Oleic acid/Linoleic acid)	Mouse; M, F 329	Diet	0, 2.25 – 3 mg/day equivalent to 112-150 mg/kg bw/day	~ 24 months	150	(Szepsenwol & Boschetti, 1975)	A NOEL was not determined.
	Mouse; NR 195-328	Diet	0, 3 mg/day equivalent to 150 mg/kg bw/day	~ 24 months	150	(Szepsenwol, 1978)	Intake of oleic/linoleic acid mixture was approximately 150 mg/kg bw/day. A NOEL was not determined.
	Rabbit; M, F 38-42	Diet		16 weeks	<4500	(Lee et al., 1986)	
	Rabbit; M, F 20	Diet		119 days	<4500	(JECFA, 1997b)	
(Ethyl crotonate [09.248])	Rat; M	Diet	373 mg/rat	35 days	>1840	(Edwards, 1975)	Only neuropathy characterized by observations on movement as

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species/Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg/day)	Reference	Comments
	4		(1840 mg/kg bw)				endpoint of neurotoxicity is reported. No histology.
(3-Methylcrotonic acid [08.070])	Rat; NR NR	Gavage		1-2 months	2500	(Schoental & Mattocks, 1960)	The study was limited to cytological examination of the liver.
(Hexyl alcohol [02.005])	Rat; M, F NR	Diet		90 days	577	(Eibert, 1992)	
	Dog; M, F 4	Diet/ gelatin capsules		90 days	>230	(Eibert, 1992)	
(2,6-Dimethyl-5-heptenal [05.074])	Rat; M, F 30	Diet		90 days	37	(Gaunt et al., 1983)	Footnote 3.
Methyl methacrylate [09.647]	Rat; M 4	Diet	410 mg/rat (1880 mg/kg bw)	35 days	>1880	(Edwards, 1975)	Only neuropathy characterized by observations on movement as endpoint of neurotoxicity is reported. No histology.
	Rat; M 8	Gavage	0, 100, 200 mg/kg bw	14 days (5 ds/week)	>200	(Ghanayem et al., 1986)	Only endpoints evaluated was forestomach carcinogenicity. Acceptable quality.
	Rat; M 30	Gavage	500 mg/kg bw	21 days	200	(Husain et al., 1985)	Investigation of behavioral effects and neurochemistry. Husain et al reported that no effect on behavior was noted at 100 and 200 mg/kg (unpublished data). Footnote 2. Insufficiently documented (CEC, 2002).
	Rat; M 20	Gavage	500 mg/kg bw	21 days	<500	(Husain et al., 1989)	Determination of effects on lipids in rat brain and sciatic nerve. Acceptable quality.
	Rat; M 20	Gavage	800 mg/kg bw	30 days	800	(Bereznowski, 1995)	Footnote 2. Histopathological examination of 7 organs including Brain but insufficiently documented. Examinations on hydrolysis sufficiently documented.
	Rat; NR 5	Gavage		70 days	940	(DuPont, 1989)	Not available for evaluation.
	Rat; 25 M, 25 F	Drinking water	0, 6, 60, 2000 ppm (equivalent to 200 mg/kg bw)	24 months	200	(Borzelleca et al., 1964)	See footnotes 1 and 2. This study was considered in the EU Risk Assessment Report (CEC, 2002) to derive the NOAEL. However, in the light of neurotoxicity data from the structurally related ethyl methacrylate, the reliablilty of this NOAEL is questionable.
	Dog; 2 M, 2 F	Oral; gelatin capsule	0, 10, 100, 1000 ppm	24 months		(Borzelleca et al., 1964)	See footnotes 1 and 2. Due to low numbers of animals the study was not used in EU Risk Assessment Report for derivation of a NOAEL.
Ethyl methacrylate [09.375]	Rat; M, 10	ip	0, 100, 200, 400, 800 mg/kg bw	60 days	< 100	(Abou-Donia et al., 2000)	Different parameters on neurotoxicity examined. Not in accordance with GLP and OECD guideline 424, but contains sufficient details. There is no NOAEL.
	Rat; M , 8	Drinking water	0.1, 0.2, or 0.5% (equivalent to 50, 100, 250 mg/kg bw)	60 days	< 50	(Abou-Donia et al., 2000)	Different parameters on neurotoxicity examined. Not in compliance with GLP, roughly in accordance with OECD guideline 424, contains sufficient details. There is no NOAEL.
(2-Methyl-1-propanol [02.001])	Rat; M, F 20	Drinking water	0, 100, 200, 400, 800 mg/kg bw	90 days	> 1450	(BASF, 1992a)	
(3-Methylbutyl alcohol [02.003])	Rat; M, F 30	Gavage	0.1, 0.2, or 0.5% (equivalent to 50, 100, 250 mg/kg bw)	119 days	> 1000	(Carpanini et al., 1973b)	
(2,4-Dimethyl-2-pentenoic acid [08.044])	Rats; NR NR	Diet		13 weeks	> 1.5	(Posternak, 1968)	
Methyl oleate [09.652]	Mouse; NR	Gavage	15 mg per	300 days		(Kiaer et al., 1975)	NQO was used as initiator in a two-stage carcinogenesis model. Slight

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species/Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg/day)	Reference	Comments
	NR		mouce/day				increase in number of papillomas but not number of mice with papillomas.

NR: Not Reported.

M: Male.

F: Female.

1 In the study of Borzelleca et al. (1964), two male and two female dogs received gelatin capsules with 10, 100 and 1000 ppm methyl methacrylate dissolved in corn oil. The high-dose was reduced to 500 ppm on day 2, 0 ppm on day 3-13 and 300 ppm on day 14 due to vomiting, and then increased to 1200 ppm at week 5 and to 1400 ppm at week 7 to 1500 ppm at week 9. 25 male and 25 female rats were administered with 6, 60 and 2000 ppm methyl methacrylate in the drinking water, the low and medium doses increased to 7 and 70 ppm after five months. The study on dogs and rats revealed no adverse effect other than a lower body weight gain in high-dose dogs and elevated kidney weights in high-dose female rats. The authors of the RAR (CEC, 2002) derived a NOAEL of 2000 ppm in drinking water (equivalent to 200 mg/kg bw/d) from the rat study. These studies on dogs and rats revealed no increase of neoplastic lesions. However the reliability of these studies is limited due to their non-conformance to current carcinogenicity test guidelines (e.g., histopathologic examination was performed on a limited number of organs) (CEC, 2002).

2 Study was considered in the EU Risk Assessment Report on methyl methacrylate (CEC, 2002).

3 Study was evaluated by JECFA during the 51th meeting (JECFA, 2000a).

Developmental and reproductive toxicity data for three candidate substances of the present flavouring group of substances from chemical groups 1 and 2, and one supporting substance of the present evaluation group evaluated at the 51st JECFA meeting (JECFA, 2000a). The supporting substance is listed in brackets.

TABLE IV.3: DEVELOPMENTAL / REPRODUCTIVE TOXICITY STUDIES

Table IV.3: Developmental / Reproductive Toxicity Studies

Chemical name [FL-no]	Study type duration	Species/sex No/group	Route	Dose levels	NOAEL mg/kg bw/day Including information on possible maternal toxicity	Reference	Comments
(4-Pentenoic acid [08.048])	Developmental toxicity; dose administered gestation day 8	Mouse; F 15	Subcutaneous injection	0, 600 mg/kg bw	600	(Nau & Löscher, 1986)	See footnote 1.
Methyl methacrylate [09.647]	Developmental toxicity; dose administered gestation days 5, 10 and 15	Rat 5	I.p.	0, 0.133, 0.266, 0.443 ml/kg bw		(Singh et al., 1972)	See footnotes 2, 3.
	Developmental toxicity; dose administered gestation days 6 to 18	Rabbit 12	I.p.	0, 0.004, 0.04, 0.4 ml/kg bw		(ICI, 1976a)	See footnotes 2, 4.
Ethyl methacrylate [09.375]	Developmental toxicity; dose administered gestation days 5, 10 and 15	Rat 5	I.p.	0, 0.122, 0.245, 0.408 ml/kg bw		(Singh et al., 1972)	See footnotes 2, 5.
Isobutyl 2-methylprop-2-enoate [09.586]	Developmental toxicity; dose administered gestation days 5, 10 and 15	Rat 5	I.p.	0, 0.140, 0.280, 0.467 ml/kg bw		(Singh et al., 1972)	See footnotes 2, 6.

