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Opinion of the Scientific Committee on Food on chemically defined flavouring substances listed in the EU register

Flavouring Group Evaluation 1 (FGE.01):
Branched-chain aliphatic saturated aldehydes, carboxylic acids and related esters of primary alcohols and branched-chain carboxylic acids from chemical groups 1 and 2
(Commission Regulation (EC) No 1565/2000 of 18 July 2000)

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FLAVOURING GROUP EVALUATION 1

BRANCHED-CHAIN ALIPHATIC SATURATED ALDEHYDES, CARBOXYLIC ACIDS AND RELATED ESTERS OF PRIMARY ALCOHOLS AND BRANCHED-CHAIN CARBOXYLIC ACIDS FROM CHEMICAL GROUPS 1 AND 2.

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Flavouring Group Evaluation 1

1. Introduction

1.1. Legal 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 others 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 2002/113/EC (EC, 2002a). The latter Decision introduced a new numbering system by attributing each flavouring substance an FL-number. Furthermore, 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 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).

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).

1.2. Description

The present Flavouring Group Evaluation (FGE.01), 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 17 branched-chain aliphatic acyclic saturated aldehydes, carboxylic acids and esters derived from aliphatic acyclic primary saturated alcohols and branched-chain aliphatic acyclic saturated carboxylic acids. These 17 flavouring substances belong to chemical groups 1 and 2 of Annex I of Regulation (EC) No 1565/2000 (EC, 2000).

The 17 flavouring substances (candidate substances) are closely related structurally to 31 flavouring substances (supporting substances) evaluated at the 49th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in the group "Esters of Aliphatic Acyclic Primary Alcohols and Branched-chain Aliphatic Acyclic Acids" (JECFA, 1998; JECFA, 1999b).

The flavouring substances under consideration in the present evaluation are listed in Tables 1 and 2, as well as their chemical names, FL-, CAS-, CoE- and FEMA-numbers and structures. Four of the 17 flavouring substances are aldehydes structurally related to aliphatic acyclic primary alcohols [FL-no: 05.164, 05.166, 05.167, and 05.169]; five are branched-chain aliphatic acyclic carboxylic acids [FL-no: 08.094 - 08.097, and 08.115] and eight are esters of aliphatic acyclic primary alcohols and branched-chain aliphatic acyclic carboxylic acids [FL-no: 09.387, 09.392, 09.499, 09.585, 09.663, 09.679, 09.698, and 09.839].

The names and structures for the 31 supporting substances are listed in Table 3, together with their evaluation status (CoE, 1992; JECFA, 1999b; SCF, 1995).

1.3. Stereoisomers

Twelve of the 17 flavouring substances in the group posses a chiral centre [FL-no: 05.164, 05.167, 08.094 - 08.097, 08.115, 09.387, 09.585, 09.663, 09.679, and 09.698]. In each of these cases, the substance has been presented without any indication that the commercial flavouring substance has dominance of one or the other isomer.

1.4. Natural food occurrence

Eleven of the substances in the present group of 17 substances from chemical groups 1 and 2 have been reported to occur naturally. Four of these 11 substances [FL-no: 08.097, 09.585, 09.663, and 09.698] occur in a wide variety of fruits, as well as in beer, cheese, meat, black tea and other foods. Quantitative data on natural occurrence have only been reported for these four substances. The levels range from trace amounts (propyl 2-methylbutyrate [FL-no: 09.698] in apples) up to 3.2 mg/kg, 0.08 mg/kg and 0.75 mg/kg of 4-methylhexanoic acid [FL-no: 08.097] in cheese, lamb and mutton, respectively (TNO, 2000).

One of the substances, 12-methyltridecanal [FL-no: 05.169] stated to be of natural/nature-identical origin (EFFA, 2000a, EFFA, 2000b) has not been reported in any food items by TNO (TNO, 2000).

2. Specifications

Purity criteria for the 17 candidate substances have been provided by the flavour industry (EFFA, 2000a, EFFA, 2000b), (Table 1).

Judged against the requirements in Annex II of Commission Regulation EC No 1565/2000 (EC, 2000), this information is adequate for seven of the 17 substances. The purity criteria for the remaining 10 substances are deficient in one or more of the parameters (Table 1).

3. Estimated Daily per Capita Intake

The total annual volume of production of the 17 candidate substances from use as flavouring substances in Europe is approximately 140 kg (EFFA, 2000c) and for the 31 supporting substances 32,000 kg (cited by JECFA (JECFA, 1999b)).

On the basis of the annual volumes of production reported for the 17 candidate substances, the daily per capita intakes for each of these flavourings have been estimated (Table 2). More than 75% of the total annual volume of production for the candidate substances (EFFA, 2000c) is accounted for by three of these flavourings: isobutyl 2-methylbutyrate [FL-no: 09.585], pentyl isovalerate [FL-no: 09.499] and pentyl 2-methylbutyrate [FL-no: 09.679]. The estimated daily per capita intake of isobutyl 2-methylbutyrate from use as a flavouring substance is 2.0 microgram/day, that of pentyl isovalerate 11 microgram/day and that of pentyl 2-methylbutyrate 2.3 microgram/day (Table 2).

According to the flavour industry (EFFA, 2000a, EFFA, 2000b) all 17 candidate substances are used in flavoured food products in the following food categories (see Annex III in the Commission Regulation 1565/2000):

Food category	Description
Category 1.	Dairy products
Category 2.	Fats and Oils
Category 3.	Edible ices
Category 4.1.	Fruits*
Category 5.	Confectionery
Category 6.	Cereals and cereal products
Category 7.	Bakery wares
Category 8.	Meat and meat products
Category 9.	Fish and fish products
Category 12.	Salts, spices, soups, sauces, salads, protein products etc.
Category 13.	Foodstuffs intended for particular nutricular uses (except: heptyl 2-methylbutyrate)
Category 14	Beverages, excluding dairy products
Category 15.	Ready-to-eat savouries
Category 16.	Composite foods, foods that could not be placed in categories 1 to 15

*Subgroup of food category 4. “Processed fruits and vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds”.

The normal use levels for the 17 candidate substances are in the range of 1 - 20 mg/kg food, with maximum use levels of up to 200 mg/kg in confectionery and ready-to-eat savouries (EFFA, 2000a, EFFA, 2000b).

4. Absorption, Distribution, Metabolism and Elimination

Data for short and medium length branched-chain aldehydes, carboxylic acids and esters (and their corresponding alcohol and carboxylic acid moieties) included in the present Flavouring Group Evaluation and general information for this class of chemicals indicate that they are rapidly absorbed from the gastrointestinal tract, metabolised and excreted. Also, in vitro hydrolysis data from studies with the supporting substances, as well as other closely related chemicals, indicate that the esters included in the present evaluation can be hydrolysed to yield corresponding alcohols and carboxylic acids (Gangolli & Shilling, 1968; Grundschober, 1977; Leegwater & Straten, 1974; Longland et al., 1977).

