ANTHRANILATE DERIVATIVES

First draft prepared by

Mrs Ir M.E.J. Pronk and Dr G.J.A. Speijers

Centre for Substances and Integrated Risk Assessment, National Institute for Public Health and the Environment, Bilthoven, Netherlands

1. EVALUATION

1.1 Introduction

The Committee evaluated a group of 19 anthranilate derivatives (Table 1) by the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1, p. 170). The Committee had previously evaluated two members of this group. Methyl anthranilate (No. 1534) was evaluated at the eleventh meeting (Annex 1, reference 14) and was assigned a conditional ADI[1] of 0–1.5 mg/kg bw. At its twenty-third meeting (Annex 1, reference 50), the Committee re-evaluated the conditional ADI of methyl anthranilate and recommended that it be converted to an (unconditional) ADI of 0–1.5 mg/kg bw. Methyl *N*-methylanthranilate (No. 1545) was evaluated at the twenty-third meeting (Annex 1, reference 50) and was assigned an ADI of 0–0.2 mg/kg bw.

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[1] 'Conditional ADI', which signifies an ADI with special considerations, is a term no longer used by JECFA.

Table 3. Summary of results of safety evaluations of anthranilate derivatives used or proposed to be used as flavouring agents

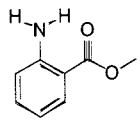
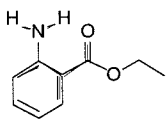
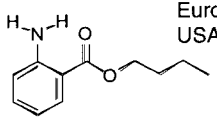
Flavouring agent	No.	CAS No. and structure	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on estimated daily intake
Structural class I							
Methyl anthranilate	1534	134-20-3 	Yes Europe: 804 USA: 3764	No	Yes. The NOEL of 150 mg/kg bw per day (Annex 1, reference 50) is > 11 000 and > 2300 times the estimated daily intakes of 13 and 63 µg/kg bw in Europe and the USA, respectively, when used as a flavouring agent.	See note 1	An ADI of 0–1.5 mg/kg bw was established for methyl anthranilate by the Committee at its twenty-third meeting (Annex 1, reference 50), which was maintained at the present meeting.
Ethyl anthranilate	1535	87-25-2 	No Europe: 14 USA: 39	NR	NR	See note 1	No safety concern
Butyl anthranilate	1536	7756-96-9 	No Europe: 0.003 USA: 14	NR	NR	See note 1	No safety concern

Table 3 /contd)

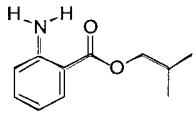
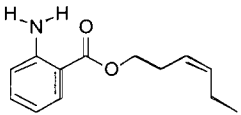
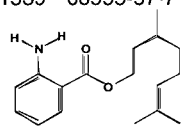
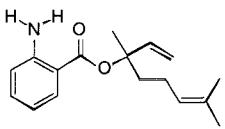
Flavouring agent	No.	CAS No. and structure	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on estimated daily intake
Isobutyl anthranilate	1537	7779-77-3 	No Europe: 1 USA: 0.4	NR	NR	See note 1	No safety concern
cis-3-Hexenyl anthranilate	1538	65405-76-7 	No Europe: ND USA: 53[b]	NR	NR	See note 1	No safety concern (conditional)
Citronellyl anthranilate	1539	68555-57-7 	No Europe: 7[b] USA: 9[b]	NR	NR	See note 1	No safety concern (conditional)
Linalyl anthranilate	1540	7149-26-0 	No Europe: 0.04 USA: 0.07	NR	NR	See note 1	No safety concern

Table 3 /contd)

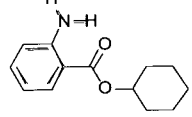
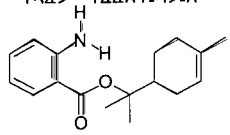
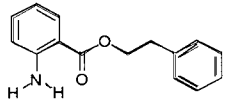
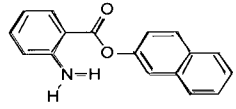
Flavouring agent	No.	CAS No. and structure	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on estimated daily intake
Cyclohexyl-anthranilate	1541	7779-16-0 	No Europe: ND USA: 0.007	NR	NR	See note 1	No safety concern
β-Terpinyl-anthranilate	1542	14481-52-8 	No Europe: 0.004 USA: 1	NR	NR	See note 1	No safety concern
Phenylethyl-anthranilate	1543	133-18-6 	No Europe: 2 USA: 7	NR	NR	See note 1	No safety concern
β-Naphthyl-anthranilate	1544	63449-68-3 	No Europe: ND USA: 2	NR	NR	See note 1	No safety concern

Table 3 /contd)

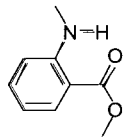
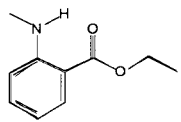
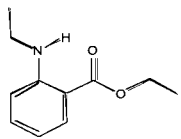
Flavouring agent	No.	CAS No. and structure	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on estimated daily intake
Methyl *N*-methyl-anthranilate	1545	85-91-6 	No Europe: 60 USA: 120	NR	NR	See note 2	An ADI of 0–0.2 mg/kg bw was established for methyl *N*-methyl-anthranilate by the Committee at its twenty-third meeting (Annex 1, reference 50), which was maintained at the present meeting.
Ethyl *N*-methyl-anthranilate	1546	35472-56-1 	No Europe: 0.03[b] USA: 0.04[b]	NR	NR	See note 2	No safety concern (conditional)
Ethyl *N*-ethyl-anthranilate	1547	38446-21-8 	No Europe: 0.07[b] USA: 0.09[b]	NR	NR	See note 3	No safety concern (conditional)

Table 3 /contd)

