FURAN-SUBSTITUTED ALIPHATIC HYDROCARBONS, ALCOHOLS, ALDEHYDES, KETONES, CARBOXYLIC ACIDS, AND RELATED ESTERS, SULFIDES, DISULFIDES AND ETHERS

First draft prepared by

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1. EVALUATION

The Committee took note of the extensive evidence for the genotoxicity of several members of this group of flavouring agents related to furan, including the clastogenicity of 2-furyl methyl ketone (No. 1503) in mouse bone marrow. Furan, which is carcinogenic, is known to undergo epoxidation and ring opening to form a reactive 2-ene-1,4-dicarbonyl intermediate. Accordingly, there is concern that the observed genotoxicity might be due to formation of a reactive metabolite. No data were available on the potential of members of this group to form reactive metabolites, and no role of metabolism has been identified in the observed genotoxicity. Moreover, there were few data on genotoxicity in vivo, and specific in-vivo assays to address potential carcinogenicity were lacking.

The Committee concluded that the Procedure could not be applied to this group, because of the above concerns. Studies that would assist in resolving the concerns include studies on metabolism and assays for DNA reactivity, mutagenicity and carcinogenic potential in vivo of members of this group with representative structures.

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

This monograph summarizes the key data relevant to the safety evaluation of 40 flavouring agents (see Table 1), comprising aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing a furan substitution. The group of flavouring agents comprises: 20 alkyl-substituted furans (Nos 1487–1496) and furyl ketone derivatives (Nos 1497, 1499, 1503–1512 and 1514–1517), 13 furyl-subtituted aldehydes (Nos 1497–1499) and related carboxylic acid esters (Nos 1513–1516), three furyl-substituted ethers (Nos 1520–1522) and four furyl-substituted compounds: two sulfides (Nos 1523 and 1525), one disulfide (No. 1524) and a thioester (No. 1526).

2.2 Intake

The total annual production volume of the 40 aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution is approximately 462 kg in Europe (International Organization of the Flavor Industry, 1995) and 457 kg in the USA (National Academy of Sciences, 1970, 1982, 1987; Lucas et al., 1999). Approximately 83% of the total annual volume in Europe and approximately 60% of that in the USA is accounted for by two substances in the group, 2-furyl methyl ketone (No. 1503) and isobutyl 3-(2-furan)propionate (No. 1514). The reported annual volumes of the other substance in this group do not exceed 50 kg. Production volumes and intake values for each substance are reported in Table 2.

Although aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution are commonly found in a variety of roasted, smoked, cooked and fermented foods, including cocoa, coffee, tea, beer, rum, roasted meats (beef, chicken, pork), and smoked fish, they also occur in a variety of fruits, vegetables, nuts and grains (e.g. peaches, berries, calamus, asparagus, cabbage, capsicums, mint, parsley, walnuts, hazelnuts and wheaten bread) (Nijssen et al., 2004). As shown in Table 2, 22 of the substances in this group have been reported to occur naturally in foods (Nijssen et al., 2004). Quantitative data on natural occurrence and consumption ratios have been reported for seven substances in the group and show that intake occurs predominantly via consumption of traditional foods (i.e. consumption ratio, > 1) (Stofberg & Kirschman, 1985; Stofberg & Grundschober, 1987) (see Table 2).

2.3 Biological data

2.3.1 Biochemical data

(a) Hydrolysis

In general, esters are expected to be hydrolysed by carboxylesterases to the corresponding alcohol and the corresponding carboxylic acid (see Figure 1), and aliphatic esters containing furyl substitution, like other aliphatic esters, undergo such hydrolysis. Furoate esters are hydrolysed to 2-furoic acid and the corresponding alcohol. Of the carboxylesterases, the most important are the A-esterases, which,