1 *4-Pentenoic acid is one of several compounds structurally related to valproic acid (VPA) and 4-en-VPA, both highly teratogenic agents, VPA even after oral administration.*

2 *These studies using the intraperitoneal route of administration produced some inconsistent results. They are of questionable significance, also since this route of administration is not considered to be an appropriate or relevant route of exposure (CEC, 2002).*

3 *Methyl methacrylate was investigated within a series of methacrylate esters. It was injected i.p. as a pure liquid compound. Maternal toxicity of the dams was not examined in this study. The following parameters of adverse effects were investigated: embryonic-fetal toxicity, as evidenced by resorptions and stillbirths; gross (external) malformations of fetuses; skeletal malformations and fetal weight. No treatment related effects in comparison to sham treated controls (distilled water or normal saline or cottonseed oil) had been revealed at termination on gestation day 20 with respect to resorptions, numbers of live or dead fetuses or mean fetal body weight. A dose-related increase of gross abnormalities (haemangiomas) was found in the fetuses, but there were no skeletal malformations. However, the results of controls are of questionable significance, (0 %, 2 % and 2.0 % gross abnormalities and 5.0 %, 7.7 % and 15.4 % skeletal abnormalities were observed with distilled water, normal saline and cottonseed oil, respectively). Additionally, there are not enough details reported.*

4 *Groups of 12 mated female Dutch rabbits were treated by intraperitoneal injections (i.p.) with doses of 0.004, 0.04, and 0.4 ml/kg bw/day from day 6 to 18 of pregnancy. Animals were weighed at intervals during the experiment and were observed daily for any change in clinical condition. On day 29, the animals were killed and their uteri examined for live fetuses and early and late resorptions. The fetuses were removed, weighed, sexed and examined for viability and abnormalities. Nine animals, distributed evenly between the groups died or were killed prematurely during the study. In addition, there was a high incidence of peritonitis probably due to the irritant properties of methyl methacrylate and an increase in respiration rate in the top dose level group. Fetal weight was significantly reduced at the 0.4 ml/kg bw/day level and an increase in the numbers of early resorptions was observed at the top dose only. There were no increases in soft tissue or skeletal abnormalities (CEC, 2002).*

5 *Ethyl methacrylate was investigated within a series of methacrylate esters. It was injected i.p. as a pure liquid compound. Maternal toxicity of the dams was not examined in this study. The following parameters of adverse effects were investigated: embryonic-fetal toxicity, as evidenced by resorptions and stillbirths; gross (external) malformations of fetuses; skeletal malformations and fetal weight. The percentage of resorptions was slightly increased and the percentage of live fetuses was slightly decreased in comparison to sham treated controls (distilled water or normal saline or cottonseed oil) at termination on gestation day 20. No treatment related effects had been revealed with respect to numbers of dead fetuses or mean fetal body weight. Gross abnormalities (up to 15.7 %) were increased compared to controls. Skeletal abnormalities (up to 11.1 %) were in the same range as observed with controls. However, the results of controls are of questionable significance (0 %, 2.0 % and 2.0 % gross abnormalities and 5.0 %, 7.7 % and 15.4 % skeletal abnormalities were observed with distilled water, normal saline and cottonseed oil, respectively). Additionally, there are not enough details reported.*

6 *Isobutyl 2-methylprop-2-enoate was investigated within a series of methacrylate esters. It was injected i.p. as a pure liquid compound. Maternal toxicity of the dams was not examined in this study. The following parameters of adverse effects were investigated: embryonic-fetal toxicity, as evidenced by resorptions and stillbirths; gross (external) malformations of fetuses; skeletal malformations and fetal weight. The percentage of resorptions (16.4 %) was slightly increased and the percentage of live fetuses (83.6 %) was slightly decreased at high dose in comparison to sham treated controls (distilled water or normal saline or cottonseed oil) at termination on gestation day 20. No treatment*

related effects had been revealed with respect to numbers of dead fetuses or mean fetal body weight. Gross abnormalities (up to 10.9 %) were increased compared to controls. Skeletal abnormalities (up to 8.0 %) were in the same range as observed with controls. However, the results of controls are of questionable significance (0%, 2.0 % and 2.0 % gross abnormalities and 5.0 %, 7.7 % and 15.4 % skeletal abnormalities were observed with distilled water, normal saline and cottonseed oil, respectively). Additionally, there are not enough details reported.

In vitro Mutagenicity / genotoxicity data are available for four candidate substances of the present flavouring group of substances from chemical groups 1 and 2, and for four supporting substances evaluated at the 51st JECFA meeting (JECFA, 2000a). The supporting substances are listed in brackets.

TABLE IV.4: GENOTOXICITY STUDIES (*IN VITRO*)

Table IV.4: Genotoxicity Studies (*In Vitro*)

Chemical Name [Fl.No.]	Test System	Test Object	Concentration	Result	Reference	Comments
(Oleic acid [08.013])	Ames	S. typh. TA1535, TA1537, TA98, TA100, TA1538. E. coli	5000 µg/plate	Neg.[1]	(Shimizu et al., 1985)	Modified Ames, reincubation.
	Ames	S. typh. TA1535, TA98, TA100, TA1537	333 µg/plate	Neg.[1]	(Mortelmans et al., 1986)	Modified Ames, reincubation.
	Rec assay	B. subtilis	1.0 mg/plate	Neg.[1]	(Osawa & Namiki, 1982)	
	Sister Chromatid Exchange	CH V79	2.5 - 10 µg/ml	Neg.	(Kinsella, 1982)	
	Chromosome aberrations	CH V79	2.5 - 10 µg/ml	Pos.	(Kinsella, 1982)	No data on cytotoxicity reported.
	6-TG resistance	CH V79	1.0 µg/ml	Neg.	(Kinsella, 1982)	
Methyl oleate [09.652]	Ames	S. typh. TA97, TA98, TA100, TA1535, TA1537	0.100, 0.333, 3.333 and 10 mg/plate	Neg.[1]	(Mortelmans et al., 1986)	
(Methyl linolenaate [09.646] & Methyl linoleate (mixture)	Ames (His reversion)	S. typh. TA100, TA98, TA102, TA97, TA1537	1.0 mg/plate	Neg.[1]	(MacGregor et al., 1985)	Tests were conducted with methyl linoleate and methyl linolenate separately, with the same result.
(2,6-Dimethyl-5-heptenal [05.074])	Ames	S. typh. TA1535, TA100, TA1537, TA1538, TA98	3.6 mg/plate	Neg.[1]	(Wild et al., 1983)	
	Ames	S. typh. TA1535, TA100, TA1537, TA1538, TA98	50 mg/plate	Neg.[1]	(Heck et al., 1989)	
(2,6-Dimethyl-5-heptenal [05.074])	UDS	Rat hepatocytes	1.0 mg/ml	Neg.[1]	(Heck et al., 1989)	
Methyl methacrylate [09.647]	Ames	S. typh. TA98, TA100, TA1535, TA1537	As part of a bonecement extract	Neg.[1]	(Jensen et al., 1991)	
	Ames	S. typh. TA98, TA100, TA1535, TA1537, TA1538	150 - 4700 µg/plate	Neg.[1]	(Hachitani et al., 1982)	The study cannot fully be evaluated as text is in Japanese, however, from the tables reported the result seems to be valid.
	Ames	S. typh. TA98, TA100, TA1535, TA1538	100, 1000 and 9000 ppm (tested as a gas)	Neg.[1]	(Anderson et al., 1979; Rohm & Haas Co., 1976a)	
	Ames	S. typh. TA97, TA98, TA100, TA1535, TA1537	10-10000 µg/plate	Neg.[1]	(Zeiger, 1990)	
	Ames	S. typh. TA98, TA100, TA1535, TA1538	4-2500 µg/plate	Neg.[1]	(ICI, 1976b)	
	Ames	S. typh. TA1535, TA1537, TA1538	10 mg/plate	Neg.[1]	(DuPont, 1975)	
	Ames	S. typh. TA98, TA100, TA1535, TA1537	1000, 2500, 5000, 7500 and 10000 µg/plate	Neg [1]	(DuPont, 1979b)	
	Ames	S. typh. TA100	10, 25 and 50 mM (liquid suspension assay)	Weak pos.[1]	(DuPont, 1979b)	Cytotoxicity at all dose levels ranging from 21 – 58 % survival at low-dose level and 18 – 36 % survival at high-dose level.
	Ames	S. typh. TA98, TA100, TA1535, TA1537, TA1538	1000 µg/plate	Neg [1]	(Lijinsky & Andrews, 1980)	
	Ames	S. typh. TA97, TA98, TA100, TA1535	33 – 10000 µg/plate	Neg [1]	(NTP, 1986b)	

Table IV.4: Genotoxicity Studies (*In Vitro*)