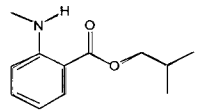
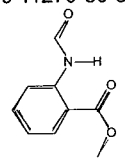
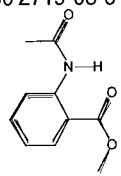
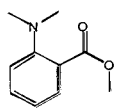
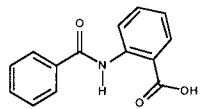
Flavouring agent	No.	CAS No. and structure	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on estimated daily intake
Isobutyl *N*-methyl-anthranilate	1548	65505-24-0 	No Europe: 0.07[b] USA: 0.09[b]	NR	NR	See note 2	No safety concern (conditional)
Methyl *N*-formyl-anthranilate	1549	41270-80-8 	No Europe: 0.1[b] USA: 0.2[b]	NR	NR	See note 4	No safety concern (conditional)
Methyl *N*-acetyl-anthranilate	1550	2719-08-6 	No Europe: 0.05[b] USA: 0.06[b]	NR	NR	See note 5	No safety concern (conditional)
Methyl *N,N*-dimethyl-anthranilate	1551	10072-05-6 	No Europe: 15[b] USA: 18[b]	NR	NR	See note 6	No safety concern (conditional)

Table 3 /contd)

Flavouring agent	No.	CAS No. and structure	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on estimated daily intake
N-Benzoylanthranilic acid	1552	579-93-1 	No Europe: 1[b] USA: 2[b]	NR	NR	See note 7	No safety concern (conditional)

CAS: Chemical Abstracts Service; ND: no intake data reported; N/R: not required for evaluation because intake of the substance was determined to be of no safety concern at Step A3 of the procedure.

Step 2: All the agents in this group can be predicted to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the Procedure.

[a] The threshold for human intake for structural class I is 1800 μg/day and 90 μg/day, respectively. All intake values are expressed in μg/day. The combined per capita intakes of flavouring agents in this group are 904 μg/day in Europe and 4030 μg/day in the USA.

[b] Intake estimate based on anticipated annual volume of production

Notes:

1. Hydrolysed to anthranilic acid, followed by rapid excretion in the urine in conjugated form with glycine (as ortho-aminohippuric acid) or glucuronic acid. The alcohols formed on hydrolysis would be oxidized or conjugated with glucuronic acid or sulfate, followed by excretion in the urine.
2. Hydrolysed to N-methylanthranilic acid, followed by excretion in the urine
3. Hydrolysed to N-ethylanthranilic acid, followed by excretion in the urine
4. Hydrolysed to N-formylanthranilic acid, followed by excretion in the urine
5. Hydrolysed to N-acetylanthranilic acid, followed by excretion in the urine
6. Hydrolyzed to N,N-dimethylanthranilic acid, followed by excretion in the urine
7. Conjugated at the carboxylic acid group to glycine and acyl-glucuronic acid conjugates, followed by excretion in the urine

Four of the 19 flavouring agents (Nos 1534, 1535, 1545 and 1546) have been reported to occur naturally in foods. They have been detected in, for example, starfruit, orange juice, grapefruit juice, strawberries and orange, mandarin and tangerine peel oils (Nijssen et al., 2003). The substance that occurs naturally most frequently is methyl anthranilate (No. 1534).

1.2 Estimated daily per capita exposure

Annual volumes of production have been reported for 10 of the 19 flavouring agents in this group (Nos 1534–1537 and 1540–1545). For the remaining nine substances (Nos 1538, 1539, 1546–1552), anticipated annual volumes of production were given for their proposed use as flavouring agents. The total reported and anticipated annual volume of production of the 19 flavouring agents in this group is about 6300 kg in Europe (International Organization of the Flavor Industry, 1995) and 30 000 kg in the USA (National Academy of Sciences, 1989; Lucas et al., 1999). Methyl anthranilate (No. 1534) accounts for approximately 89% of the total reported and anticipated annual volume of production in Europe and 94% in the USA. The estimated daily exposure to methyl anthranilate in Europe and the USA is approximately 800 and 3800 μg/person, respectively. Ethyl anthranilate (No. 1535) and methyl *N*-methylanthranilate (No. 1545) account for most of the remaining total reported and anticipated annual volume of production (approximately 8% in Europe and 4% in the USA). The estimated daily exposure to ethyl anthranilate is 14 μg per person in Europe and 39 μg per person in the USA; that of methyl *N*-methylanthranilate is 60 μg per person in Europe and 120 μg per person in the USA; and that of the remaining flavouring agents in this group is 0.003–15 μg per person in Europe and 0.007–53 μg per person in the USA. The estimated daily per capita exposure to each agent is reported in Table 2.

1.3 Absorption, distribution, metabolism and elimination

The 11 anthranilic acid esters (Nos 1534–1544) and the five *N*-alkyl anthranilic acid esters (Nos 1545–1548 and 1551) in this group are expected to be readily absorbed, either unchanged or in hydrolysed form. Once absorbed, the unchanged esters are hydrolysed in the liver to their corresponding alcohols and carboxylic acids (anthranilic acid, *N*-methylanthranilic acid, *N*-ethylanthranilic acid or *N*,*N*-dimethylanthranilic acid). These anthranilic acid derivatives are then rapidly excreted in the urine.

Given the relative resistance of the amide bond to hydrolysis, the two combined amides–esters in this group (Nos 1549 and 1550) are expected to be hydrolysed at the methyl ester bond, with rapid excretion of the corresponding carboxylic acids (*N*-formylanthranilic acid or *N*-acetylanthranilic acid) in the urine, either unchanged or in conjugated form. Rather than undergoing hydrolysis at the amide bond, *N*-benzoylanthranilic acid (No. 1552) will be conjugated with glycine or glucuronic acid at the free carboxylic acid group, before excretion in the urine.

1.4 Application of the Procedure for the Safety Evaluation of Flavouring Agents

In applying the Procedure to flavouring agents for which both a reported and an anticipated volume of production were given, the Committee based its evaluation

Table 2. Annual volumes of production of anthranilate derivatives used or proposed for use as flavouring agents in Europe and the USA