Table 1. Aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution

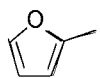
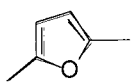
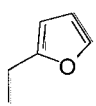
Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class II						
2-Methylfuran	1487	534-22-5 	No Europe: 0.2 USA: 0.3	Yes. The NOEL of 25.6 mg/kg bw per day for the related substance 2-pentylfuran (Shellenberger, 1971a,b) is 5 120 000 times the estimated daily intake of 2-methylfuran when used as a flavouring agent.	See notes 1 and 2	No safety concern
2,5-Dimethylfuran	1488	625-86-5 	No Europe: 0.01 USA: 0.02	Yes. The NOEL of 25.6 mg/kg bw per day for the related substance 2-pentylfuran (Shellenberger, 1971a,b) is 5 120 000 times the estimated daily intake of 2,5-dimethylfuran when used as a flavouring agent.	See note 2	No safety concern
2-Ethylfuran	1489	3208-16-0 	No Europe: 0.07 USA: 0.5	Yes. The NOEL of 25.6 mg/kg bw per day for the related substance 2-pentylfuran (Shellenberger, 1971a,b) is 5 120 000 times the estimated daily intake of 2-ethylfuran when used as a flavouring agent.	See note 2	No safety concern

Table 1 (contd)

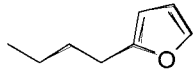
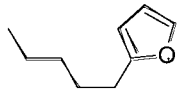
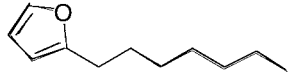
Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
2-Butylfuran	1490	4466-24-4 	No Europe: 0.3 USA: 0.4	Yes. The NOEL of 25.6 mg/kg bw per day for the related substance 2-pentylfuran (Shellenberger, 1971a,b) is 5 120 000 times the estimated daily intake of 2-buthylfuran when used as a flavouring agent.	See note 2	No safety concern
2-Pentylfuran	1491	3777-69-3 	No Europe: 0.2 USA: 0.03	Yes. The NOEL of 25.6 mg/kg bw per day (Shellenberger, 1971a,b) is > 6 400 000 times the estimated daily intake of 2-pentylfuran when used as a flavouring agent.	See note 2	No safety concern
2-Heptylfuran	1492	3777-71-7 	No Europe: 0.01 USA: 0.9	Yes. The NOEL of 25.6 mg/kg bw per day for the related substance 2-pentylfuran (Shellenberger, 1971a,b) is > 1 280 000 times the estimated daily intake of 2-heptylfuran when used as a flavouring agent.	See note 2	No safety concern

Table 1 (contd)

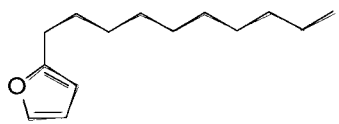
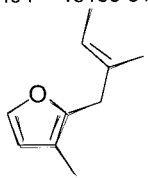
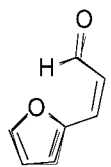
Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
2-Decylfuran	1493	83469-85-6 	No Europe: 0.001 USA: 0.002	Yes. The NOEL of 25.6 mg/kg bw per day for the related substance 2-pentylfuran (Shellenberger, 1971a,b) is > 853 333 300 times the estimated daily intake of 2-decylfuran when used as a flavouring agent.	See note 2	No safety concern
3-Methyl-2-(3-methyl-but-2-enyl) furan	1494	15186-51-3 	No Europe: 0.1 USA: 0.2	Yes. The NOEL of 45 mg/kg bw per day for the related substance 3-(2-furyl)acrolein (Lough et al., 1985) is > 15 000 000 times the estimated daily intake of 2-methyl-2-(3-methylbut-2-enyl)-furan when used as a flavouring agent.	See note 2	No safety concern
3-(2-Furyl)acrolein	1497	623-30-3 	No Europe: 0.04 USA: 0.4	Yes. The NOEL of 45 mg/kg bw per day (Lough et al., 1985) is > 6 428 000 times the estimated daily intake of 3-(2-furyl)acrolein when used as a flavouring agent.	See note 4	No safety concern

Table 1 (contd)

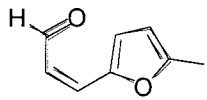
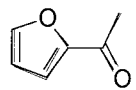
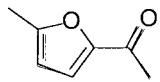
Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
3-(5-Methyl-2-furyl)-prop-2-enal	1499	5555-90-8 	No Europe: 0.001 USA: 0.002	Yes. The NOEL of 45 mg/kg bw per day for the related substance 3-(2-furyl)acrolein (Lough et al., 1985) is > 1 500 00 000 times the estimated daily intake of 3-(5-methyl-2-furyl)prop-2-enal when used as a flavouring agent.	See note 4	No safety concern
2-Furyl methyl ketone	1503	1192-62-7 	No Europe: 54 USA: 13	Yes. The NOEL of 25 mg/kg bw per day (Lough et al., 1985) is > 27 770 times the estimated daily intake of 2-furyl methyl ketone when used as a flavouring agent.	See note 3	No safety concern
2-Acetyl-5-methylfuran	1504	1193-79-9 	No Europe: 0.4 USA: 0.1	Yes. The NOEL of 10 mg/kg bw per day for the related substance 3-acetyl-2.5-dimethylfuran (Van Miller & Weaver, 1987) is > 1 428 000 times the estimated daily intake of 2-acetyl-5-methyl-furan when used as a flavouring agent.	See note 3	No safety concern