Chemical Name [Fl.No.]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames	S. typh. TA98, TA1535, TA1537, TA1538	40 - 10000 µg/plate	Neg [1]	(Waegemaekers & Bensink, 1984)	
	Ames	S. typh. TA100	100 - 10000 µg/2 ml	Neg [1]	(Waegemaekers & Bensink, 1984)	
	Ames	S. typh. TA97a, TA98, TA100, TA102, TA104	0.005 – 25 mg/plate (tested eluates in DMSO and saline; 100 µl of eluate is expressed as 5 mg/plate)	Neg.	(Schweikl et al., 1994)	
	Ames	S. typh. TA98, TA100, TA1537	0.08 - 2.5%	Neg.[1]	(DuPont, 1979a)	
	Ames	S. typh. TA1535	0.08 - 2.5%	Neg.[2] Weak pos.[3]	(DuPont, 1979a)	Weak pos.: The dose levels selected for the test were nontoxic or only slightly toxic.
	Ames	S. typh. TA100	25 mM (suspension assay)	Neg.[2] Weak pos.[3]	(DuPont, 1979a)	Survival at 25 mM was 28 – 29 %.
	Forward mutation	S. typh. TM677	10 - 100 mM	Weak pos.[2] Neg.[3]	(Poss et al., 1979)	Relative survival was 0.50 at 10 mM and 0.10 at 100 mM.
	Forward Mutation	S. typh. TM677	25 – 50 mM	Weak pos.[2]	(Haskell Laboratory, 1989)	Slight increase in mutagenicity, but percent survival was only 20 – 22 % at low-dose level and 12 – 17 % at high-dose level.
	Chromosome aberrations	CHO	5000 µg/ml (50 mM)	Weak pos.[2]	(Anderson et al., 1990; NTP, 1986b)	Increase in percentage of aberrant cells was only at concentrations above 10mM; no cytotoxicity data reported.
	Chromosome aberrations	CHO	1600 µg/ml (16 mM)	Pos.	(Anderson et al., 1990; NTP, 1986b)	Increase in percentage of aberrant cells was only at concentrations above 10mM; no cytotoxicity data reported.
	Chromosome aberrations	L5178Y TK+/- cells	2200 – 3000 µg/ml	Weak pos.[3]	(Doerr et al., 1989)	Survival was 26 % at 2200 microgram/ml and 12 % at 3000 microgram/ml.
	Mouse lymphoma	L5178Y TK+/- cells	500 µg/ml (5 mM)	Pos.[2]	(Amtower et al., 1986)	No data on cytotoxicity available.
	Mouse lymphoma	L5178Y TK+/- cells	1000 - 3000 µg/ml (10 – 30 mM)	Pos.[3]	(Moore et al., 1988)	Mutagenic responses and increases of small colonies were small, not clearly dose-related and observed only at concentrations above 10 mM. Dose-dependent effects on survival, with 60 % survival at 1000 microgram/ml; approximately 15 % survival at 3000 microgram/ml.

Table IV.4: Genotoxicity Studies (*In Vitro*)

Chemical Name [Fl.No.]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mouse lymphoma	L5178Y TK+/- cells	2200 – 3000 µg/ml (22 – 30 mM)	Pos.[3]	(Doerr et al., 1989)	Increases of mutation frequencies occurred only at concentrations above 10 mM. There was a higher than normal level of small colonies in the control cultures. Dose-dependent effects on survival, with 53 % survival at 1000 microgram/ml and 12 % at 3000 microgram/ml.
	Mouse lymphoma	L5178Y TK+/- cells	500 – 1000 µg/ml (5 – 10 mM)	Pos.[2]	(Dearfield et al., 1991)	Percent survival was approximately 80 % at 500 microgram/ml and approximately 40 % at 1000 microgram/ml.
	Mouse lymphoma	L5178Y TK+/- cells	1500 – 3000 µg/ml (15 – 30 mM)	Pos.[3]	(Dearfield et al., 1991)	Percent survival was approximately 50 % at 1500 microgram/ml and approximately 15 % at 3000 microgram/ml.
	Mouse lymphoma	L5178Y TK+/- cells	300 nl/ml (cytotoxic concentration) 100 nl/ml	Pos.[2] Neg.[3]	(Rohm & Haas Co., 1985)	
	Mouse lymphoma	L5178Y TK+/- cells	0.125 µl/ml - 1 µl/ml	Pos.[2] Ambiguous[3]	(NTP, 1986b)	Ambiguous: Small but dose-related increases in mutant frequencies and numbers, but dose-related cytotoxicity was observed.
	Mouse lymphoma	L5178Y TK+/- cells	≥ 200 nl/ml 500 – 1500 nl/ml	Pos.[1]	(Myhr et al., 1990)	Treatments of 1500 nl/ml (without activation) and 2000 nl/ml (with activation) considered extremely toxic and/or lethal. No other cytotoxicity data available.
	SCE	Human lymphocytes	0.1 µg/ml	Neg.[3]	(Cannas et al., 1987; Bigatti et al., 1989)	
	SCE	CHO	16 – 5000 µg/ml	Ambiguous[1]	(Anderson et al., 1990)	Small increases in SCE frequency were reported.
	HRPT	CH V79 B cells	10 - 20 µg/ml	Very weak pos.[3]	(Schweikl et al., 1998)	Survival was 71 and 49 % at 10 and 20 microgram/ml, respectively.
	Cell transformation	BHK21/C13 cells	0.01 - 0.000001 M	Neg.	(Anderson et al., 1979)	
	Micronucleus	Bi-nucleated L5178Y cells	2200 – 3000 µg/ml (22 - 30 mM)	Ambiguous	(Doerr et al., 1989)	Small but dose-related increases in mutant frequencies and numbers, but dose-related cytotoxicity was observed Increases in frequencies of micro-nucleated cells were small and not clearly dose-related. No cytotoxicity data reported.
Ethyl methacrylate [09.375]	Ames	S. typh. TA98, TA100, TA1535, TA1537	33-10000 µg/plate	Neg.[1]	(Zeiger et al., 1987)	

Table IV.4: Genotoxicity Studies (*In Vitro*)

Chemical Name [Fl.No.]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames	S. typh. TA98, TA100, TA1535, TA1537, TA1538	40 – 2500 µg/plate	Neg.[1]	(Waegemaekers & Bensink, 1984)	
	Mouse lymphoma	L5178Y TK+/- cells	>1400 µg/ml	Weak Pos.[3]	(Moore et al., 1988)	Negative at 1400 microgram/ml and below; survival at 1400 microgram/ml and above ranged from 2 to 33 %, with cytotoxicity appearing to reach a plateau at concentrations above 1500 microgram/ml.
	SCE	CHO	NR	Pos.	(NTP, 1987b)	Abstract in table format only, study report not available for re-evaluation.
Isobutyl 2-methylprop-2-enoate [09.586]	Ames	S. typh. TA98, TA100, TA1535, TA1537	100, 333, 1000 and 10000 µg/plate	Neg.[1]	(Zeiger et al., 1987)	

1 With and without metabolic activation.

2 With metabolic activation.

3 Without metabolic activation.

In vivo Mutagenicity / genotoxicity data available for two candidate substances of the present flavouring group of substances from chemical groups 1 and 2, and one supporting substance evaluated at the 51st JECFA meeting (JECFA, 2000a). The supporting substances are listed in brackets.

TABLE IV.5: GENOTOXICITY STUDIES (*IN VIVO*)

Table IV.5: Genotoxicity Studies (*In Vivo*)

Chemical Name [FL-no]	Test system	Test Object	Route	Dose	Result	Reference	Comments
(2,6-Dimethyl-5-heptenal [05.074])	Micronucleus	Mouse (bone marrow)	Intraperitoneal injection	Single dose of 0, 420, 980, and 1540 mg/kg	Neg.	(Wild et al., 1983)	Limited quality since only a single sampling time (30 h after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not clear whether the substance had reached the bone marrow.
	Basc test (Sex-linked recessive lethal mutation)	Drosophila melanogaster	Diet	25 mM	Neg.	(Wild et al., 1983)	A single dose was tested in two experiments. Method not described in detail.
Methyl methacrylate [09.647]	Micronucleus	Mouse (bone marrow)	Gavage	Single dose of 1130 to 4520 mg/kg or 4 doses of 1130 mg/kg	Neg.	(Hachitani et al., 1982)	The study cannot be evaluated as text is in Japanese. Thus, e.g. it is not clear if samples were taken at different sampling times after single treatment and if sampling time was adequate after multiple treatment. Frequency of reticulocytes only slightly changed compared to control. Therefore it is not clear whether the substance had reached the bone marrow.
	Micronucleus	Mouse (bone marrow)	Intraperitoneal injection	Single dose of methacrylate bone cement mixture	Neg.	(Jensen et al., 1991)	Not relevant since an extract of a mixture (containing some additives used as accelerator, stabilizer, colourings etc.) was tested.
	Sex-linked recessive lethal mutation	Drosophila melanogaster	Inhalation	1400 ppm	Neg.	(Foureman et al., 1994)	Sufficient experimental details reported. Result is considered as valid.
	Sex-linked recessive lethal mutation	Drosophila melanogaster	Inhalation.	14000 ppm	Neg.	(Foureman et al., 1994)	Sufficient experimental details reported. Result is considered as valid.