Agent (No.)	Reported[a] / anticipated annual volume (kg)	Intake[b]		Annual volume in naturally occurring foods (kg)[c]	Consumption ratio[d]
		µg/day	µg/kg bw per day		
Methyl anthranilate (1534)					
Europe	5 635	804	13		
USA	28 576	3 764	63	61 731	2
Ethyl anthranilate (1535)					
Europe	101	14	0.2		
USA	299	39	0.7	7	000
Butyl anthranilate (1536)					
Europe	000	0.003	0.00005		
USA	109	14	0.2	–	NA
Isobutyl anthranilate (1537)					
Europe	7	1	000		
USA	3	0.4	0.006	–	NA
cis-3-Hexenyl anthranilate (1538)					
Europe	NR	NA	NA		
USA[e]	300	53	0.9	–	NA
Citronellyl anthranilate (1539)					
Europe[e]	50	7	0.1		
USA[e]	50	9	0.1	–	NA
Linalyl anthranilate (1540)					
Europe	0.3	000	0.0007		
USA[f]	0.4	000	0.001	–	NA
Cyclohexyl anthranilate (1541)					
Europe	NR	NA	NA		
USA	000	0.007	0.0001	–	NA
β-Terpinyl anthranilate (1542)					
Europe	000	0.004	0.00007		
USA[f]	7	1	000	–	NA
Phenylethyl anthranilate (1543)					
Europe	11	2	000		
USA	54	7	0.1	–	NA
β-Naphthyl anthranilate (1544)					
Europe	NR	NA	NA		
USA[f]	11	2	000	–	NA
Methyl *N*-methylanthranilate (1545)					
Europe	417	60	1		
USA	912	120	2	+	NA
Ethyl *N*-methylanthranilate (1546)					
Europe[e]	0.2	000	0.0005		
USA[e]	0.2	000	0.0006	+	NA

Table 2 (contd)

Agent (No.)	Reported[a] / anticipated annual volume (kg)	Intake[b] µg/day	µg/kg bw per day	Annual volume in naturally occurring foods (kg)[c]	Consumption ratio[d]
Ethyl *N*-ethylanthranilate (1547)					
Europe[e]	0.5	000	0.001		
USA[e]	0.5	000	0.001	–	NA
Isobutyl *N*-methylanthranilate (1548)					
Europe[e]	0.5	000	0.001		
USA[e]	0.5	000	0.001	–	NA
Methyl *N*-formylanthranilate (1549)					
Europe[e]	1	0.1	0.002		
USA[e]	1	0.2	0.003	–	NA
Methyl *N*-acetylanthranilate (1550)					
Europe[e]	0.3	000	0.0008		
USA[e]	0.3	000	0.0009	–	NA
Methyl *N*,*N*-dimethylanthranilate (1551)					
Europe[e]	102	15	0.2		
USA[e]	102	18	0.3	–	NA
N-Benzoylanthranilic acid (1552)					
Europe[e]	10	1	000		
USA[e]	10	2	000	–	NA
Total					
Europe	6 336				
USA	30 436				

NA, not available; ND, no intake data reported; + reported to occur naturally in foods (Njissen et al., 2004), but no quantitative data; –. not reported to occur naturally in foods

[a] From International Organization of the Flavor Industry (1995) and Lucas et al. (1999) or National Academy of Sciences (1970, 1982, 1987)

[b] Intake (µg/person per day) calculated as follows: [(annual volume, kg) x (1×10^9 µg/kg)/(population x survey correction factor x 365 days)], where population (10%, 'eaters only') = 32×10^6 for Europe and 26×10^6 for the USA; where survey correction factor = 0.6 for Europe and 0.8 for the USA, representing the assumption that only 60% and 80% of the annual flavour volume, respectively, was reported in poundage surveys (International Organization of the Flavor Industry, 1995; Lucas et al., 1999; National Academy of Sciences, 1982) or in the anticipated annual volume.
Intake (µg/kg bw per day) calculated as follows: [(µg/person per day)/body weight], where body weight = 60 kg. Slight variations may occur from rounding.

[c] Quantitative data for the USA reported by Stofberg and Grundschober (1987)

[d] The consumption ratio is calculated as follows: (annual consumption from food, kg)/(most recent reported volume as a flavouring substance, kg)

[e] The volume cited is the anticipated annual volume, which was the maximum amount of flavour estimated to be used annually by the manufacturer at the time the material was proposed for flavour use. National surveys (National Academy of Sciences, 1970, 1982, 1987; Lucas et al., 1999), if applicable, revealed no reported use as a flavour agent.

[f] Annual volume reported in previous surveys in the USA (National Academy of Sciences, 1970, 1982)

on the reported volume of production if the exposure estimated from it exceeded the exposure estimated from the anticipated volume of production and applied no conditions to its decision on safety. If the exposure estimated from the anticipated volume of production exceeded the exposure estimated from the reported volume of production, the Committee based its evaluation on the anticipated volume of production but considered its decision on safety to be 'conditional', pending receipt of information on use levels or poundage data by December 2007. In applying the Procedure to flavouring agents for which only anticipated volumes of production were given, the decision was likewise made conditional.

Step 1. In applying the Procedure, the Committee assigned all 19 flavouring agents in this group to structural class I (Cramer et al., 1978).

Step 2. All the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all the agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. The estimated daily intakes of 18 of the 19 flavouring agents (Nos 1535–1552) are below the threshold of concern (i.e. 1800 μg per person per day for class I). Nine of these 18 substances (Nos 1535–1537 and 1540–1545) are reported to be used as flavouring agents; according to the Procedure, use of these nine flavouring agents raises no safety concern at estimated current exposure. The other nine substances (Nos 1538, 1539 and 1546–1552) are proposed for use as flavouring agents. Although, according to the Procedure, use of these nine flavouring agents raises no safety concern at the estimated exposure on the basis of anticipated annual volumes of production, more reliable estimates are needed. The estimated daily exposure to the remaining agent in this group, methyl anthranilate (No. 1534), which is 804 μg per person in Europe and 3764 μg per person in the USA, exceeds the threshold of concern for class I. Accordingly, the evaluation of methyl anthranilate proceeded to step A4.

Step A4. Methyl anthranilate is not endogenous in humans. Therefore, its evaluation proceeded to step A5.

Step A5. At its twenty-third meeting, the Committee established an ADI of 0–1.5 mg/kg bw for methyl anthranilate on the basis of a NOEL of 150 mg/kg bw per day in a short-term study in rats (Annex 1, reference 50). This NOEL is > 11 000 and > 2300 times greater than the estimated daily exposure to methyl anthranilate from its use as a flavouring agent in Europe (13 μg/kg bw) and the USA (63 μg/kg bw), respectively. The Committee therefore concluded that methyl anthranilate would not present a safety concern at the estimated daily exposure.