General discussions of the biotransformations of branched-chain aliphatic carboxylic acids and aliphatic linear alcohols, as well as specific discussions of the metabolic pathways for isobutyric acid, isovaleric acid, 2-methylbutyric acid, 2-methylpentanoic acid, 4-methylpentanoic acid and 3-methylpentanoic acid as well as hydrolysis of their esters, are provided in Annex II.

In summary it is anticipated that the eight esters [FL-no: 09.387, 09.392, 09.499, 09.585, 09.663, 09.679, 09.698, and 09.839] will undergo hydrolysis to yield their corresponding aliphatic alcohols and branched-chain carboxylic acids. The resulting aliphatic alcohols and branched-chain carboxylic acids, as well as the four branched-chain aldehydes [FL-no: 05.164, 05.166, 05.167, and 05.169] and five branched-chain carboxylic acids [FL-no: 08.094 - 08.097, and 08.115], are expected to be completely oxidised to carbon dioxide via the fatty acid pathway followed by the tricarboxylic acid cycle. The alcohols can also be conjugated, especially the branched ones, and excreted via the urine. See Annex II.

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

For the safety evaluation of the 17 candidate substances from chemical groups 1 and 2 the Procedure (Annex I) was applied. The stepwise evaluations of the 17 substances are summarized in Table 2.

Step 1.

All the 17 substances from chemical groups 1 and 2 are classified in structural class I (Cramer et al., 1978) suggesting a low order of oral toxicity.

Step 2.

Step 2 requires consideration of whether detoxification pathways are available to safely metabolise the substances, at the expected levels of intake, to innocuous products.

It is anticipated that the eight esters [FL-no: 09.387, 09.392, 09.499, 09.585, 09.663, 09.679, 09.698, and 09.839] will be readily hydrolysed to their corresponding alcohols and carboxylic acids. The resulting linear and branched-chain aliphatic alcohols and branched-chain carboxylic acids, as well as four of the aldehydes [FL-no: 05.164, 05.166, 05.167, and 05.169] and five branched-chain carboxylic acids [FL-no: 08.094 - 08.097, and 08.115] are expected to be rapidly absorbed from the gastrointestinal tract and to be oxidised to carbon dioxide via the fatty acid pathway followed by the tricarboxylic acid cycle.

Alcohols can also be conjugated, especially the branched ones, and excreted via the urine (Browning, 1965), see also Annex II.

Three branched-chain carboxylic acids resulting from the hydrolysis of the candidate esters [FL-no: 09.387, 09.392, 09.499, 09.585, 09.663, 09.679, 09.698, and 09.839] are endogenous in humans as intermediates in amino acid metabolism (Voet & Voet, 1990): isobutyric acid, isovaleric acid and 2-methylbutyric acid.

At current levels of intake from use as flavouring substances the 17 substances that have been classified in structural class I from chemical groups 1 and 2 would not be expected to saturate available detoxification pathways.

Therefore the response to Step 2 for each of these 17 substances is "Yes", and accordingly they all proceed via the A-side of the Procedure scheme (Annex I).

Step A3

The 17 candidate substances which have all been assigned to class I have current estimated European daily per capita intakes from < 0.01 to 11 microgram (EFFA, 2000c). These intakes are below the threshold of concern of 1,800 microgram/person/day for class I.

Based on the results of the safety evaluation sequence all 17 flavouring substances from chemical groups 1 and 2 do not pose a safety concern when used at current estimated levels of intake as flavouring substances.

6. Considerations of Combined Intakes from Use as Flavouring Substances

On the basis of the reported annual volumes of production in Europe (EFFA, 2000c) the total estimated daily per capita intake of the 17 flavouring substances from chemical groups 1 and 2 as

flavouring substances is 19 microgram/day, which does not exceed the threshold of concern for a compound belonging to class I of 1,800 microgram per person per day.

The 17 candidate substances are structurally related to 31 flavouring substances evaluated by JECFA at its 49th session (JECFA, 1998; JECFA, 1999b) where it was noted that, in the unlikely event that all these 31 substances are consumed at the same time, the estimated combined intake (in Europe) was 4,600 microgram per person per day. This exceeds the threshold of concern for a compound belonging to structural class I. However, at the level of exposure resulting from the use as flavourings, all 17 candidate and 31 supporting substances are expected to be efficiently metabolised and would not be expected to saturate the metabolic pathways, even if they were ingested together. For these reasons and in the light of toxicological data on supporting substances (Annex III), the combined intake of these substances would not be expected to be of safety concern.

7. Toxicity

7.1. Acute toxicity Studies

Data are available for one candidate substance and for several supporting substances (see Annex III, Table III.1).

7.2. Subacute, subchronic, chronic toxicity and carcinogenicity studies

There are no studies available on the candidate substances in the present flavouring group, but several subacute/subchronic studies on supporting substances, which are summarised in Annex III, Table III.2.

7.3. Developmental/reproductive toxicity studies

Data are available for two candidate substances (see Annex III, Table III.3).

7.4. Genotoxicity studies

There are no data for any of the flavouring substances of this group and only in-vitro data on a single structurally related substance, ethyl isovalerate [FL-no: 09.447]. Two negative and one positive result were obtained in the recombination assays with B. subtilis, however, the tests for mutagenicity in bacteria and mammalian cells were negative. The combined intake of the four aldehydes of the group [FL-no: 05.164, 05.166, 05.167, and 05.169] amounts to about 1 microgram per person per day. At this current level of intake, it is expected that the aldehydes are rapidly oxidised to the corresponding carboxylic acids, which are further partly oxidised, partly conjugated and excreted via urine. Neither the genotoxicity data available nor the chemical structures of the eight esters, the five carboxylic acids and the four carboxylic acids formed by oxidation of the four aldehydes of the group of flavouring substances, raise concerns about genotoxicity. More detailed information on genotoxicity is given Annex III, Table III.4.

It was noted, that where toxicity data were available on single candidate and supporting substances, they were consistent with the conclusions in the Flavouring Group Evaluation using the Procedure.

8. Summary and Conclusions

The 17 flavouring substances and 31 structurally related flavouring substances are all saturated aliphatic aldehydes, carboxylic acids, or esters.

Twelve of the 17 substances in the group posses a chiral centre [FL-no: 05.164, 05.167, 08.094 - 08.097, 08.115, 09.387, 09.585, 09.663, 09.679, and 09.698]. In each of these cases, the substance has been presented without any indication whether the commercial flavouring substance has dominance of one or the other isomer. The substances have been evaluated irrespective of their chirality.