Table 1 (contd)

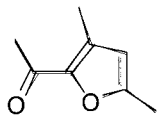
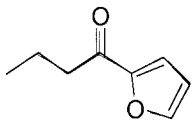
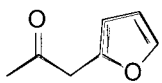
Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
2-Acetyl-3,5-dimethylfuran	1505	22940-86-9 	No Europe: 0.001 USA: 0.002	Yes. The NOEL of 10 mg/kg bw per day for the related substance 3-acetyl-2.5-dimethylfuran (Van Miller & Weaver, 1987) is > 333 333 300 times the estimated daily intake of 2-acetyl-3,5-dimethylfuran when used as a flavouring agent.	See note 3	No safety concern
2-Butyrylfuran	1507	4208-57-5 	No Europe: 0.1 USA: 0.2	Yes. The NOEL of 25 mg/kg bw per day for the related substance 2-furyl methyl ketone (Lough et al., 1985) is > 8 333 300 times the estimated daily intake of 2-butyryl-furan when used as a flavouring agent.	See note 3	No safety concern
(2-Furyl)-2-propanone	1508	6975-60-6 	No Europe: 0.04 USA: 0.02	Yes. The NOEL of 25 mg/kg bw per day for the related substance 2-furyl methyl ketone (Lough et al., 1985) is > 35 700 000 times the estimated daily intake of (2-furyl)-2-propanone when used as a flavouring agent.	See note 3	No safety concern

Table 1 (contd)

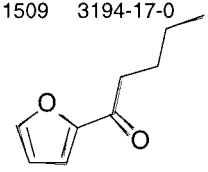
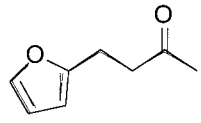
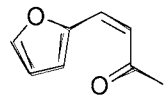
Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
2-Pentanoylfuran	1509	3194-17-0 	No Europe: 0.07 USA: 0.09	Yes. The NOEL of 25 mg/kg bw per day for the related substance 2-furyl methyl ketone (Lough et al., 1985) is 25 000 000 times the estimated daily intake of 2-pentanoylfuran when used as a flavouring agent.	See note 3	No safety concern
1-(2-Furyl)butan-3-one	1510	699-17-2 	No Europe: 3 USA: 3	Yes. The NOEL of 25 mg/kg bw per day for the related substance 2-furyl methyl ketone (Lough et al., 1985) is 500 000 times the estimated daily intake of 1-(2-furyl)butan-3-one when used as a flavouring agent.	See note 3	No safety concern
4-(2-Furyl)-3-buten-2-one	1511	623-15-4 	No Europe: 2 USA: 1	Yes. The NOEL of 30 mg/kg bw per day (Gill & Van Miller, 1987) is > 1 000 000 times the estimated daily intake of 4-(2-furyl)-3-buten-2-one when used as a flavouring agent.	See note 3	No safety concern

Table 1 (contd)

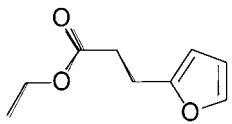
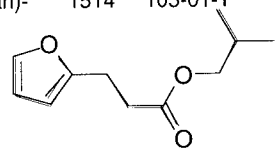
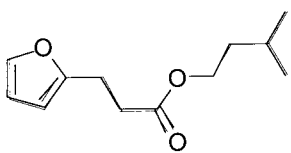
Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Ethyl 3-(2-furyl)-propanoate	1513	10031-90-0 	No Europe: 0.01 USA: 0.07	Yes. The NOEL of 875 mg/kg bw per day for the related substance isobutyl 3-(2-furan)propionate (Lough et al., 1985) is > 875 000 000 times the estimated daily intake of ethyl 3-(2-furyl)-propanoate when used as a flavouring agent.	See note 5	No safety concern
Isobutyl 3-(2-furan)-propionate	1514	105-01-1 	No Europe: 0.1 USA: 24	Yes. The NOEL of 875 mg/kg bw per day (Lough et al., 1985) is > 2 187 500 times the estimated daily intake of isobutyl 3-(2-furan)-propionate when used as a flavouring agent.	See note 5	No safety concern
Isoamyl 3-(2-furan)-propionate	1515	7779-67-1 	No Europe: NR USA: 0.09	Yes. The NOEL of 875 mg/kg bw per day for the related substance isobutyl 3-(2-furan)propionate (Lough et al., 1985) is 437 500 000 times the estimated daily intake of isoamyl 3-(2-furan)propionate when used as a flavouring agent.	See note 5	No safety concern