The exposure considerations and other information used to evaluate the 19 anthranilate derivatives in this group are summarized in Table 1.

1.5 Consideration of secondary components

All 19 flavouring agents in this group have minimum assay values of ≥ 95%. Hence, it is not necessary to consider secondary components.

1.6 Consideration of combined exposure from use as flavouring agents

In the unlikely event that all 19 agents in this group were to be consumed concurrently on a daily basis, the estimated combined exposure would exceed the human intake threshold of 1800 μg per person per day for class I. All these agents are, however, expected to be efficiently metabolized and would not saturate metabolic pathways. Overall evaluation of the data indicated that combined exposure to these agents would not raise a safety concern.

1.7 Conclusions

The Committee maintained the previously established ADIs of 0–1.5 mg/kg bw for methyl anthranilate and 0–0.2 mg/kg bw for methyl *N*-methylanthranilate (Annex 1, reference 50). The Committee concluded that use of the flavouring agents in this group of anthranilate derivatives would not present a safety concern at the estimated exposure level. For nine flavouring agents (Nos 1538, 1539 and 1546–1552), the evaluation was conditional because the estimated exposure was based on anticipated annual volumes of production. The conclusions of the safety evaluations of these agents will be revoked if use levels or poundage data are not provided before December 2007. The Committee noted that the available data on the toxicity and metabolism of the anthranilate derivatives were consistent with the results of the safety evaluation conducted with the Procedure.

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

The relevant background information summarizes the key scientific data applicable to the safety evaluation of 19 anthranilate derivatives used or proposed for use as flavouring agents (see Table 1).

2.2 Additional considerations on intake

The production volumes and exposure values for each flavouring agent are reported in Table 2. Four of the 19 flavouring agents in the group have been reported to occur naturally in traditional foods (Nijssen et al., 2003). Quantitative data on natural occurrence have been reported for two of them (Stofberg & Grundschober, 1987): exposure to methyl anthranilate (No. 1534) is due predominately to its presence in traditional foods (i.e. it has a consumption ratio ≥ 1), whereas exposure to ethyl anthranilate (No. 1535) is due predominantly to its use as a flavouring agent (i.e. it has a consumption ratio < 1).

2.3 Biological data

2.3.1 Biochemical data

(a) *Hydrolysis and absorption*

Ester derivatives

The group contains 11 anthranilic acid esters (Nos 1534–1544) and five *N*-alkyl anthranilic acid esters (Nos 1545–1548 and 1551). These esters are expected

to be hydrolysed to the corresponding alcohols and carboxylic acids (anthranilic acid, *N*-methylanthranilic acid, *N*-ethylanthranilic acid or *N,N*-dimethylanthranilic acid), catalysed by classes of enzymes known as carboxylesterases or esterases, the most important of which are the B-esterases. In mammals, these enzymes occur in most tissues, but they predominate in hepatocytes. The substrate specificity of B-esterases has been correlated with the structure of the alcohol and carboxylic moieties (Heymann, 1980).

Data on hydrolysis in vitro have been provided for a series of benzoate esters and for two esters of the present group of anthranilate derivatives, methyl anthranilate (No. 1534; synonym, methyl *ortho*-aminobenzoate) and methyl *N*-methylanthranilate (No. 1545; synonym, methyl *ortho*-methylaminobenzoate).

The hydrolysis of a number of alkyl benzoate esters (including methyl, ethyl, butyl and phenylethyl benzoate) in human blood plasma followed first-order kinetics, with half-lives ranging from 15 min to 3.5 h (Nielson & Bundgaard, 1987).

The hydrolysis of methyl anthranilate (No. 1534) and methyl *N*-methylanthranilate (No. 1545) has been studied in vitro with pancreatin (Leegwater & van Straten, 1974a; Grundschober, 1977), artificial gastric and pancreatic juices (Gangolli & Shilling, 1968; Longland et al., 1977), freshly prepared rat liver and small intestine homogenates (Longland et al., 1977) and freshly prepared pig liver and small intestine homogenates (Leegwater & van Straten, 1974b; Grundschober, 1977). After incubation of methyl anthranilate and methyl *N*-methylanthranilate with pancreatin (in 0.5 mol/l phosphate buffer at pH 7.5 and 37 °C), no hydrolysis was observed after 2 h (Leegwater & van Straten, 1974a; Grundschober, 1977). Little hydrolysis was observed when methyl anthranilate was incubated with artificial pancreatic juice (in phosphate buffer at pH 7.5 and 37 °C) or with artificial gastric juice (at pH 1.2 and 37 °C). In artificial gastric juice, only 3% was hydrolysed within 4 h (Gangolli & Shilling, 1968), and the time required for 50% hydrolysis ($t_{0.5}$) was calculated to be 5950 min (Longland et al., 1977). In artificial pancreatic juice, hydrolysis was somewhat faster, 4% being hydrolysed within 4 h (Gangolli & Shilling, 1968) and a t0.5 of 4150 min (Longland et al., 1977). In contrast, preparations of rat and pig tissue homogenates were much more efficient in hydrolysing methyl anthranilate and methyl *N*-methylanthranilate. Whereas hydrolysis was still relatively low (15% within 2 h for methyl anthranilate and 20% for methyl *N*-methylanthranilate) after incubation with pig intestinal mucosa homogenate (in 0.1 mol/l phosphate buffer at pH 7.5 and 37 °C), hydrolysis was almost complete (> 99% for both esters within 2 h) after incubation with pig liver homogenate (in 0.1 mol/l phosphate buffer at pH 7.5 and 37 °C) (Leegwater & van Straten, 1974b; Grundschober, 1977). Methyl anthranilate was also efficiently hydrolysed by rat liver and intestinal mucosal homogenates, following first-order kinetics, with half-lives of 26.7 and 2.5 min, respectively (Longland et al., 1977).

The hydrolysis of methyl *N*-methylanthranilate has been confirmed in vivo in rats and humans (Morgareidge, 1963). Three adult male rats were given methyl *N*-methylanthranilate at a single dose of 1, 5 or 50 mg by stomach tube, after which the urine was collected for 24 h and analysed for the hydrolysis product, *N*-methylanthranilic acid, and the hydrolysed *N*-demethylated product, anthranilic acid. At all three doses, the ratio of *N*-methylanthranilic acid to anthranilic acid was approximately 20:1. *N*-Methylanthranilic acid was also the main metabolite excreted in the 7-h urine of a volunteer given a capsule containing 150 mg of methyl *N*-methylanthranilate, and the ratio of this metabolite to anthranilic acid was also approximately 20:1.