Table IV.5: Genotoxicity Studies (*In Vivo*)

Chemical Name [FL-no]	Test system	Test Object	Route	Dose	Result	Reference	Comments
	Dominant lethal	Mouse	Inh.alation, 6hrs/day for 5 days	100, 1000, and 9000 ppm	Neg.	(ICI, 1976c)	Unpublished non-GLP study. Report contains sufficient details. Result is considered as valid.
	SCE	Human (38 male workers)	Inhalation; 8 hrs/day	0.9 - 71.9 ppm	Neg.	(Seiji et al., 1994)	Exposure period was not reported. 11 unexposed subjects were used as controls. A marginal increase was found (6.11 vs. 4.91 SCEs/cell). However, this effect was considered to be age-related (and not dependent on MMA exposure). Result is considered as valid.
	Chromosome aberrations	Human (38 male workers)	Inhalation; 8 hrs/day	0.9 - 71.9 ppm	Neg.	(Seiji et al., 1994)	Exposure period was not reported. 11 unexposed subjects were used as controls. Result is considered as valid.
	Chromosome aberrations	Rat (bone marrow)	Inhalation, single 2hrs exposure or 5h/day for 5 days	100 - 9000 ppm	Weak pos.	(Rohm & Haas Co., 1976b; Rohm & Haas Co., 1979)	"Both studies suffer from inadequate description; esp. the second study demonstrates severe methodological problems, e.g., analysis of 50 metaphases was not possible for 10 out of 27 animals in the acute and 10 out 26 in the subacute test. Altogether, a clear conclusion cannot be drawn from these studies." (CEC 2002).
Isobutyl 2-methyl-prop-2-enoate [09.586]	Micronucleus	Mouse	Gavage	5000 mg/kg	Neg.	(Roehm GmbH., 1989)	Reported to be in accordance with OECD 474, however, the study cannot be re-evaluated as only a summary of the EU-IUCLID database is available. According to this summary, a decrease of PCE/NCE ratio was observed. This indicates that the substance had reached the target cells.

REFERENCES

Abou-Donia, M.B., Abdel-Rahman, A.A., Kishk, A.M., Walker,D., Markwiese, B.J., Acheson, S.K., Reagan, K.E., Swartzwelder, S., Jensen, K. F., 2000. Neurotoxicity of ethyl methacrylate in rats. J. Toxicol. Environ. Health (part A) 59, 97-118.

Abumrad, N.A., Park, J.H., Park, C.R., 1984. Permeation of long-chain fatty acid into adipocytes. J. Biol. Chem. 259(14), 8945-8953.

ACGIH, 1986. Documentation of the threshold limit values and biological exposure indices, 5 th Ed. Cincinnati, Ohio, USA. 83, 406-407.

ACGIH, 1991. Documentation of the threshold limit values (TLVs) and biological exposure indices (BEIs), 6 th Ed., Methyl methacrylate. Cincinatti, USA.

Albro, P.W., 1975. The metabolism of 2-ethylhexanol in rats. Xenobiotica 5(10), 625-636.

Amtower, A.L., Brock, K.H., Doerr, C.D., Dearfeld, K.L., Moore, M.M., 1986. Genotoxicity of three acrylate compounds in L5178Y mouse lymphoma cells. Environ. Mutag. 8(suppl. 6), 4.

Anderson, D., Longstaff, E., Ashby, J., 1979. An assessment of the carcinogenic and mutagenic potential of methylmethacrylate. Toxicol. Appl. Pharmacol. 48(1- Part 2), A29.

Anderson, B.E., Zeiger, E., Shelby, M.D., Resnick, M.A., Gulati, D.K., Ivett, J.L., Loveday, K.S., 1990. Chromosome aberration and sister chromatid exchange test results with 42 chemicals. Environ. Mol. Mutag. 16(Suppl. 18), 55-137.

Arndt, R., Krisch, K., 1973. Catalytic properties of an unspecific carboxylesterase (E1) from rat-liver microsomes. Eur. J. Biochem. 36, 129-134.

Autian, J., 1975. Structure-toxicity relationship of acrylic monomers. Environ. Health Perspect. 11, 141-152.

Babish, J.G., 1978c. Acute oral toxicity (LD50) of 78-001-2 in albino mice (BLU: Ha (ICR)). Ethyl 2-methyl-3,4-pentadienoate. Food and Drug Research Laboratories, Inc. Lab. no. 5724b, study no. 78-001, may 12, 1978. Unpublished data submitted by EFFA to SCF.

Bailey, D.E., 1976d. Acute oral toxicity in rats. Dermal toxicity in rabbits. Ethyl oleate. Food and Drug Research Laboratories, Inc. Lab. no. 2782, May 21, 1976. Unpublished data submitted by EFFA to SCF.

Basak, A., Bhattacharya, B., Palit, S.K., 1997. Novel regioselective ester hydrolysis by pig-liver esterase. Bull. Chem. Soc. Jap. 70, 2509-2513.

BASF, 1992a. Study on the oral toxicity of 2-methyl-1-propanol in rats via the drinking water over three months. Unpublished report submitted by EFFA to SCF.

Baxter, J.H., Steinberg, D., Mize, C.E., Avigan, J., 1967. Absorption and metabolism of uniformly 14C-labeled phytol and phytanic acid by the intestine of the rat studied with thoracic duct cannulation. Biochim. Biophys. Acta 137(2), 277-290.

Beedham, C., 1988. Molybdenum hydroxylases. In: Gorrod, J.W., Oelschlager, H., Caldwell, J., (Eds.). Metabolism of xenobiotics. Taylor and Francis, London, pp. 51-58.

Bell, G.H., Emslie-Smidt, D., Paterson, C.R., 1976. Textbook of physiology and biochemistry. Churchill Livingstone, Edingburg. pp 193-194.

Bereznowski, Z., 1995. In vivo assessment of methyl methacrylate metabolism and toxicity. Int. J. Biochem. Cell. Biol. 27(12), 1311-1316.

Bigatti, M.P., Lamberti, L., Cannas, M., Rossi, E., 1989. Lack of sister-chromatid exchange induction by polymethyl methacrylate bone cement in human lymphocytes cultured in vitro. Mutat. Res. 227, 21-24.

Blair, A.H., Bodley, F.H., 1969. Human liver aldehyde dehydrogenase: partial purification and properties. Can. J. Biochem. Cell Biol. 47, 265-272.

Blomstrand, R., Rumpf, J.A., 1954. The conversion of [I-14C] cetyl alcohol into palmitic acid in the intestinal mucosa of the rat. Acta Physiol. Scand. 32, 374-383.

Borgström, B., 1974. Fat digestion and absorption. In: Smyth, D.H. (Ed.). Biomembranes- Intestinal Absorption. vol. 4B. Plenum Press, London - New York, 556-620.

Borzelleca, J.F., Larson, P.S., Hennigar Jr, G.R., Huf, E.G., Crawford, E.M., Smith Jr, R.B., 1964. Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. Toxicol. Appl. Pharmacol. 6, 29-36.

Bosron, W.F., Li, T.K., 1980. Alcohol dehydrogenase. In: Jakoby, W.B. (Ed.). Enzymatic Basis of Detoxification vol. 1. Academic Press, New York, 231-248.

Brabec, M.J., 1993. Aldehydes and Acetals. In: Clayton, G.D., Clayton, F.E. (Eds.). Patty's Industrial Hygiene and Toxicology. 4th Ed. vol. 2A. John Wiley & Sons Inc., New York, 283-327.

Bratt, H., Hathway, D.E., 1977. Fate of methyl methacrylate in rats. Br. J. Cancer 36, 114-119.

Briggs, G.B., Doyle, R.L., Young, J.A., 1976. Safety studies on a series of fatty acids. Am. Ind. Hyg. Assoc. J. 37(4), 251-253.

Cannas, M., Bigatti, P., Rossi, E., Rossi, P., 1987. In vitro research on the possibility of chromosomal damage caused by polymethyl methacrylate in orthopaedics. A preliminary report. Ital. J. Orthop. Traumatol. 13(3), 387-391.

Carpanini, F.M.B., Gaunt, I.F., Kiss, I.S., Grasso, P., Gangolli, S.D., 1973b. Short-term toxicity of isoamyl alcohols in rats. Food Cosmet. Toxicol. 11, 713-724.

CEC, 2002. European Union Risk Assessment Report, methyl methacrylate, vol. 22.