All seventeen flavouring substances belong to structural class I suggesting a low order of oral toxicity. All of them are expected to share similar routes of absorption, distribution and metabolism and to exhibit similar toxicological properties. Data for short and medium length branched-chain aldehydes and carboxylic acids included in the present Flavouring Group Evaluation and general information for this class of chemicals indicate that they are rapidly absorbed from the gastrointestinal tract, metabolised and excreted. Based on published data on compounds structurally related to the esters of the present group of flavourings it can be expected that the eight esters of the group will be hydrolysed to their corresponding acids and alcohols in humans within a relatively short time.

The four aldehydes of the group of flavouring substances (FL-no: 05.164, 05.166, 05.167, and 05.169) are all expected to be rapidly oxidised and excreted at the current level of estimated intake (taken together about 1 microgram per person per day). So, though the genotoxicity data are very limited, they do not raise concerns about genotoxicity of the flavouring substances of this group.

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

The 17 candidate substances have European daily per capita intakes from < 0.01 to 11 microgram/person/day, at current estimated levels of intake, which are below the threshold of concern value for class I of 1,800 microgram/person/day.

All 17 candidate and 31 supporting substances are expected to be efficiently metabolised and the combined level of intakes of 19 microgram and 4,600 microgram per day, taken together, is not expected to saturate the metabolic pathways.

In conclusion, these 17 flavouring substances per se are not regarded as giving rise to safety concerns at the current estimated levels of intake arising from their use as flavourings.

In order to determine whether this conclusion can be applied to the material of commerce, it is necessary to consider the available specifications of purity:

- Adequate specifications including complete purity criteria have been provided for seven materials of commerce [FL-no: 05.164, 05.169, 08.096, 08.097, 09.499, 09.663, and 09.698] and these are regarded as presenting no safety concern at the current estimated levels of intake.
- The specifications of purity for the remaining 10 substances [FL-no: 05.166, 05.167, 08.094, 08.095, 08.115, 09.387, 09.392, 09.585, 09.679, and 09.839] are deficient in one or more of the parameters, and the final evaluation of the material of commerce cannot be performed, pending further information on purity.

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

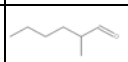
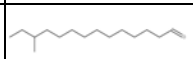
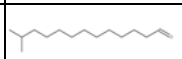
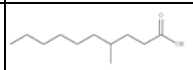
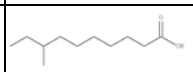
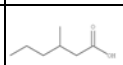
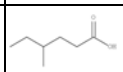
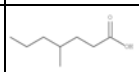
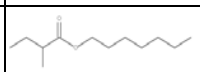
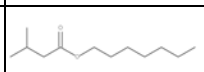
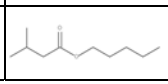
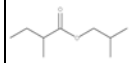
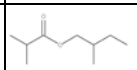
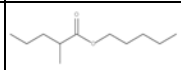
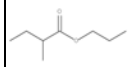
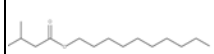
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water Solubility in ethanol	Boiling point, °C 3) Melting point, °C	ID test Assay min.	Refrac. Index 4) Spec.gravity 5)	Specification comments
05.164	2-Methylhexanal 1)		 925-54-2	Liquid $C_7H_{14}O$ 114.19	Insoluble in water 1 ml in 1 ml 95% Ethanol	141	IR NMR MS 95 %	1.411-1.417 0.801-0.807	
05.166	4-Methylpentanal		 10369 1119-16-0	Liquid $C_6H_{12}O$ 100.16	Insoluble in water 1 ml in 1 ml 95% Ethanol	116	IR NMR MS 95 %	1.399-1.415	SG 2)
05.167	12-Methyltetradecanal 1)		 75853-50-8	Liquid $C_{15}H_{30}O$ 226.40	Insoluble in water 1 ml in 1 ml 95% Ethanol	291	NMR 95 %	1.425-1.448	SG 2)
05.169	12-Methyltridecanal		4005 75853-49-5	Liquid $C_{14}H_{28}O$ 212.38	Insoluble in water 1 ml in 1 ml 95% Ethanol	85 (1.3 hPa)	IR NMR 98 %	1.420-1.446 0.833-0.843	
08.094	4-Methyldecanoic acid 1)		 24323-24-8	Liquid $C_{11}H_{22}O_2$ 186.29	Insoluble in water 1 ml in 1 ml 95% Ethanol	152 (13 hPa)	 95 %	1.436-1.442	ID, SG 2)
08.095	8-Methyldecanoic acid 1)		 5601-60-5	Liquid $C_{11}H_{22}O_2$ 186.29	Insoluble in water 1 ml in 1 ml 95% Ethanol	162 (15 hPa)	 95 %	1.443-1.449	ID, SG 2)
08.096	3-Methylhexanoic acid 1)		 3780-58-3	Liquid $C_7H_{14}O_2$ 130.19	slightly soluble in water 1 ml in 1 ml 95% Ethanol	212	NMR 95 %	1.419-1.425 0.916-0.922	
08.097	4-Methylhexanoic acid 1)		 1561-11-1	Liquid $C_7H_{14}O_2$ 130.19	slightly soluble in water 1 ml in 1 ml 95% Ethanol	218	IR NMR MS 95 %	1.418-1.424 0.917-0.923	
08.115	4-Methylheptanoic acid 1)		 3302-03-2	Liquid $C_8H_{16}O_2$ 144.21	slightly soluble in water 1 ml in 1 ml 95% Ethanol	131 (20 hPa)	 95 %	1.427-1.433 0.879-0.885	ID 2)
09.387	Heptyl 2-methylbutyrate 1)		 10668 50862-12-9	Liquid $C_{12}H_{24}O_2$ 200.32	Insoluble in water 1 ml in 1 ml 95% Ethanol	109 (13 hPa)	MS 95 %	1.418-1.424	SG 2)
09.392	Heptyl isovalerate		 10667 56423-43-9	Liquid $C_{12}H_{24}O_2$ 200.32	Insoluble in water 1 ml in 1 ml 95% Ethanol	108 (13 hPa)	 95 %	1.422-1.428 0.862-0.868	ID 2)
09.499	Pentyl isovalerate		 2224 25415-62-7	Liquid $C_{10}H_{20}O_2$ 172.27	Insoluble in water 1 ml in 1 ml 95% Ethanol	108 (7 hPa)	NMR 98 %	1.413-1.415 0.856-0.862	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 1, continued