Information on absorption was available only for methyl *N*-methylanthranilate (No. 1545). The intestinal absorption of this compound was examined after injection of various concentrations ranging from 25 to 260 ppm (in physiological isotonic saline) into the duodenal lumen of male Dunkin-Hartley guinea-pigs at a rate of 6 ml/min. Samples of portal blood taken up to 30 min after administration revealed rapid absorption at all concentrations; however, the form in which methyl *N*-methylanthranilate was absorbed varied according to the concentration. At 25 ppm, no unhydrolysed ester was detected in the blood at any time, indicating that methyl *N*-methylanthranilate was absorbed as the hydrolysed form, *N*-methylanthranilic acid. No unhydrolysed ester was observed after 10 min at 40 ppm or after 20 min at 120 ppm. At 260 ppm, the unhydrolysed ester was detected at all times, peaking at 5 min (Pelling et al., 1980).

Amide derivatives

The remaining agents in this group of flavouring agents are two combined amides–esters (Nos 1549 and 1550) and a benzoyl amide (No. 1552). No data were available on their hydrolysis or kinetics; however, it is known that amides are more resistant to hydrolysis than esters. Hence, methyl *N*-formylanthranilate (No. 1549) and methyl *N*-acetylanthranilate (No. 1550) are expected to undergo hydrolysis of the ester bond more rapidly than hydrolysis of the amide bond, resulting in methanol and either *N*-formylanthranilic acid or *N*-acetylanthranilic acid. Likewise, it is expected that *N*-benzoylanthranilic acid (No. 1552) will be hydrolysed to only a limited extent.

(b) Metabolism

Ester derivatives

Upon hydrolysis, the 11 anthranilic acid esters in this group (Nos 1534–1544) are hydrolysed, principally in the liver, to anthranilic acid and the corresponding alcohols (methanol, ethanol, (iso)butanol, *cis*-3-hexenol, citronellol, linalool, cyclohexanol, β-terpinol, phenylethyl alcohol or β-naphthol). In its previous review of methyl anthranilate (Annex 1, references 50 and 51), the Committee noted that anthranilic acid is endogenous in humans (being an intermediate in the metabolism of tryptophan) and that anthranilic acid is excreted in the urine, mainly as *ortho*-aminohippuric acid and to a lesser extent as anthranilic acid glucuronide. The Committee previously reviewed data on the metabolism of the corresponding alcohols, with the exception of β-naphthol (Annex 1, references 23, 132, 138, 161 and 167). General aspects of their metabolism have been described (Annex 1, references 23, 131, 137, 160 and 166). β-Naphthol is excreted in the urine, mainly in conjugated form, as the glucuronide or sulfate, but also in unchanged form (BG Chemie, 1995).

The metabolism of the five *N*-alkyl anthranilic acid esters in this group (Nos 1545–1548 and 1551) is consistent with that of the anthranilic acid esters. The ester function undergoes hydrolysis, principally in the liver, followed by excretion of the *N*-alkylanthranilic acid (*N*-methylanthranilic acid, *N*-ethylanthranilic acid or *N,N*-dimethylanthranilic acid) in the urine. In rats and humans, the main reaction of methyl *N*-methylanthranilate is hydrolysis to *N*-methylanthranilic acid, with little *N*-demethylation, to yield anthranilic acid (ratio of *N*-methylanthranilic acid:anthranilic acid, approximately 20:1); the metabolites are eliminated in the urine (Morgareidge,

1963; see Figure 1). The metabolism of the corresponding alcohols (methanol, ethanol and isobutanol) has been reviewed by the Committee, and general aspects of their metabolism have been described (Annex 1, references 23, 131 and 132).

Amide derivatives

No data were available on the metabolism of the three amide derivatives in this group of flavouring agents. After hydrolysis of the methyl ester group in methyl *N*-formylanthranilate (No. 1549) and methyl *N*-acetylanthranilate (No. 1550), however, the corresponding carboxylic acids will be rapidly excreted in the urine, either unchanged or in conjugated form. The metabolism of *N*-benzoylanthranilic acid (No. 1552) is expected to proceed rapidly by conjugation with glycine or glucuronic acid at the free carboxylic acid group, with or without amide hydrolysis. Besides, any amide hydrolysis occurring would result in innocuous benzoic acid and anthranilic acid. Hence, the three amide derivatives can be predicted to be metabolized to innocuous products.

2.3.2 Toxicological studies

(a) Acute toxicity

Oral LD_{50} values have been reported for eight of the 19 substances in this group, one having been tested in mice, rats as well as guinea-pigs, and the other seven only in rats (see Table 3). In mice and guinea-pigs, the oral LD_{50} values were 3900 and 2780 mg/kg bw, respectively (Jenner et al., 1964). In rats, the oral LD_{50} values ranged from 2910 to 5825 mg/kg bw (Jenner et al., 1964; Gaunt et al., 1970; BASF, 1973; Russel, 1973; Levenstein, 1974; Wohl, 1974; Moreno, 1975a,b, 1978;

Figure 1. Metabolism of anthranilic acid derivatives

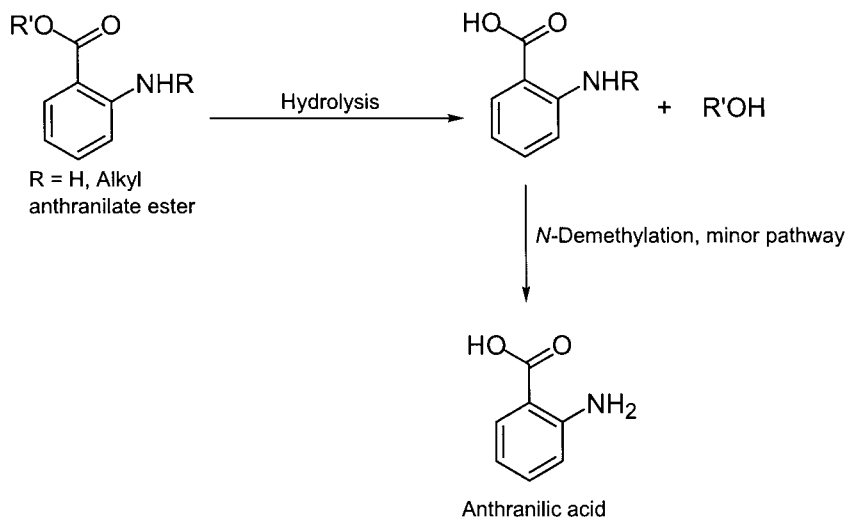


Table 3. Results of studies of acute toxicity with anthranilate derivatives administered orally