Chiappe, C., De Rubertis, A., Amato, G., Gervasi, P.G., 1998. Stereochemistry of the biotransformation of 1-hexene and 2-methyl-1-hexene with rat liver microsomes and purified P450s of rats and humans. Chem. Res. Toxicol., 11, 1487-1493.

CoE, 1992. Flavouring substances and natural sources of flavourings. 4th Ed. vol. I. Chemically defined flavouring substances. Council of Europe, partial agreement in the social and public health field. Strasbourg.

Corkill, J.A., Lloyd, E.J., Hoyle, P., Crout, D.H.G., Ling, R.S.M., James, M.L., Piper, R.J., 1976. Toxicology of methyl methacrylate: The rate of disappearance of methyl methacrylate in human blood in vitro. Clin. Chim. Acta 68, 141-146.

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard - a decision tree approach. Food Cosmet. Toxicol. 16(3), 255-276.

Crout, D.H.G., Lloyd, E.J., Singh, J. 1982. Metabolism of methyl methacrylate: Evidence for metabolism by the valine pathway of catabolism in rat and in man. Xenobiotica 12(12), 821-829.

CSTEE, 2000. Available: www.europa.eu.int/comm/health/ph_risk/committees/sct/docshtml/sct_out90_en.htm. 2 April, 2004.

Dawson, A.M., Holdsworth, C.D., Webb, J., 1964b. Absorption of short chain fatty acids in man. Proc. Soc. Exp. Biol. Med. 117, 97-100.

Dearfield, K.L., Harrington-Brock, K., Doerr, C.L., Rabinowitz, J.R., Moore, M.M., 1991. Genotoxicity in mouse lymphoma cells of chemicals capable of Michael addition. Mutagenesis 6(6), 519-525.

Deichmann, W., 1941. Toxicity of methyl, ethyl and n-butyl methacrylate. J. Ind. Hyg. Toxicol. 23(7), 343-351.

Delbressine, L.P.C., Seutter-Berlage, F., Seutter, E. 1981a. Identification of urinary mercapturic acids formed from acrylate, methacrylate and crotonate in the rat. Xenobiotica 11(4), 241-247.

DFG (Deutsche Forschungsgemeinschaft, Senatskommission zur Pruefung gesundheitsschaedlicher Arbeitsstoffe), 1984. Blagodatin et al., 1970, as quoted in: Henschler, D.(Ed). Toxikologischarbeitsmedizinische Begruendung von MAK-Werten (Methyl Methacrylat). (In German)

Dhopeshwarkar, G.A., Mead, J.F., 1973. Uptake and transport of fatty acids into the brain and the role of the blood-brain barrier system. In: Paoletti, R., Kritchevsky, D. (Eds.). Advances in Lipid Research. vol. 11. Academic Press, pp. 109-142.

DiVincenzo, G.D., Hamilton, M.L., 1979. Fate of n-butanol in rats after oral administration and its uptake by dogs after inhalation or skin application. Toxicol. Appl. Pharmacol. 48, 317-325.

Doerr, C., Harrington-Brock, K., Moore, M.M., 1989. Micronucleus, chromosome aberration, and small-colony TK mutant analysis to quantitate chromosomal damage in L5178Y mouse lymphoma cells. Mutat. Res. 222, 191-203.

Dow Chemical Company, 1957. Results of range finding toxicological test on methyl methacrylate (sanitized). EPA Doc 86-890001194S, microfiche no. OTS0520706. Unpublished report submitted by EFFA to SCF.

DuPont, E.I., 1975. In vitro microbiological mutagenicity studies on methyl methacrylate monomer with attachments, cover sheets and letter dated 092484 (sanitized). Barsky, F.C. EPA Doc 86-8900008145, microfiche no. OTS0520931. May 3, 1975. Unpublished report submitted by EFFA to SCF.

DuPont, E.I., 1979a. Mutagenicity evaluation in Salmonella/typhimurium with attachments, cover sheets and letter dated 092484 (sanitized). Russell, J.F. EPA Doc 86-890000804S, microfiche no. OTS0520921. May 9, 1979. Unpublished report submitted by EFFA to SCF.

DuPont, E.I., 1979b. Mutagenic activity in the Salmonella/microsome assay with attachments, cover sheets and letter dated 092484 (sanitized). Sippel, M.E. EPA Doc 86-890000815S, microfiche No, OTS0520932. February 28,1979. Unpublished report submitted by EFFA to SCF.

DuPont, E.I., 1989. The toxicity and potential dangers of methyl methacrylate (monomer) with attachments and cover sheet dated 061289. EPA Doc 86-890000818, microfiche no. OTS0520934. Unpublished report submitted by EFFA to SCF.

Eastman Kodak Co., 1965. Toxicity and health hazard summary, MSDS, and environmental safety data sheet for methyl methacrylate with cover letter dated 041989. EPA Doc 86-890000209, microfiche no. OTS0516746. September 17, 1965. Unpublished data submitted by EFFA to SCF.

EC, 1996. Regulation No 2232/96 of the European Parliament and of the Council of 28 October 1996. Official Journal of the European Communities 23.11.1996, L 299, 1-4.

EC, 1999a. Commission Decision 1999/217/EC of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs. Official Journal of the European Communities 27.3.1999, L 84, 1-137.

EC, 2000. Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) no. 2232/96. Official Journal of the European Communities 19.7.2000, L 180, 8-16.

EC, 2002b. Commission Regulation No 622/2002 of 11 April 2002 establishing deadlines for the submission of information for the evaluation of chemically defined flavouring substances used in or on foodstuffs. Official Journal of the European Communities 12.4.2002, L 95, 10-11.

EC, 2006. Commission Decision 2006/252/EC of 27 March 2006 amending Decision 1999/217/EC as regards the register of flavouring substances used in or on foodstuffs. Official Journal of the European Union 29.3.2006, L 91, 48.

Edwards, P.M., 1975. Neurotoxicity of acrylamide and its analogues and effects of these analogues and other agents on acrylamide neuropathy. Br. J. Ind. Med. 32, 31-38.

EFFA, 2001c. Submission 2000-3. Flavouring group evaluation of 24 flavouring substances (candidate chemicals) of the chemical groups 1 and 2 (Annex I of 1565/2000/EC), structurally related to linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids, and related esters from FAO/WHO JECFA 42/51. November 20, 2001. SCOOP/FLAV/8.7.

EFFA, 2001e. Submission 2000-3. Flavouring group evaluation of 24 flavouring substances (candidate chemicals) of the chemical groups 1 and 2 (Annex I of 1565/2000/EC), structurally related to linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids, and related esters from FAO/WHO JECFA 42/51. November 20, 2001. SCOOP/FLAV/8.7. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to SCF.

EFFA, 2002i. Letter from EFFA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.

EFFA, 2004e. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.

EFFA, 2007a. E-mail from Jan Demyttenaere, EFFA to Flavis Secretariat, National Foodinstitute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages FLAVIS/8.70.

EFSA, 2004a. Minutes of the 7th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12-13 July 2004. Brussels, 28 September 2004. [Online]. Available: http://www.efsa.eu.int/science/afc/afc_meetings/502_en.html

Eibert Jr., J., 1992. Thirteen week dietary study in rats (hexanol, hexadecanol). Vista Chemical Company. Unpublished report submitted by EFFA to SCF.

El-Khatib, S.M., Cora, E.M., 1981. Role of high-fat diet in tumorigenesis in C57BL/1 mice. J. Natl. Cancer. Inst. 66(2), 297-301.

Elleman, P.N., 1979. Acute oral toxicity in rats (single dose LD50) of hexyl 2-methyl-3 and 4-pentenoate. Cosmopolitan Safety Evaluation. Study no. 0179, November 26, 1979. Unpublished data submitted by EFFA to SCF.

Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. Available: http://europa.eu.int/comm/eurostat/newcronos/queen/display.do?screen=detail&language=en&product=YES&root=YES/yearlies/c/ca/caa/caa10000. August 13, 2004.

Feldman, R.I., Weiner, H., 1972. Horse liver aldehyde dehydrogenase. I. Purification and characterization. J. Biol. Chem. 247(1), 260-266.

Flavour Industry, 2006a. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-05.

Foureman, P., Mason, J.M., Valencia, R., Zimmering, S., 1994. Chemical mutagenesis testing in Drosophila. X. Results of 70 coded chemicals tested for the National Toxicology Program. Environ. Mol. Mutag. 23, 208-227.

Gaillard, D., Derache, R., 1965. Métabolisation de différents alcools, présents dans les boissons alcooliques, chez le rat. [Metabolism of different alcohols, present in different alcoholic beverages in rat]. Trav. Soc. Pharm. Montp. 25(1), 51-62. (In French)

Gangolli, S.D., Shilling, W.H., 1968. Hydrolysis of esters by artificial gastric and pancreatic juices. Research report no. 11/1968. Unpublished report submitted by EFFA to SCF.