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water Solubility in ethanol	Boiling point, °C 3) Melting point, °C	ID test Assay min.	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.585	Isobutyl 2-methylbutyrate 1)		10710 2445-67-2	Liquid $C_9H_{18}O_2$ 158.24	Insoluble in water 1 ml in 1 ml 95% Ethanol	166 (96 hPa)	MS 95 %	1.403-1.409 0.845-0.870	SG range
09.663	2-Methylbutyl isobutyrate 1)		10770 2445-69-4	Liquid $C_9H_{18}O_2$ 158.24	Insoluble in water 1 ml in 1 ml 95% Ethanol	170	MS 95 %	1.408-1.410 0.856 - 0.862	
09.679	Pentyl 2-methylbutyrate 1)		10875 68039-26-9	Liquid $C_{10}H_{20}O_2$ 172.27	Insoluble in water 1 ml in 1 ml 95% Ethanol	102 (5 hPa)	99 %	1.412-1.416 0.858-0.864	ID 2)
09.698	Propyl 2-methylbutyrate 1)		10891 37064-20-3	Liquid $C_8H_{16}O_2$ 144.21	Insoluble in water 1 ml in 1 ml 95% Ethanol	156 (97 hPa)	MS 99 %	1.399-1.406 0.862-0.868	
09.839	Decyl 3-methylbutyrate		72928-48-4	Liquid $C_{15}H_{30}O_2$ 242.40	Insoluble in water 1 ml in 1 ml 95% Ethanol	287	NMR 95 %	1.422-1.434	SG 2)

1) Stereoisomeric purity not specified

2) A: missing minimum assay value, BP: missing boiling point, ID: missing identification test, RI: missing refractive index, SG: missing specific gravity

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

Table 2: Summary of Safety Evaluation Results

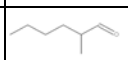
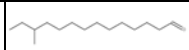
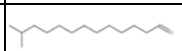
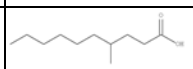
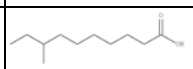
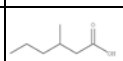
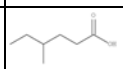
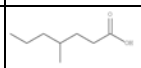
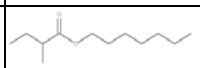
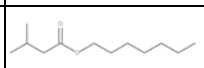
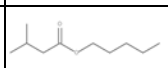
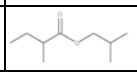
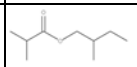
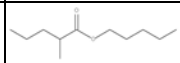
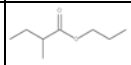
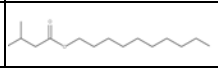
FL-no	EU Register name	Structural formula	MSDI 1) (µg/person/day)	Class 2)	Evaluation procedure path 3)	Conclusion on the named compound 4) or 5)	Conclusion on the material of commerce 6), 7), or 8)
05.164	2-Methylhexanal		0.71	Class I	A3: Intake below threshold	No safety concern 4)	6)
05.166	4-Methylpentanal		<0.005	Class I	A3: Intake below threshold	No safety concern 4)	7)
05.167	12-Methyltetradecanal		0.14	Class I	A3: Intake below threshold	No safety concern 4)	7)
05.169	12-Methyltridecanal		0.29	Class I	A3: Intake below threshold	No safety concern 4)	6)
08.094	4-Methyldecanoic acid		0.29	Class I	A3: Intake below threshold	No safety concern 4)	7)
08.095	8-Methyldecanoic acid		0.71	Class I	A3: Intake below threshold	No safety concern 4)	7)
08.096	3-Methylhexanoic acid		0.29	Class I	A3: Intake below threshold	No safety concern 4)	6)
08.097	4-Methylhexanoic acid		0.86	Class I	A3: Intake below threshold	No safety concern 4)	6)
08.115	4-Methylheptanoic acid		0.29	Class I	A3: Intake below threshold	No safety concern 4)	7)
09.387	Heptyl 2-methylbutyrate		<0.005	Class I	A3: Intake below threshold	No safety concern 4)	7)
09.392	Heptyl isovalerate		<0.005	Class I	A3: Intake below threshold	No safety concern 4)	7)
09.499	Pentyl isovalerate		11	Class I	A3: Intake below threshold	No safety concern 4)	6)
09.585	Isobutyl 2-methylbutyrate		2.0	Class I	A3: Intake below threshold	No safety concern 4)	7)

Table 2: Summary of Safety Evaluation Results, continued

FL-no	EU Register name	Structural formula	MSDI 1) (µg/person/day)	Class 2)	Evaluation procedure path 3)	Conclusion on the named compound 4) or 5)	Conclusion on the material of commerce 6), 7), or 8)
09.663	2-Methylbutyl isobutyrate		0.86	Class I	A3: Intake below threshold	No safety concern 4)	6)
09.679	Pentyl 2-methylbutyrate		2.3	Class I	A3: Intake below threshold	No safety concern 4)	7)
09.698	Propyl 2-methylbutyrate		0.14	Class I	A3: Intake below threshold	No safety concern 4)	6)
09.839	Decyl 3-methylbutyrate		0.01	Class I	A3: Intake below threshold	No safety concern 4)	7)

1) 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/person/day

2) Threshold 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 at estimated current level of intake 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 current level of intake of the material of commerce meeting the specification of Table 1

7) Tentatively regarded as presenting no safety concern pending further information on the purity of the material of commerce.

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

Table 3: Supporting Substances Summary

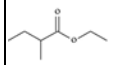
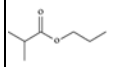
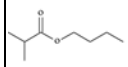
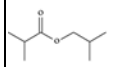
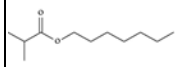
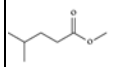
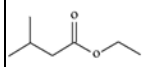
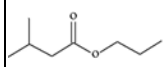
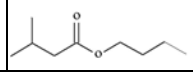
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no	Specification available	MSDI (EU) 1) (μg/person/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.409	Ethyl 2-methylbutyrate		2443 265 7452-79-1	206	JECFA specification c)	2,200	- No safety concern a) Category B b)	Metabolites are endogenous
09.412	Methyl isobutyrate		2694 287 547-63-7	185	JECFA specification c)	23	- No safety concern a) Category A b)	
09.413	Ethyl isobutyrate		2428 288 97-62-1	186	JECFA specification c)	750	- No safety concern a) Category A b)	
09.414	Propyl isobutyrate		2936 289 644-49-5	187	JECFA specification c)	15	- No safety concern a) Category A b)	
09.416	Butyl isobutyrate		2188 291 97-87-0	188	JECFA specification c)	2.7	- No safety concern a) Category A b)	
09.417	Isobutyl isobutyrate		2189 292 97-85-8	194	JECFA specification c)	65	- No safety concern a) Category A b)	
09.420	Heptyl isobutyrate		2550 295 2349-13-5	190	JECFA specification c)	0 (EU)	- No safety concern a) Category A b)	
09.432	Methyl 4-methylvalerate		2721 322 2412-80-8	216	JECFA specification c)	0.03	- No safety concern a) Category B b)	
09.447	Ethyl isovalerate		2463 442 108-64-5	196	JECFA specification c)	760	- No safety concern a) Category B b)	
09.448	Propyl isovalerate		2960 443 557-00-6	197	JECFA specification c)	2.0	- No safety concern a) Category B b)	
09.449	Butyl isovalerate		2218 444 109-19-3	198	JECFA specification c)	94	- No safety concern a) Category B b)	