Table 1 (contd)

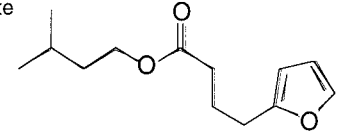
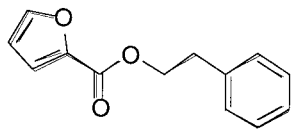
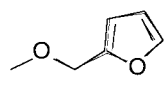
Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Isoamyl 4-(2-furan)-butyrate 	1516	7779-66-0	No Europe: NR USA: 0.09	Yes. The NOEL of 875 mg/kg bw per day for the related substance isobutyl 3-(2-furan)propionate (Lough et al., 1985) is 437 500 000 times the estimated daily intake of isoamyl 4-(2-furan)butyrate when used as a flavouring agent.	See note 5	No safety concern
Phenethyl 2-furoate 	1517	7149-32-8	No Europe: NR USA: 0.2	Yes. The NOEL of 50 mg/kg bw per day for the related substance furfural (National Toxicology Program, 1990) is 16 666 600 times the estimated daily intake of phenethyl 2-furoate when used as a flavouring agent.	See note 5	No safety concern
Furfuryl methyl ether 	1520	13679-46-4	No Europe: NR USA: 0.09	Yes. The NOEL of 27 mg/kg bw per day (Van Miller & Weaver, 1987) is 27 000 000 times the estimated daily intake of furfuryl methyl ether when used as a flavouring agent.	See note 6	No safety concern

Table 1 (contd)

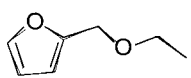
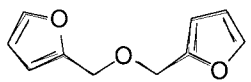
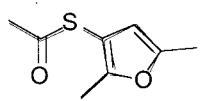
Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Ethyl furfuryl ether	1521	6270-56-0 	No Europe: 0.001 USA: 0.002	Yes. The NOEL of 27 mg/kg bw per day for the related substance furfuryl methyl ether (Van Miller & Weaver, 1987) is 900 000 000 times the estimated daily intake of ethyl furfuryl ether when used as a flavouring agent.	See note 6	No safety concern
Difurfuryl ether	1522	4437-22-3 	No Europe: NR USA: 0.09	Yes. The NOEL of 27 mg/kg bw per day for the related substance furfuryl methyl ether (Van Miller & Weaver, 1987) is 13 500 000 times the estimated daily intake of difurfuryl ether when used as a flavouring agent.	See note 6	No safety concern
2,5-Dimethyl-3-furanthiol acetate	1523	55764-22-2 	No Europe: 4 USA: 4	Yes. The NOEL of 0.73 mg/kg bw per day for the related substance 2,5-dimethyl-3-furan thioisovalerate (Morgareidge & Oser, 1974) is > 10 400 times the estimated daily intake of 2,5-dimethyl-3-furanthiol acetate when used as a flavouring agent.	See note 7	No safety concern

Table 1 (contd)

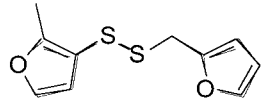
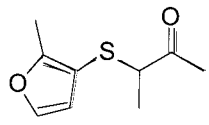
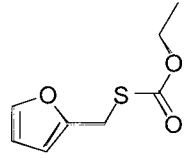
Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Furfuryl 2-methyl-3-furyl disulfide	1524	109537-55-5 	No Europe: NR USA: 0.2	Yes. The NOEL of 5 mg/kg bw per day for the related substance 2-methyl-3-furanthiol (Oser, 1970) is 1 666 600 times the estimated daily intake of furfuryl 2-methyl-3-furyl disulfide when used as a flavouring agent.	See note 8	No safety concern
3-[(2-Methyl-3-furyl)-thio]-2-butanone	1525	61295-44-1 	No Europe: 0.01 USA: 0.02	Yes. The NOEL of 5 mg/kg bw per day for the related substance 2-methyl-3-furanthiol (Oser, 1970) is > 16 666 600 times the estimated daily intake of 3-[(2-methyl-3-furyl)thio]-2-butanone when used as a flavouring agent.	See note 7	No safety concern
O-Ethyl *S*-(2-furyl-methyl)thiocarbonate	1526	376595-42-5 	No Europe: 0.7 USA: 0.9	Yes. The NOEL of 8 mg/kg bw per day (van Otterdijk & Frieling, 2001) is > 533 300 times the estimated daily intake of *O*-ethyl *S*-(2-furylmethyl)thiocarbonate when used as a flavouring agent.	See note 7	No safety concern