Gaunt, I.F., Colley, J., Grasso, P., Lansdown, A.B.G., Gangolli, S.D., 1969. Acute (rat and mouse) and short term (rat) toxicity studies on cis-3-hexen-1-ol. Food Cosmet. Toxicol. 7(5), 451-459.

Gaunt, I.F., Wright, M.G., Cottrell, R., Gangolli, S.D., 1983. Short-term toxicity of 2,6-dimethylhept-5-en-1-al in rats. Food Chem. Toxicol. 21(5), 543-549.

Ghanayem, B.I., Maronpot, R.R., Matthews, H.B., 1986. Association of chemically induced forestomach cell proliferation and carcinogenesis. Cancer Lett. 32, 271-278.

Gibson, G.G., Orton, T.C., Tamburini, P.P., 1982. Cytochrome P-450 induction by clofibrate. Purification and properties of a hepatic cytochrome P-450 relatively specific for the 12- and 11- hydroxylation of dodecanoic acid (lauric acid). Biochem. J. 203, 161-168.

Gigiena i Sanitariia, 1976. 41(4), 6-11. (In Russian)

Grundschober, F., 1977. Toxicological assessment of flavouring esters. Toxicology 8, 387-390.

Hachitani, N., Takeya, A., Takazawa, Y., 1982. [Studies on mutagenicity of life-related environmental agents. III. Ames and mouse bone marrow micronucleus assays of acryl resin monomers and major additives]. Jap. J. Public Health 29(5), 236-239. (In Japanese)

Harris, P., Gloster, J.A., Ward, B.J., 1980. Transport of fatty acids in the heart. Arch. Mal. Coeur 73(6), 593-598.

Hart, E.R., Wong, L.C.K., 1971. Acute oral toxicity studies in rats, acute dermal toxicity and primary skin irritation studies in rabbits of 17 fragrance materials. Bionetics Research Laboratories. July 30, 1971. Report submitted by EFFA to SCF.

Haskell Laboratory, 1989. Submission sheet from Haskell Laboratory submitting a mutagenicity study in Salmonella and a glove permeation study with methyl methacrylate with attachments. EPA Doc 86-890000816, microfiche no. OTS0544277. June 12, 1989. Unpublished report submitted by EFFA to SCF.

Heck, J.D., Vollmuth, T.A., Cifone, M.A., Jagannath, D.R., Myhr, B., Curren, R.D., 1989. An evaluation of food flavoring ingredients in a genetic toxicity screening battery. Toxicologist 9(1), 257-272.

Hedlund, S.G., Kiessling, K.H., 1969a. The physiological mechanism involved in hangover. I. Oxidation of some lower aliphatic fusel alcohols and aldehydes in rat liver and their effect on the mitochondrial oxidation of various substrates. Acta Pharmacol. Toxicol. 27, 381-396.

Heymann, E., 1980. Carboxylesterases and amidases. In: Jakoby, W.B. (Ed.). Enzymatic basis of detoxication. 2nd Ed. Academic Press, New York, pp. 291-323.

Husain, R., Srivastava, S.P., Seth, P.K., 1985. Methyl methacrylate induced behavioural and neurochemical changes in rats. Arch. Toxicol. 58, 33-36.

Husain, R., Khan, S., Husain, I., Seth, P.K., Pandya, K.P. 1989. Effect of methyl methacrylate on selected lipids in rat brain and sciatic nerve. Ind. Health 27, 121-124.

ICI, 1976a. Methyl methacrylate monomer teratogenicity in the rabbit with attachments and cover sheet dated 071789 (sanitized). EPA Doc 86-890001392S, microfiche no. OTS0521015. September 30, 1976. Unpublished data submitted by EFFA to SCF.

ICI, 1976b. Methyl methacrylate: Short-term predictive tests for carcinogenicity: Results with methyl methacrylate monomer (MMM) in mammal with cover letter dated 071789 (sanitized). EPA Doc 86-8900013938, microfiche no. OTS0544294. Unpublished data submitted by EFFA to SCF.

ICI, 1976c. Methyl methacrylate monomer dominant lethal study in the mouse with attachments and cover sheet dated 071789 (sanitized). EPA Doc 86-890001375S, microfiche no. OTS0521010. November 16, 1976. Unpublished data submitted by EFFA to SCF.

IOFI, 1995. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995.

JECFA, 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 859. Geneva.

JECFA, 1996a. Toxicological evaluation of certain food additives. The forty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA, 1997a. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.

JECFA, 1997b. Compendium of food additive specifications. Addendum 5. Joint FAO/WHO Expert Commitee of Food Additives 49th session. Rome, 17-26 June 1997. FAO Food and Nutrition paper 52 Add. 5.

JECFA, 1998b. Compendium of food additive specifications. Addendum 6. Joint FAO/WHO Expert Committee of Food Additives 51st session. Geneva, 9-18 June 1998. FAO Food and Nutrition paper 52 Add. 6.

JECFA, 1999a. Safety evaluation of certain food additives. The fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food Additives Series: 42. IPCS, WHO, Geneva.

JECFA, 1999b. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.

JECFA, 2000a. Evaluation of certain food additives. Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 9-18 June 1998. WHO Technical Report Series, no. 891. Geneva.

JECFA, 2000d. Compendium of food additive specifications. Addendum 8. Joint FAO/WHO Expert Commitee of Food Additives. 55th meeting. Geneva, 6-15 June 2000. FAO Food and Nutrition paper 52 Add. 8.

JECFA, 2001c. Compendium of food additive specifications. Addendum 9. Joint FAO/WHO Expert Commitee of Food Additives 57th session. Rome, 5-14 June 2001. FAO Food and Nutrition paper 52 Add. 9.

JECFA, 2003b. Compendium of food additive specifications. Addendum 11. Joint FAO/WHO Expert Commitee of Food Additives 61st session. Rome, 10-19 June 2003. FAO Food and Nutrition paper 52 Add. 11.

JECFA, 2004a. Evaluation of certain food additives. Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 922. Rome, 10-19 June 2003.

JECFA, 2004b. Safety evaluation of certain food additives and contaminants. Sixty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 52. IPCS, WHO, Geneva.

JECFA, 2005c. Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 928. Geneva, 8-17 June 2004.

JECFA, 2007c. Joint FAO/WHO Expert Committee on Food Additives. Sixty-eight meeting, Geneva, 19-28 June 2007. Summary and conclusions issued 12 July 2007.

Jenner P.M., Hagan E.C., Taylor J.M., Cook E.L., Fitzhugh O.G., 1964. Food flavorings and compounds of related structure. I. Acute oral toxicity. Food Cosmet. Toxicol. 2, 327-343.

Jensen, J.S., Sylvest, A., Trap, B., Jensen, J.C., 1991. Genotoxicity of acrylic bone cements. Pharmacol. Toxicol. 69, 386-389.

Junge, W., Heymann, E., 1979. Characterization of the isoenzymes of pig liver esterase. II. Kinetic studies. Eur. J. Biochem. 95, 519-525.

Kamil, I.A., Smith, J.N., Williams, R.T., 1953a. Studies in detoxication. 46. The metabolism of aliphatic alcohols. The glucuronic acid conjugation of acyclic aliphatic alcohols. Biochem. J. 53(1), 129-136.

Kiaer, H.W., Glavind, J., Arffmann, E., 1975. Carcinogenicity in mice of some fatty acid methyl esters. 2. Peroral and subcutaneous application. Acta Pathol. Microbiol. Immunol. Scand. Section A, 83, 550-558.

Kinsella, A.R., 1982. Elimination of metabolic co-operation and the induction of sister chromatid exchanges are not properties common to all promoting or co-carcinogenic agents. Carcinogenesis 3(5), 499-503.

Klesov, A.A., Lange, L.G., Sytkowski, A.J., Vallee, B.L.,1977. Unusual nature of the substrate specificity of alcohol dehydrogenases of different origins. (Translated from paper in Russian: Bioorganich. Khim. 3(8), 1141-1144).

Krasavage, W.J., Terhaar, C.J., 1981. Unpublished data, Eastman Kodak Co., Corporate Health and Environment Laboratories, Report no. TX-81-38. In: Clayton, G.D., Clayton F.E. (Eds.), Patty's 4th Ed., vol. II, Part D, 32 Esters. John Wiley & Sons, Inc., pp. 2967-3118.

Lawrence, W.H., Malik, M., Autian, J., 1974. Development of a toxicity evaluation program for dental materials and products. II. Screening for systemic toxicity. J. Biomed. Mater. Res. 8, 11-34.

Lee, S.P., Tasman-Jones, C., Carlisle, V., 1986. Oleic acid-induced cholelithiasis in rabbits: Changes in bile composition and gallbladder morphology. Am. J. Pathol. 124(1), 18-24.