Table 3: Supporting Substances Summary, continued

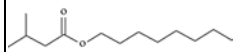
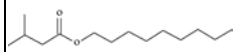
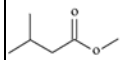
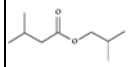
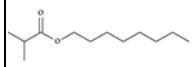
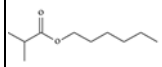
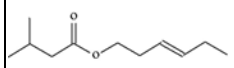
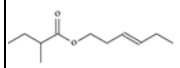
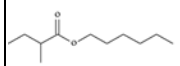
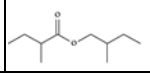
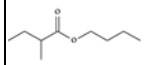
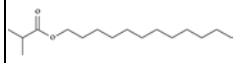
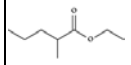
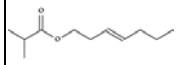
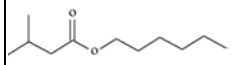
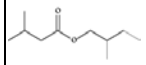
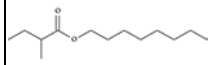
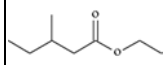
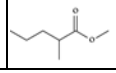
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no	Specification available	MSDI (EU) 1) (μg/person/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.451	Octyl isovalerate		2814 446 7786-58-5	200	JECFA specification c)	7.3	- No safety concern a) Category B b)	
09.452	Nonyl isovalerate		2791 447 7786-47-2	201	JECFA specification c)	0.01	- No safety concern a) Category B b)	
09.462	Methyl isovalerate		2753 457 556-24-1	195	JECFA specification c)	7.8	- No safety concern a) Category B b)	
09.472	Isobutyl isovalerate		3369 568 589-59-3	203	JECFA specification c)	78	- No safety concern a) Category B b)	
09.473	Octyl isobutyrate		2808 593 109-15-9	192	JECFA specification c)	11	- No safety concern a) Category B b)	
09.478	Hexyl isobutyrate		3172 646 2349-07-7	189	JECFA specification c)	3.0	- No safety concern a) Category B b)	
09.483	Methyl 2-methylbutyrate		2719 2085 868-57-5	205	JECFA specification c)	390	- No safety concern a) Category B b)	
09.505	Hex-3-enyl isovalerate		3498 2344 10032-11-8	202	JECFA specification c)	9.4	- No safety concern a) Category B b)	
09.506	Hex-3-enyl 2-methylbutyrate		3497 2345 10094-41-4	211	Tentative JECFA spec. c)	5	- No safety concern a) Category B b)	
09.507	Hexyl 2-methylbutyrate		3499 4132 10032-15-2	208	JECFA specification c)	4.9	- No safety concern a) Category B b)	
09.516	2-Methylbutyl 2-methylbutyrate		3359 10773 2445-78-5	212	JECFA specification c)	3.6	- No safety concern a) -	

Table 3: Supporting Substances Summary, continued

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no	Specification available	MSDI (EU) 1) (μg/person/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.519	Butyl 2-methylbutyrate		3393 10534 15706-73-7	207	JECFA specification c)	26	- No safety concern a) -	
09.523	Dodecyl isobutyrate		3452 10563 6624-71-1	193	JECFA specification c)	50	- No safety concern a) -	
09.526	Ethyl 2-methylvalerate		3488 10616 39255-32-8	214	JECFA specification c)	7.6	- No safety concern a) -	
09.528	trans-3-Heptenyl isobutyrate		3494 10663	191	JECFA specification c)	0.01	- No safety concern a) -	
09.529	Hexyl isovalerate		3500 10692 10032-13-0	199	JECFA specification c)	2.3	- No safety concern a) -	
09.531	2-Methylbutyl isovalerate		3506 10772 2445-77-4	204	Tentative JECFA spec. c)	0.86	- No safety concern a) -	
09.537	Octyl 2-methylbutyrate		3604 10866 29811-50-5	209	JECFA specification c)	0.01	- No safety concern a) -	
09.541	Ethyl 3-methylvalerate		3679 5870-68-8	215	Tentative JECFA spec. c)	0.31	- No safety concern a) -	
09.549	Methyl 2-methylvalerate		3707 2177-77-7	213	Tentative JECFA spec. c)	0.17	- No safety concern a) -	

1) 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/person/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 current levels of intake

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

a) (JECFA, 1999b)

b) (CoE, 1992)

c) (JECFA, 1997b)

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) that are not considered to present a safety concern have been specified.

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 μg/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[1] (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[2] (Step A4)?
- does a NOEL 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.