No.	Flavouring agent	Species; sex	LD_{50} (mg/kg bw)	Reference
1534	Methyl anthranilate	Mouse; NR	3900	Jenner et al. (1964)
1534	Methyl anthranilate	Rat; M, F	2910	Jenner et al. (1964)
1534	Methyl anthranilate	Rat; NR	5 ml/kg bw (5825[a])	BASF (1973)
1534	Methyl anthranilate	Guinea-pig; M, F	2780	Jenner et al. (1964)
1535	Ethyl anthranilate	Rat; NR	3750	Moreno (1975a)
1536	Butyl anthranilate	Rat; NR	> 5000	Wohl (1974)
1538	*cis*-3-Hexenyl anthranilate	Rat; NR	> 5000	Moreno (1978)
1540	Linalyl anthranilate	Rat; NR	4250	Russel (1973)
1543	Phenylethyl anthranilate	Rat; NR	> 5000	Moreno (1975b)
1545	Methyl *N*-methylanthranilate	Rat; F	2250–3380[b]	Gaunt et al. (1970)
1545	Methyl *N*-methylanthranilate	Rat; NR	3.7 ml/kg bw (4177[c])	Levenstein (1974)
1549	Methyl *N*-formylanthranilate	Rat; M, F	> 5000	Collier & Jones (1986)

F, female; M, male; NR, not reported
a Calculated from a density of methyl anthranilate = 1.165 (1.161–1.169) g/ml (Lewis, 1999)
b LD50 between 2250 (0 death) and 3380 (100% death) mg/kg bw
c Calculated from a density of methyl N-methylanthranilate = 1.129 (1.126–1.132) g/ml (Lewis, 1999)

Collier & Jones, 1986). These LD_{50} values indicate that the acute toxicity of orally administered anthranilate derivatives is low.

(b) Short-term studies of toxicity

Short-term studies of toxicity were available for only two of the 19 substances in this group (Oser et al., 1965; Hagan et al., 1967; Gaunt et al., 1970). The results of these studies are summarized in Table 4 and described below.

Methyl anthranilate (No. 1534)

Groups of 10 male and 10 female weanling Osborne-Mendel rats were given diets containing methyl anthranilate at a concentration of 0, 1000 or 10 000 mg/kg of diet, calculated (Food & Drug Administration, 1993) to provide average daily intakes of 0, 50 and 500 mg/kg bw, respectively, for 13 weeks. Body weight, food intake and general condition were recorded weekly. Haematological examinations carried out at termination of the study, included leukocyte and erythrocyte counts, haemoglobin and erythrocyte volume fraction. At necropsy, all animals were examined macroscopically, and liver, kidneys, heart, spleen and testes were weighed. These organs and the remaining abdominal and thoracic viscera and bone, bone marrow and muscle from one hind leg were taken from three to four rats of each sex in the control and highest dose group, preserved and examined histopathologically. No treatment-related effects on growth, haematological end-points or organ weights were observed,

Table 4. Results of short-term studies of toxicity with in male and female rats given anthranilate derivatives in the diet

No.	Substance	No. test groups[a]/ no. per group[b]	Duration	NOEL (mg/kg bw per day)	Reference
1534	Methyl anthranilate	2/20	13 weeks	500[c]	Hagan et al. (1967)
1545	Methyl *N*-methyl-anthranilate	1/30	90 days	19.9[c] (M) 22.2[c] (F)	Oser et al. (1965)
1545	Methyl *N*-methyl-anthranilate	3/30	13 weeks	21 (M) 24 (F)	Gaunt et al. (1970)

[a] Total number of test groups does not include control animals.
[b] Total number per test group includes both male and female animals.
[c] Study performed with either a single dose or multiple doses. The dose(s) tested had no adverse effects.

and there were no macroscopic or microscopic changes in the tissues. The NOEL was 500 mg/kg bw per day, the highest dose tested (Hagan et al., 1967).

In a 115-day study of toxicity that was not available to the present Committee but was reviewed by the Committee at its twenty-third meeting (Annex 1, references 50 and 51; Dow, 1967), groups of 10 male and 10 female weanling rats were given diets containing methyl anthranilate at a concentration of 0, 3000 or 10 000 mg/kg of diet, stated to be approximately equivalent to 0, 150–300 and 500–1000 mg/kg bw per day, respectively. It was reported that there was no evidence of adverse effects at 3000 mg/kg, as judged by appearance, behaviour, growth, mortality, terminal haematological examination, final body weights and gross and microscopic examination. The only effects observed at 10 000 mg/kg were increased average weights of the liver and kidneys and slight (minimal) histological changes in the kidneys. The NOEL was 150 mg/kg bw per day and formed the basis for the ADI for methyl anthranilate (Annex 1, references 50 and 51).

Methyl N*-methylanthraniline (No. 1545)*

In a 90-day study of toxicity, groups of 15 male and 15 female weanling rats of the FDRL strain were given diets containing methyl *N*-methylanthranilate (dissolved in cottonseed oil) at an intended dose of 0 or 20.3 mg/kg bw per day. The actual average daily intake was 19.9 mg/kg bw for males and 22.2 mg/kg bw for females. Measurements of body weight and food consumption revealed no treatment-related effects on growth. Limited haematological and clinical chemistry analyses performed on eight rats of each sex per group at week 6 and on all rats at week 12 revealed no differences between treated and control animals. At necropsy, all animals were examined macroscopically, liver and kidney weights were recorded, and tissue samples from approximately 20 major organs and tissues of half the animals per group were obtained for histological examination. There were no treatment-related effects on liver or kidney weights, and there was no evidence of gross pathological or histopathological alterations. The NOEL was 20 mg/kg bw per day, the only dose tested (Oser et al., 1965).