Leegwater, D.C., Straten, S., 1974a. In vitro study of the hydrolysis of twenty-six organic esters by pancreatin. Central Institute for Nutrition and Food Research. Report no. R 4319. Project no. 8.33.01. February, 1974.

Lehninger, A.L., 1982. Principles of biochemistry. Worth Publisers, Inc., New York.

Levenstein, I., 1974a. Acute oral toxicity (rat - 5 gms./kg. body weight dose). Dermal toxicity (rabbit - 5 gms./kg. body weight dose). Acetate C-10. Leberco Laboratories, Inc. Assay no. 41758. March 18, 1974. Unpublished report submitted by EFFA to SCF.

Levi, P.E., Hodgson, E., 1989. Metabolites resulting from oxidative and reductive processes. In: Hutson, D. H., Caldwell, J., Paulson, G.D. (Eds.). Intermediary Xenobiotic Metabolism in Animals. Taylor and Francis, London, pp. 119-138.

Lewis, R.J., 1996a. Sax's dangerous properties of industrial materials. 9th Ed. vol. 3. Van Nostrand Reinhold, New York, p. 1957.

Lijinsky, W., Andrews, A.W., 1980. Mutagenicity of vinyl compounds in Salmonella typhimurium. Teratog. Carcinog. Mutag. 1, 259-267.

Longland, R.C., Shilling, W.H., Gangolli, S.D., 1977. The hydrolysis of flavouring esters by artificial gastrointestinal juices and rat tissue preparations. Toxicology 8, 197-204.

MacGregor, J.T., Wilson, R.E., Neff, W.E., Frankel, E.N., 1985. Mutagenicity tests of lipid oxidation products in Salmonella typhimurium: Monohydroperoxides and secondary oxidation products of methyl linoleate and methyl linolenate. Food Chem. Toxicol. 23(12), 1041-1047.

Masoro, E.J., 1977. Lipids and lipid metabolism. Annu. Rev. Physiol. 39, 301-321.

Mayyasi, S.A., Calkins, J.E., Shanahan, R.W., Gray, W.D., 1981. Acute oral toxicity and pharmacotoxic screen in rats of 81-301-01. Biosphere Research Center, Inc. J.E. Project no. 81-049. May, 28, 1981. Unpublished data submitted by EFFA to SCF.

McCarthy, T.J., Witz, G., 1997. Structure-activity relationships in the hydrolysis of acrylate and methacrylate esters by carboxylesterase in vitro. Toxicology 116, 153-158.

Melnick, R.L., 2002. Carcinogenicity and mechanistic insights on the behavior of epoxides and epoxide-forming chemicals. Ann. N.Y. Acad. Sci. 982, 177-189.

MFRM, 1979. Special Issue V. Methyl crotonate. Food Cosmet. Toxicol. 17(Suppl.), 865.

Mondino, A., 1979. TT 193 Acute toxicity study. Instituto Di Ricerche Biomediche " Antoine Marxer " S.P.A. Exp. no. 887. September 18, 1979. Unpublished data submitted by EFFA to SCF.

Moore, M.M., Amtower, A., Doerr, C.L., Brock, K.H., Dearfield, K.L., 1988. Genotoxicity of acrylic acid, methyl acrylate, ethyl acrylate, methyl methacrylate, and ethyl methacrylate in L5178Y mouse lymphoma cells. Environ. Mol. Mutag. 11, 49-63.

Moran, E.J., Easterday, D.D., Oser, B.L., 1980. Acute oral toxicity of selected flavor chemicals. Drug Chem. Toxicol. 3(3), 249-258.

Moreno, O.M., 1973b. Acute oral toxicity study in rats. Dermal toxicity in rabbits. Beta gamma hexenol. MB Research Laboratories, Inc. Project no. MB 72-18. Date 2/1/73. Unpublished report submitted by EFFA to SCF.

Moreno, O.M., 1976g. Acute oral toxicity in rats. Dermal toxicity in rabbits. Cis-3-hexenyl tiglate. MB Research Laboratories, Inc. Project no. MB 76-1041. March 13, 1976. Unpublished report submitted by EFFA to SCF.

Moreno, O.M., 1977b. Acute oral toxicity in rats. Dermal toxicity in rabbits. MB Research Laboratories, Inc. Oleic acid, project no. MB 76-1451, January, 24, 1977. Butyl undecylenate, project no. MB 77-1693, July 22, 1977. Ethyl undecylenate, project no. MB 77-1877, September 29, 1977. Methyl undecylenate, project no. MB 77-1885, October 7, 1977. Unpublished data submitted by EFFA to SCF.

Moreno, O.M., 1978b. Acute oral toxicity in mice. Acute dermal toxicity in guinea pigs. Cis-6-nonen-1-al. MB Research Laboratories, Inc. Project no. 78-2641. Unpublished report submitted by EFFA to SCF.

Moreno, O. M., 1978o. Acute oral toxicity of Hexyl Crotonate in rats. MB Research Laboratories. Project no. MB-77-2198. 2/01/78. Unpublished report submitted by EFFA to FLAVIS Secretariat

Moreno, O.M., 1980a. Report to RIFM. March 17. Cited in Ford, R.A., Letizia, C., Api, A.M., 1988. Methyl tiglate. Food Chem. Toxciol., 26(4), 387.

Morgott, D.A., 1993. Acetone. In: Clayton, G.D., Clayton, F.E. (Eds.). Patty's Industrial Hygiene and Toxicology, 4th Ed. Vol. II, Part A, John Wiley & Sons, New York, pp. 149-281.

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., Zeiger, E., 1986. Salmonella mutagenicity tests II. Results from the testing of 270 chemicals. Environ. Mol. Mutag. 8(Suppl. 7), 1-119.

Myhr, B., McGregor, D., Bowers, L., Riach, C., Brown, A.G., Edwards, I., McBride, D., Martin, R., Caspary, W.J., 1990. L5178Y mouse lymphoma cell mutation assay results with 41 compounds. Environ. Mol. Mutag. 16 Suppl. 18, 138-167.

Nakayasu, H., Mihara, K., Sato, R., 1978. Purification and proporties of a membrane-bound aldehyde dehydrogenase from rat liver microsomes. Biochem. Biophys. Res. Commun. 83(2), 697-703.

Narotsky, M.G., Francis, E.Z., Kavlock, R.J., 1994. Developmental toxicity and structure-activity relationships of aliphatic acids, including dose-response assessment of valproic acid in mice and rats. Fundam. Appl. Toxicol. 22, 251-265.

Nau, H., Löscher, W., 1986., Pharmacologic evaluation of metabolites and analogs of valproic acid: Teratogenic potencies in mice. Fundam. Appl. Toxicol. 6, 669-676.

Newell, G.W., Petretti, A.K., Reiner, L., 1949. Studies of the acute and chronic toxicity of undecylenic acid. J. Invest. Dermatol. 13, 145-149.

NTP, 1986b. NTP technical report on the toxicology and carcinogenesis studies of methyl methacrylate (CAS no. 80-62-6) in F344/N rats and B6C3F1 mice (inhalation studies). October 1986. NTP-TR 314. NIH Publication no. 87-2570.

NTP, 1987b. National Toxicology Program annual plan for fiscal year 1987. May, 1987. NTP-87-001.

Osawa, T., Namiki, M., 1982. Mutagen formation in the reaction of nitrite with the food components analogous to sorbic acid. Agric. Biol. Chem. 45, 2299-2304.

Ouyang, G., Shi, T., Fan, Z., Zhang, B., Yu, T., Hao, A., Tang, G. 1988. [Acute toxicity and toxicokinteics of methyl methacrylate]. Zhonghua Laodong Weisheng Zhiyebing Zazhi. 6, 211-214. (In Chinese)

Palanker, A.L., Lewis, C.A., 1979. Acute oral toxicity (rat). Acute dermal toxicity (rabbit). Oral LD50 (rat). cis-3-hexenal, 50% in triacitin, 09033. Consumer Product Testing. Experiment Reference no. 79104-17. May 31, 1979. Unpublished report submitted by EFFA to SCF.

Pang, S.N.J., 1995. Final report on ethyl methacrylate. J. Am. Coll. Toxicol. 14(6), 452-467.

Pietruszko, R., Crawford, K., Lester, D. 1973. Comparison of substrate specificity of alcohol dehydrogenases from human liver, horse liver and yeast towards saturated and z-enoil alcohols and aldehydes. Arch. Biochem. Biophys. 159, 50-60.

Poss, R., Thilly, W.G., Kaden, D.A., 1979. Methylmethacrylate is a mutagen for Salmonella typhimurium. J. Bone Jt. Surg. 61-A(8), 1203-1207.