[1] *"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).*

[2] *"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).*

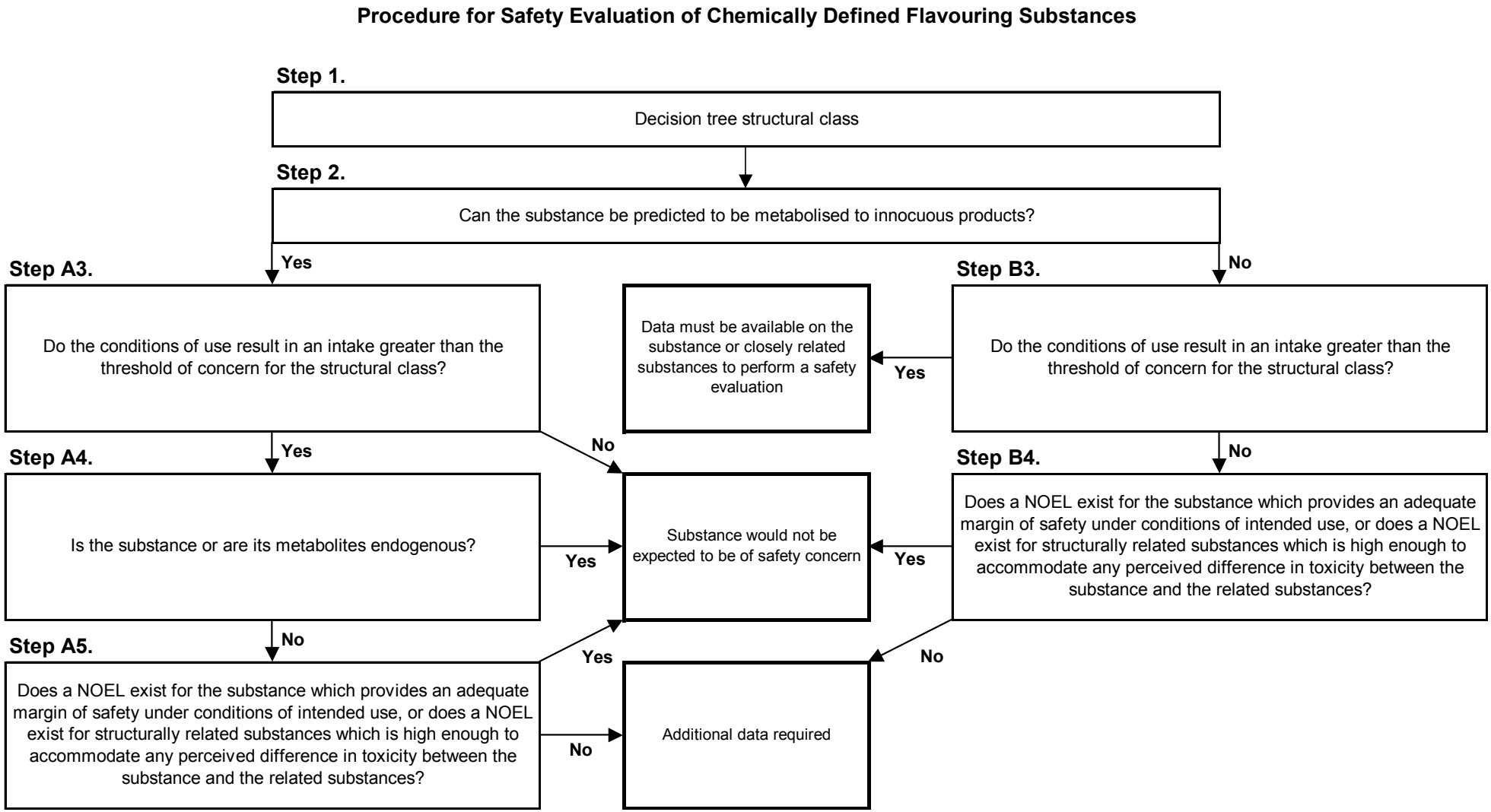


Figure I.1 *Procedure for Safety evaluation of Chemically Defined Flavouring Substances*

Annex II: Metabolism

II.1. Absorption, distribution and excretion

Data for short- and medium-length branched-chain aldehydes, esters and carboxylic acids (and their component alcohols and carboxylic acids) included in this evaluation and more general information for this class of chemicals indicate that they are rapidly absorbed from the gastrointestinal tract (JECFA, 1998; JECFA, 1999b). Information on distribution and excretion of these compounds has not been submitted, but as these flavouring substances can be expected to be extensively metabolised (see below), accumulation of metabolic intermediates and excretion of parent substances is not likely to be of relevance.

II.2. Biotransformation

A general discussion of the biotransformation of branched-chain aliphatic acids and aliphatic linear alcohols, as well as specific discussions of the metabolic pathways for isobutyric acid, isovaleric acid, 2-methylbutyric acid, 2-methylpentanoic acid, 4-methylpentanoic acid and 3-methylpentanoic acid are provided by JECFA (JECFA, 1998; JECFA, 1999b). These discussions and conclusions apply equally well to the candidate substances as they do to the supporting substances.

The following additional discussion on the metabolism of the short- and medium-length branched-chain aldehydes, esters and carboxylic acids is structured according to the general metabolic reactions that have been demonstrated for these or similar chemicals. The likelihood that the candidate substances undergo these metabolic reactions depends on their chain length, degree of branching and functional groups. It is likely that multiple metabolic reactions will occur for some chemicals. The probable metabolic reactions are the following:

II.2.1. Oxidation of alcohols and aldehydes to acids
II.2.2. Reduction of aldehydes to alcohols
II.2.3. Metabolism to glucuronides and sulphates
II.2.4. Ester hydrolysis
II.2.5. beta-Oxidation of carboxylic acids
II.2.6. omega-Oxidation of carboxylic acids
II.2.7. Oxidation of branched-chain carboxylic acids

II.2.1. Oxidation of alcohols and aldehydes to acids

Linear and branched chain alcohols and aldehydes are oxidised to corresponding carboxylic acids by high capacity NAD+/NADH-dependent enzymes (Feron et al., 1991; Parkinson, 1996a; Voet & Voet, 1990).

Alcohol dehydrogenase (ADH) enzymes are cytosolic enzymes that are primarily responsible for the oxidation of alcohols to their corresponding aldehydes. Alcohols also can be oxidised to aldehydes by non-ADH enzymes present in the microsomes and peroxisomes, but these are generally quantitatively less important than ADH. Aldehyde dehydrogenases (ALDH) oxidise aldehydes to their corresponding carboxylic acids. Of the several ALDH enzymes involved in the oxidation of aldehydes, Class I ALDH enzymes are responsible for the oxidation of the widest variety of aldehydes and are expected to be responsible for the oxidation of the candidate

substances (Parkinson, 1996a). Branched-chain aliphatic alcohols and aldehydes also are excellent substrates for ADH and ALDH (Albro, 1975; Blair & Bodley, 1969; Hedlund & Kiessling, 1969a).

II.2.2. Reduction of aldehydes to alcohols

Aldehydes also may be reduced to alcohols, but reduction has a minor overall impact on aldehyde metabolism. Reduction is a reversible reaction, while oxidation is irreversible and the Km of ALD is substantially lower (higher affinity) than the Km of the reductases for the aldehydes (Sladek, 1989).

II.2.3. Metabolism to glucuronides and sulphates

Hydroxyl and carboxyl functional groups may undergo conjugation reactions forming glucuronides and sulphates (Parkinson, 1996a). Conjugation of hydroxyl and carboxyl groups with glucuronide and sulphate and subsequent urinary excretion is expected to compete with the other metabolic reactions described in this annex. Based on the metabolic profiles available, these conjugation reactions apparently comprise a small fraction of the overall metabolic disposition of short- and medium- length branched-chain alcohols, acids, aldehydes and esters. Conjugation with glucuronide may account for the elimination of up to 10% of the dose, for linear aliphatic alcohols with a chain length of about 6 to 8 carbon atoms. For linear alcohols with shorter chain length, this conjugation with glucuronic acid is even less important (Kamil et al., 1953a).

II.2.4. Ester hydrolysis

The esters included in this monograph are expected to be hydrolysed enzymatically to carboxylic acids and alcohols via carboxylesterases found in most tissues throughout the body, the most important of which are the beta-esterases (Heymann, 1980).