Groups of 15 male and 15 female weanling CFE rats were given diets containing methyl *N*-methylanthranilate at a concentration of 0, 300, 1200 or 3600 mg/kg for 13 weeks. These concentrations provided average daily intakes of 21, 82 and 244 mg/kg bw for males and 24, 95 and 280 mg/kg bw for females. Body weight and food intake were recorded weekly. Haematological, clinical chemistry and urinary analyses were performed on randomly selected rats from all groups at weeks 6 and 13. At necropsy, the weights of the liver, kidneys, brain, heart, spleen, stomach, small intestine, caecum, adrenals, gonads, pituitary and thyroid were recorded, and gross and histopathological examinations were performed. No treatment-related effects on growth, clinical chemistry or urine parameters were observed. Haematology revealed a slight but significant leukocytopenia and anaemia in animals given 1200 or 3600 mg/kg for 6 weeks (leukocytopenia only in males, anaemia in males and females), but these effects were no longer present at week 13. A statistically significant but very small (< 10%) increase was observed in absolute and relative kidney weights in animals at 1200 and 3600 mg/kg. Other organ weights were not affected, and no gross abnormalities were seen at necropsy or on the histological examination of any organ, including the kidneys. Although the NOEL for methyl *N*-methylanthranilate in this study was 20 mg/kg bw per day, the toxicological significance of the effects observed at higher doses is doubtful (Gaunt et al., 1970).

(c) Long-term studies of toxicity and carcinogenicity

No long-term studies of toxicity and carcinogenicity were available for any of the substances in this group of flavouring agents; however, studies of carcinogenicity in mice and rats were available for a related substance, anthranilic acid (National Cancer Institute, 1978). These studies were considered by the Committee at its twenty-third meeting (Annex 1, references 50 and 51) in the safety evaluation of methyl anthranilate. In these studies, groups of 35 male and 35 female $B6C3F_1$ mice were given diets containing anthranilic acid at a concentration of 25 000 or 50 000 mg/kg, calculated (Food & Drug Administration, 1993) to provide average daily intakes of 3750 and 7500 mg/kg bw, respectively, 5 days per week for 78 weeks. The animals were then observed for an additional 26–27 weeks, during which they received a basal diet. A control group of 15 male and 15 female mice received the basal diet for 106 weeks. With the same study protocol, groups of 35 male and 35 female Fischer 344 rats received diets containing anthranilic acid at a concentration of 15 000 or 30 000 mg/kg, calculated (Food & Drug Administration, 1993) to provide average daily intakes of 750 and 1500 mg/kg bw, respectively. The Committee concluded that, under the conditions of the study, anthranilic acid was not carcinogenic to either mice or rats.

(d) Genotoxicity

Ten of the 19 flavouring agents in this group (Nos 1534–1537, 1540, 1541, 1543, 1545, 1549, 1552) have been tested for genotoxicity. The results of these tests are summarized in Table 5 and described below.

In vitro

No evidence of reverse mutation was observed in standard or modified (pre-incubation method) Ames assays when methyl anthranilate (No. 1534; up to

Table 5. Results of studies of genotoxicity with anthranilate derivatives

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
In vitro						
1534	Methyl anthranilate	Reverse mutation	*S. typhimurium* TA98, TA100	0.05–500 µg/plate	Negative[a]	Kasamaki et al. (1982)
1534	Methyl anthranilate	Reverse mutation	*S. typhimurium* TA97, TA102	10–1000 µg/plate	Negative[a]	Fujita & Sasaki (1987)
1534	Methyl anthranilate	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33–1800 µg/plate	Negative[a,b]	Mortelmans et al. (1986)
1534	Methyl anthranilate	Mutation	*E. coli* WP2 uvrA	250–2000 µg/plate	Negative	Yoo (1986)
1534	Methyl anthranilate	DNA repair	*Bacillus subtilis* H17 and M45	23 µg/disc	Negative	Oda et al. (1979)
1534	Methyl anthranilate	DNA repair	*Bacillus subtilis* H17 and M45	20 µl/disc (23 300 µg/disc)[c]	Weakly positive	Yoo (1986)
1534	Methyl anthranilate	Chromosomal aberration	Chinese hamster cell line B241	50 nmol/l (0.008 µg/ml)[d]	Positive[a]	Kasamaki et al. (1982)
1534	Methyl anthranilate	Unscheduled DNA synthesis	Rat hepatocytes	10–6–10–3 mol/l (0.15–151 µg/ml)d[d]	Negative	Yoshimi et al. (1988)
1535	Ethyl anthranilate	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	10–500 µg/plate –S9; 10–800 µg/plate +S9	Negative[a,e]	Mortelmans et al. (1986)
1536	Butyl anthranilate	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	1–50 µg/plate –S9; 3.3–280 µg/plate +S9	Negative[a,f]	Zeiger et al. (1987)
1537	Isobutyl anthranilate	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	1–67 µg/plate –S9; 3.3–333 µg/plate +S9	Negative[a,e]	Mortelmans et al. (1986
1540	Linalyl anthranilate	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	1–100 µg/plate –S9; 10–666 µg/plate +S9	Negative[a,g]	Zeiger et al. (1987)
1541	Cyclohexyl anthranilate	Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535	0.3–20 µg/plate –S9; 0.3–67 µg/plate +S9	Negative[a,h]	Zeiger et al. (1992)
1543	Phenylethyl anthranilate	Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535	0.1–10 µg/plate –S9; 1–100 µg/plate +S9	Negative[a,i]	Zeiger et al. (1988)
1545	Methyl *N*-methyl-anthranilate	Reverse mutation	*S. typhimurium* TA98, TA100	3–5000 µg/plate	Negative[a,j]	Verspeek-Rip (2003)
1545	Methyl *N*-methyl-anthranilate	Reverse mutation	*S. typhimurium* TA102, TA1535, TA1537	10–3330 µg/plate	Negative[a,k]	Verspeek-Rip (2003)