Posternak, J.M., 1968. Subacute toxicity (90 days) of chemical 2,4-dimethyl-2-pentenoic acid (TT 118). Firmenich & Cie. May 1968. Unpublished report submitted by EFFA to SCF.

Rijke, A.M., Johnson, R.A., Oser, E.R. 1977. On the fate of methyl methacrylate in blood. J. Biomed. Mater. Res. 11, 211-221.

Roehm GmbH., 1977. Unpublished Report no. 77-012. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, CAS no. 97-86-9. Section 5 Toxicity.

Roehm GmbH., 1989. Unpublished Report no. 89-001. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, CAS no. 97-86-9. Section 5 Toxicity.

Rohm & Haas Co., 1976a. Methylmethacrylate monomer (MMM): Estimation of mutagenic potential in the Salmonella typhimurium plate incorporation mutagenicity assay with cover letter dated 071789 (sanitized). EPA Doc 86-890001382S, microfiche no. OTS0544286. November 30, 1976. Unpublished data submitted by EFFA to SCF.

Rohm & Haas Co., 1976b. Methylmethacrylate monomer: Cytogenetic study in rats with cover letter dated 071789 (sanitized). EPA Doc 86-890001381S, microfiche no. OTS0544285. November 30, 1976. Unpublished data submitted by EFFA to SCF.

Rohm & Haas Co., 1979. Methyl methacrylate: A second cytogenetic study in rats with cover letter dated 071789 (sanitized). EPA Doc 86-890001380S, microfiche no. OTS0544284. May 22, 1979. Unpublished data submitted by EFFA to SCF.

Rohm & Haas Co., 1982. Acute range finding toxicity studies with methyl methacrylate in rats and rabbits with cover letter dated 071789 (sanitized). EPA Doc 86-890001378S, microfiche no. OTS0544282. Juni 15, 1982. Unpublished data submitted by EFFA to SCF.

Rohm & Haas Co., 1985. Mutagenicity evaluation of TD-80-254 in the mouse lymphoma forward mutation assay, Rohm & Haas protocol no. 81 P-259 final report Rohm & Haas report no. 81RC-136. Methyl methacrylate. EPA Doc FYI-OTS-0785-0367 FW, microfiche no. 0367. April 1, 1985. Unpublished ata submitted by EFFA to SCF.

Samren, E.B., van-Duijn, C.M., Koch, S., Hiilesmaa, V.K., Klepel, H., Bardy, A.H., Mannagetta, G.B., Deichl, A.W., Gaily, E., Granstrom, M.L., Meinardi, H., Grobbee, D.E., Hofman, A., Janz, D., Lindhout, D., 1997. Maternal use of antiepileptic drugs and the risk of major congenital malformations: a joint European propective study of human teratogenesis associated with maternal epilepsy. Epilepsia 38(9), 981-990.

SCF, 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev.2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.

SCF, 1999. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

SCF, 2002a. Opinion of the Scientific Committee on Food on the 19th additional list of monomers and additives for food contact materials (expressed on 26 September 2002). (4.4'(1,3,6,8-tetrahydro-1,3,6,8-tetraoxobenzo[lmn][3,8]phenanthroline-2,7-diyl)bisbenzoic acid, diethyl ester), crotonic acid, ethylene carbonate. SCF/CS/PM/GEN/M90 Final. 3 October, 2002. European Commission, Health & Consumer Protection Directorate-General.

Schoental, R., Mattocks, A.R., 1960. Hepatotoxic activity of semi-synthetic analogues of pyrrolizidine alkaloids. Nature 185, 842-843.

Schulthess, G., Werder, M., Hauser, H., 2000. Receptor-mediated lipid uptake at the smallintestinal brush border membrane. In: Christophe, A.B., DeVriese, S. (Eds.). Fat Digestion and Absorption. AOCS Press, Champaign, Illinois, pp. 60-95.

Schulz, H., 1983. Metabolism of 4-pentenoic acid and inhibition of thiolase by metabolites of 4-pentenoic acid. Biochem. 22(8), 1827-1832.

Schwach, G.W., Hofer, H., 1978. [Determination of the oral acute toxicity of methacrylates and vinylpyrrolidone in mouse]. Berichte der Oesterreicheischen Studiengesellschaft für Atomenergie Ges. m.b.H. Forchungszentrum Seibersdorf. (In Austrian)

Schweikl, H., Schmalz, G., Bey, B., 1994. Mutagenicity of dentin bonding agents. J. Biomed. Mater. Res. 28, 1061-1067.

Schweikl, H., Schmalz, G., Rackebrandt, K., 1998. The mutagenic activity of unpolymerized resin monomers in Salmonella typhimurium and V79 cells. Mutat. Res. 415, 119-130.

Scimeca, J.A., 1998. Toxicological evaluation of dietary conjugated linoleic acid in male Fischer 344 rats. Food Chem. Toxicol. 36, 391-395.

Seiji, K., Inoue, O., Kawai, T., Mizunuma, K., Yasugi, T., Moon, C.-S., Takeda, S., Ikeda, M., 1994. Absence of mutagenicity in peripheral lymphocytes of workers occupationally exposed to methyl methacrylate. Ind. Health 32, 97-105.

Senior, A.E., Sherratt, H.S.A., 1969. A comparison of the effect on blood glucose and ketone-body levels, and of the toxicities of pent-4-enoic acid and four simple fatty acids. J. Pharm. Pharmacol. 21(2), 85-92.

Shimizu, H., Suzuki, Y., Takemura, N., Goto, S., Matsushita, H., 1985. The results of microbial mutation test for forty-three industrial chemicals. Jap. J. Ind. Health 27, 400-419.

Singh, A.R., Lawrence, W.H., Autian, J., 1972. Embryonic-fetal toxicity and teratogenic effects of a group of methacrylate esters in rats. J. Dent. Res. 51(6), 1632-1638.

Smyth Jr., H.F., Carpenter, C.P., 1944. The place of the range finding test in the industrial toxicology laboratory. J. Ind. Hyg. Toxicol. 26(8), 269-273.

Spealman, C.R., Main, R.J., Haag, H.B., Larson, P.S., 1945. Monomeric methyl methacrylate studies on toxicity. Ind. Med. 14(4), 292-298.

Stryer, L., 1988. Biochemistry. Freeman, W.H. and Co., New York. pp 469-491, 459-514.

Szepsenwol, J., Boschetti, N.V., 1975. Primary and secondary heart tumors in mice maintained on various diets. Oncology 32, 58-72.

Szepsenwol, J., 1978. Gastro-intestinal tumors in mice of three strains maintained on fat-enriched diets. Oncology 35, 143-152.

Tanii, H., Hashimoto, K., 1982. Structure-toxicity relationship of acrylates and methacrylates. Toxicol. Lett. 11, 125-129.

Tislow, R., Margolin, S., Foley, E.J., Lee, S.W., 1950. Toxicity of undecylenic acid. J. Pharmacol. Exp. Ther. 98(1), 31-32.

TNO, 2000. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

Voet, D., Voet, J.G., 1990. Biochemistry. Chapter 19: Citric Acid Cycle. Chapter 23: Lipid Metabolism, beta-oxidation, cholesterol biosynthesis. Chapter 24: Amino Acid Metabolism, tetrahydrofolate pathway. John Wiley & Sons, New York, pp. 506- 527, 623-633, 645- 651, 686- 700, 761-763.

Waegemaekers, T.H.J.M., Bensink, M.P.M., 1984. Non-mutagenicity of 27 aliphatic acrylate esters in the Salmonella-microsome test. Mutat. Res. 137, 95-102.

Wakil, S.J., Barnes, E.M., 1971. Fatty Acid Metabolism. In: Florkin, M., Stotz, E. (Eds.). Comprehensive Biochemistry. vol. 18S. Pyrovate and Fatty Acid Metabolism. Elsevier Publishing Co., New York, p. 91.

Wild, D., King, M.T., Gocke, E., Eckhard, K., 1983. Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, BASC and micronucleus tests. Food Chem. Toxicol. 21(6), 707-719.

Williams, R.T., 1959. Detoxication mechanisms. The metabolism and Detoxification of Drugs, Toxic Substances, and Other Organic Compounds. 2nd Ed. Chapman and Hall Ltd, London.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., 1987. Salmonella mutagenicity tests. 3. Results from the testing of 255 chemicals. Environ. Mol. Mutag. 9(Suppl. 9), 1-110.

Zeiger, E., 1990. Mutagenicity of 42 chemicals in Salmonella. Environ. Mol. Mutag. 16(Suppl. 18), 32-54.

Zubay, G., 1988. Biochemistry 2nd. Ed. Chapter 19: Catabolism of amino acids. Macmillan Publishing Company, New York, pp. 635-639.