Results of in vitro studies indicate that the affinity of the esterases for their substrates increases as the chain length of the ester increases and that the rate of hydrolyses of the straight-chain esters is approximately 100 times faster than the rate of hydrolysis of the branched-chain esters (Arndt & Krisch, 1973; Junge & Heymann, 1979).

While no hydrolysis data have been provided for the esters of the present group of flavourings, there are in vitro hydrolysis data for some structurally related esters.

Structurally related esters were shown to be hydrolysed rather slowly in artificial gastric juice (half life T1/2 146 - 1390 min) (Gangolli & Shilling, 1968; Longland et al., 1977). Hydrolysis in artificial pancreatic juice / pancreatin was found to be faster than in artificial gastric juice (Gangolli & Shilling, 1968; Leegwater & Straten, 1974; Longland et al., 1977). However, there is a variation in the rate of hydrolysis between different structurally related esters. Hydrolysis by artificial pancreatic juice was rather fast for some esters (T1/2 of isoamyl butyrate, benzyl isobutyrate and isoamyl isovalerate 11, 18 and 10 min, respectively) and relatively slow for other esters (T1/2 of ethyl isovalerate and isoamyl caproate 198 and 38 min, respectively). Rat liver homogenate and small intestinal mucosa preparation were found to be much more efficient in hydrolysing esters. While half lives of ethyl isovalerate were 24 and 133 seconds for hydrolysis by liver homogenate and intestinal mucosa preparation, respectively, half lives of isoamyl butyrate and benzyl isobutyrate were less than one second in liver homogenate or intestinal mucosa preparation (Longland et al., 1977).

Based on these data on substances structurally related to the esters of the present group of flavourings it can be expected that the eight esters of the present group will be hydrolysed to their corresponding acids and alcohols in humans within a relatively short time.

II.2.5. beta-Oxidation of carboxylic acids

beta-Oxidation is a major route of metabolism for the candidate chemicals included in this monograph. beta-Oxidation of fatty acids results in acetate and the sequential removal of two-carbon units from carboxylic acids. The process is repeated until the end products are acetate, propionate or butyrate. The products of fatty acid oxidation depend on the chain length of the chemical. Acetate and butyrate are utilized for energy via the citric acid cycle or converted to acetoacetate and subsequently to other ketone bodies. Ketone bodies may be oxidised or excreted in the urine. Propionate originates from odd numbered-chain acids and is converted to carbohydrate and into lipids (Voet & Voet, 1990). The candidate chemicals included in this monograph are prime candidates for fatty acid beta-oxidation since the esters are expected to be hydrolysed to acids and alcohols, and the alcohols and aldehydes will be oxidised to carboxylic acids.

II.2.6. omega-Oxidation of carboxylic acids

Medium-chain length acids also may be partly oxidised via omega-oxidation, producing dicarboxylic acids, which may be attacked from either end by beta-oxidation. omega-Oxidation may occur when capacity for beta-oxidation is either exceeded because of a large dose of the chemical or blocked because of substitution in the alpha- or beta-position. Short-chain acids, such as butyric, caproic and caprylic acids may be converted to longer-chain fatty acids for incorporation into normal intermediary metabolism (Voet & Voet, 1990; Williams, 1959).

II.2.7. Oxidation of branched-chain carboxylic acids

Short-chain branched carboxylic acids (<6 C atoms) can be oxidised via beta-oxidation mainly in the longer chain, followed by cleavage to yield short-chain linear fragments that are metabolised via the normal pathways. Longer branched-chain fatty acids may undergo omega-oxidation to diacids, which may be further oxidised. Cleaved acids with a methyl substituent can be metabolised to carbon dioxide via alfa- and/or beta-oxidation (JECFA, 1998; JECFA, 1999b).

II.3. Conclusion

The information in JECFA (JECFA, 1998; JECFA, 1999b) and that discussed above allow the following assessment to be made:

It is anticipated that the eight candidate esters [FL-no: 09.392, 09.499, 09.585, 09.663, 09.679, 09.698, and 09.839] will undergo hydrolysis, either before or after absorption from the gastrointestinal tract, to yield their corresponding aliphatic alcohols and branched-chain carboxylic acids.

The hydrolysis products, any remaining non-hydrolysed candidate esters, the four candidate branched-chain aldehydes [FL-no: 05.164, 05.166, 05.167, and 05.169] and the five candidate branched-chain carboxylic acids [FL-no: 08.094-08.097, and 08.115], are all expected to be absorbed rapidly from the gastrointestinal tract.

Linear alcohols, resulting from ester hydrolysis, would be oxidised to their corresponding aldehydes and further to their corresponding linear carboxylic acids, which can be assumed to be metabolised to carbon dioxide via the fatty acid pathways and the tricarboxylic acid cycle.

Branched-chain alcohols, resulting from ester hydrolysis, would be oxidised to their corresponding aldehydes and these and the four candidate branched-chain aldehydes would be oxidised to their

corresponding branched carboxylic acids, which can be assumed to be metabolised to carbon dioxide via the fatty acid pathways and the tricarboxylic acid cycle.

Similarly, branched-chain carboxylic acids, resulting from ester hydrolysis, and the five candidate branched-chain aliphatic carboxylic acids can be assumed to be metabolised to carbon dioxide via the fatty acid pathways and the tricarboxylic acid cycle.

Annex III: Toxicity

Oral acute toxicity data are available for one candidate substance of the present flavouring group of 17 substances from chemical groups 1 and 2, and for several supporting substances evaluated at the 49th JECFA meeting (JECFA, 1998). The supporting substances are listed in brackets.