Table 5 (contd)

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
1545	Methyl *N*-methyl-anthranilate	Reverse mutation	*S. typhimurium* TA102, TA1535, TA1537	3–1000 µg/plate	Negative[a,l]	Verspeek-Rip (2003)
1545	Methyl *N*-methyl-anthranilate	Unscheduled DNA synthesis	Rat hepatocytes (0.16–165 µg/ml)[m]	10–6–10–3 mol/l	Negative	Yoshimi et al. (1988)
1549	Methyl *N*-formyl-anthranilate	Reverse mutation	*S. typhimurium* TA98, TA100, TA1538, TA1535, TA1537	100–10 000 µg/plate	Negative[a,n]	Blowers (1987)
1552	*N*-Benzoylanthranilic acid	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	15–5000 µg/plate	Negative[a,o]	King (2004)

S9, 9000 x *g* supernatant of rat liver homogenate

[a] Without and with metabolic activation (–/+S9)

[b] Cytotoxicity observed at highest dose (–/+S9) in all strains tested and at 1000 µg/plate –S9 in strains TA100 and TA1535

[c] Calculated from a density of methyl anthranilate of 1.165 (1.161–1.169) g/ml (Lewis, 1999)

[d] Calculated from a relative molecular mass of methyl anthranilate = 151.17

[e] Cytotoxicity observed at highest dose (–/+S9) in all strains tested

[f] Cytotoxicity observed at highest dose (–/+S9) in all strains tested and at next highest dose (i.e. 33 µg/plate –S9 and 100 µg/plate +S9) in strain TA100

[g] Cytotoxicity observed at highest dose (–/+S9) in all strains tested and at 333 µg/plate +S9 in all strains tested

[h] Cytotoxicity observed at highest dose (–/+S9) in all strains tested and at 33 µg/plate +S9 in strain TA1535

[i] Cytotoxicity observed at 100 µg/plate +S9 in TA97, TA100 and TA1535 and at 33 µg/plate +S9 in strain TA97

[j] Test carried out with both direct plate assay and pre-incubation assay. In the direct plate assay, cytotoxicity observed at 3330 and 5000 µg/plate (–/+S9) in both strains tested. In the pre-incubation assay, cytotoxicity observed at 333–5000 µg/plate (–/+S9) in both strains tested, except in strain TA98 at 333 µg/plate +S9.

[k] Test carried out with direct plate assay; cytotoxicity observed at 3330 µg/plate (–/+S9) in all strains tested

[l] Test carried out with pre-incubation assay; cytotoxicity observed at 333 and 1000 µg/plate (–/+S9) in all strains tested, except in strain TA102 at 333 µg/plate +S9

[m] Calculated from a relative molecular mass of methyl *N*-methylanthranilate = 165.19

[n] Cytotoxicity observed at highest dose (–/+S9) in strains TA98 and TA1535

[o] Without metabolic activation, cytotoxicity observed at 1500 and 5000 µg/plate in strains TA98, TA100 and TA1537 and at 5000 µg/plate in strains TA102 and TA1535. With metabolic activation, cytotoxicity observed at 1500 and 5000 µg/plate in strains TA98, TA100, TA1535 and TA1537 and at 5000 µg/plate in strain TA102

1800 μg/plate), ethyl anthranilate (No. 1535; up to 800 μg/plate), butyl anthranilate (No. 1536; up to 280 μg/plate), isobutyl anthranilate (No. 1537; up to 333 μg/plate), linalyl anthranilate (No. 1540; up to 666 μg/plate), cyclohexyl anthranilate (No. 1541; up to 67 μg/plate), phenylethyl anthranilate (No. 1543; up to 100 μg/plate), methyl *N*-methylanthranilate (No. 1545; up to 5000 μg/plate), methyl *N*-formylanthranilate (No. 1549; up to 10 000 μg/plate) or *N*-benzoylanthranilic acid (No. 1552; up to 5000 μg/plate) was incubated with *Salmonella typhimurium* strain TA97, TA98, TA100, TA102, TA1535, TA1537 or TA1538, with and without metabolic activation (Kasamaki et al., 1982; Mortelmans et al., 1986; Blowers, 1987; Fujita & Sasaki, 1987; Zeiger et al., 1987, 1988, 1992; Verspeek-Rip, 2003; King, 2004). Negative results were reported in a test for mutation in which up to 2000 μg/plate of methyl anthranilate were incubated with *Escherichia coli* WP2 uvrA (Yoo, 1986).

Rec assays for DNA repair in *Bacillus subtilis* strains M45 and H17 were performed with methyl anthranilate (No. 1534). In one study, methyl anthranilate gave a negative result at a concentration of 23 μg/disc (Oda et al., 1979). In another study, methyl anthranilate gave a weakly positive result (Yoo, 1986), but only at a 1000 times higher concentration (23 300 μg/disc).

In a non-standard assay designed to maximize the frequency of chromosomal aberrations in a Chinese hamster B241 cell line, methyl anthranilate at a concentration of 0.008 μg/ml gave a positive result with and without metabolic activation (Kasamaki et al., 1982).

Methyl anthranilate (No. 1534; up to 151 μg/ml) and methyl *N*-methylanthranilate (No. 1545; up to 165 μg/ml) did not induce unscheduled DNA synthesis in rat hepatocytes (Yoshimi et al., 1988).

Conclusion

Ten substances in this group of flavouring agents have been tested and found not to induce forward mutation in bacteria in vitro. In addition, methyl anthranilate gave negative results in an assay for mutagenicity in *E. coli*. There was some evidence of DNA damage caused by methyl anthranilate in a rec assay with *B. subtilis*, but only at very high concentrations; a rec assay performed with lower concentrations of methyl anthranilate gave negative results.

In mammalian cell systems, methyl anthranilate showed evidence of clastogenicity in a non-standard assay for chromosomal aberrations, but gave negative results in an assay for unscheduled DNA synthesis. Methyl *N*-methylanthranilate also gave negative results when tested for unscheduled DNA synthesis.

On the basis of the results of available studies of genotoxicity, albeit limited in the number of end-points investigated, the Committee concluded that the flavouring agents in this group of anthranilate derivatives present no significant genotoxic potential.

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