Table III.1: Acute Toxicity Studies

Chemical Name [FL-no]	**Species**	**Sex**	**LD50 (mg/kg bw)**	**Reference**	**Comments**
(Methyl isobutyrate [09.412])	Rat	NR	16000	(Patty, 1982)	1
(Ethyl isobutyrate [09.413])	Rat	NR	>5000	(Moreno, 1975b)	1
(Propyl isobutyrate [09.414])	Rat	NR	15000	(Jenner et al., 1964)	1
(Butyl isobutyrate [09.416])	Rat	NR	>5000	(Levenstein, 1974)	1
(Hexyl isobutyrate [09.478])	Rat	NR	>5000	(Moreno, 1977b)	1
(Octyl isobutyrate [09.473])	Rat	NR	>5000	(Levenstein, 1974)	1
(Isobutyl isobutyrate [09.417])	Rat, Mouse	NR	12800	(Patty, 1982)	1
(Methyl isovalerate [09.462])	Rabbit	NR	5690	(Munch, 1972)	1
(Ethyl isovalerate [09.447])	Rat	NR	>5000	(Levenstein, 1976)	1
	Rabbit	NR	7030	(Munch, 1972)	1
(Propyl isovalerate [09.448])	Rabbit	NR	8220	(Munch, 1972)	1
(Butyl isovalerate [09.449])	Rat	NR	>5000	(Moreno, 1978b)	1
	Rabbit	NR	8230	(Munch, 1972)	1
(2-Methylpropyl isovalerate [09.472])	Rabbit	NR	>5000	(Munch, 1972)	1
	Rat	NR	6970	(Moreno, 1978b)	1
(2-Methylbutyl isovalerate [09.531])	Rat	NR	>5000	(Moreno, 1978b)	1
(Hexyl 2-methylbutanoate [09.507])	Rat	NR	>5000	(Moreno, 1977b)	1
(3-Hexenyl 2-methylbutanoate [09.506])	Rat	NR	>5000	(Moreno, 1977b)	1
4-Methylpentanal [05.166]	Rat	NR	5660	(Smyth et al., 1962)	Assumes a density of 1 g/ml
(Ethyl 3-methylpentanoate [09.541])	Rat	NR	>4000	(Biosphere Res Center Inc., 1981)	1

NR: sex not reported

1. Summarised by JECFA, 49th meeting (JECFA, 1998).

Subacute / subchronic / chronic toxicity data are only available for supporting substances of the present flavouring group. They were all evaluated at the 46th, 49th and 51st JECFA meetings. No carcinogenicity data are available (JECFA, 1997a; JECFA, 1998; JECFA, 1999b).

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

Chemical Name [FL-no]	Species/Sex	Route	Duration	NOEL (mg/kg/day)	Reference	Comments
(Ethyl isobutyrate [09.413])	Rat/ M, F	Oral	90 days	29.2	(Mecler & Craig, 1980)	1, 2
(Isobutyl isobutyrate [09.417])	Rat/ M, F	Gavage	18 weeks	1000	(Drake et al., 1978)	1, 2
(Ethyl isovalerate [09.447])	Rat/ M, F	Oral	13 weeks	12.1	(Mecler & Craig, 1980)	1, 2
(Isovaleric acid [08.008])	Rat/ NR	Oral	90 days	2500	(Amoore et al., 1978)	1, 2
(3-Methylbutyl 3-methylbutyrate [09.463])	Rat/ M, F	Diet	90 days	219	(Damske et al., 1980)	1, 4
(Propyl alcohol [02.002])	Rat/ M	Oral	4 months	60	(Hillbom et al., 1974b)	1, 2
(Butyl alcohol [02.004])	Rat/ M	Oral	13 weeks	5.6	(Wakabayashi et al., 1984)	1, 2
	Rat/ M, F	Oral	14 days	1380	(PPG, 1991)	2
	Rat/ NR	Oral	28 days	940	(Bio-Fax Industrial Bio-test Lab. Inc., 1969)	1, 2
(Hexyl alcohol [02.005])	Beagle/ M, F	Oral	13 weeks	230	(Eibert, 1992)	2
	Rat/M, F	Oral	13 weeks	577	(Eibert, 1992)	2
(Octanol [02.006])	Mouse/ NR	Oral	1 month	179	(Voskoboinikova, 1966)	1, 2
(cis-3-Hexen-1-ol [02.056])	Rat/ M, F	Oral	98 days	150	(Gaunt et al., 1982)	3
(Isobutanol [02.001])	Rat/ M, F	Oral	90 days	1450	(BASF, 1992)	1, 2
	Rat/ M, F	Drinking water	53-56 weeks	200	(Johannsen & Purchase, 1969)	1, 2
(Isoamyl alcohol [02.003])	Rat/ M, F	Gavage	17 weeks	1000	(Carpanini et al., 1973)	1, 4
	Rat/ M, F	Drinking water	53-56 weeks	2000	(Johannsen & Purchase, 1969)	1, 4

NR = sex not reported

M = Male

F = Female

1. The study was performed at a single dose level or multiple dose levels that produced no effects and, therefore, a NOAEL was not determined.

2. Summarised by JECFA, 49th meeting (JECFA, 1998).

3. Summarised by JECFA, 51st meeting (JECFA, 1999a).

4. Summarised by JECFA 46th meeting (JECFA, 1997a).

Developmental and reproductive toxicity data are only available for 2 candidate substances of the present flavouring group evaluation from chemical groups 1 and 2.

Table III.3: Developmental and Reproductive Toxicity Studies

Chemical name	**Study type duration**	**Species/ sex**	**Route**	**NOEL mg/kg/day**	**Reference**	**Comments**
4-Methylhexanoic acid [08.097]	Dev. tox./ day 12 of gestation	Rat/ F	Gavage	Offspring 1833 Maternal <1833	(Scott et al., 1994)	Isomer administered was not specified. No embryotoxicity observed despite severe maternal toxicity.
3-Methylhexanoic acid [08.096]	Dev. tox./ day 12 of gestation	Rat/ F	Gavage	Offspring1833 Maternal<1833	(Scott et al., 1994)	Isomer administered was not specified. No embryotoxicity observed despite severe maternal toxicity.
	Dev. tox./ dosed during organogenesis	Rat/ F	Gavage	Offspring 500 Maternal <375	(Narotsky et al., 1994)	Chernoff/Kavlock assay. No signs of developmental toxicity were observed despite maternal mortality, respiratory toxicity and decreased maternal body weights.

F = Female

In vitro mutagenicity / genotoxicity data are only available for one supporting substance from a group of 31 flavourings evaluated at the 49th JECFA meeting (JECFA, 1999b). No *in vivo* genotoxicity data were available.

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

Chemical Name [Fl.No.]	Test System	Test Object	Concentration	Result	Reference	Comments
(Ethyl isovalerate [09.413])	Ames Test	S.typh. TA 92, TA 1535, TA 100, TA 1537, TA 94, TA 98, TA 2637	0.01-1 mg/plate	Negative	(Fujita & Sasaki, 1987)	With and without metabolic activation
	Ames Test	S.typh. TA 92, TA 1535, TA 100, TA 1537, TA 94, TA 98, TA 2637	10 mg/plate	Negative	(Ishidate et al., 1984)	With and without metabolic activation
	Rec. Assay	B. subtilis. H17 & M45	100-200 microlitre/ml	Positive	(Kuroda et al., 1984)	
	Rec. Assay	B. subtilis. H17 (rec+) & M45 (rec-)	up to 20 microlitre/disc	Negative	(Yoo, 1986)	
	Chrom. Abs.	CHO fibroblasts	up to 2 mg/ml	Negative	(Ishidate et al., 1984)	
	Rec. Assay	B. subtilis. H17 & M45	17 microgram/plate	Negative	(Oda et al., 1978)	

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