Table 1 (contd)

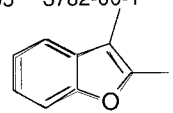
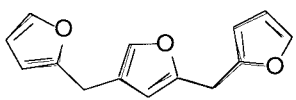
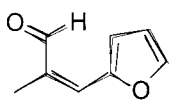
Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class III						
2,3-Dimethylbenzofuran	1495	3782-00-1 	No Europe: 0.6 USA: 0.01	Yes. The NOEL of 0.6 mg/kg bw per day (Long, 1997a) is 60 000 times the estimated daily intake of 2,3-dimethylbenzofuran when used as a flavouring agent.	See note 2	No safety concern
2,4-Difurfurylfuran	1496	64280-32-6 	No Europe: 0.001 USA: 0.002	Yes. The NOEL of 0.6 mg/kg bw per day for the related substance 2,3-dimethylbenzofuran (Long, 1997a) is > 20 000 000 times the estimated daily intake of 2,4-difurfurylfuran when used as a flavouring agent.	See note 2	No safety concern
2-Methyl-3(2-furyl)-acrolein	1498	874-66-8 	No Europe: 0.3 USA: 6	Yes. The NOEL of 45 mg/kg bw per day for the related substance 3-(2-furyl)acrolein (Lough et al., 1985) is > 450 000 times the estimated daily intake of 2-methyl-3(2-furyl)acrolein when used as a flavouring agent.	See note 4	No safety concern

Table 1 (contd)

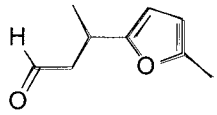
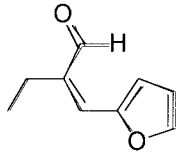
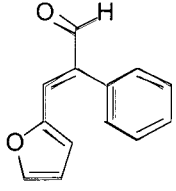
Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
3-(5-Methyl-2-furyl)-butanal	1500	31704-80-0 	No Europe: 0.001 USA: 0.5	Yes. The NOEL of 45 mg/kg bw per day for the related substance 3-(2-furyl)acrolein (Lough et al., 1985) is > 5 000 000 times the estimated daily intake of 3-(5-methyl-2-furyl)butanal when used as a flavouring agent.	See note 4	No safety concern
2-Furfurylidene-butyraldehyde	1501	770-27-4 	No Europe: NR USA: 0.007	Yes. The NOEL of 45 mg/kg bw per day for the related substance 3-(2-furyl)acrolein (Lough et al., 1985) is 450 000 000 times the estimated daily intake of 2-furfurylidene-butyraldehyde when used as a flavouring agent.	See note 4	No safety concern
2-Phenyl-3-(2-furyl)-prop-2-enal	1502	65545-81-5 	No Europe: NR USA: 0.007	Yes. The NOEL of 45 mg/kg bw per day for the related substance 3-(2-furyl)acrolein (Lough et al., 1985) is 450 000 000 times the estimated daily intake of 2-phenyl-3-(2-furyl)prop-2-enal when used as a flavouring agent.	See note 4	No safety concern

Table 1 (contd)

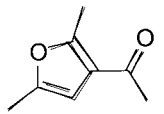
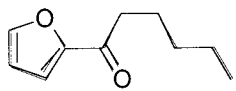
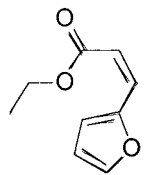
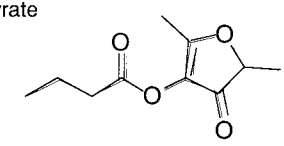
Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
3-Acetyl-2,5-dimethylfuran	1506	10599-70-9 	No Europe: NR USA: 2	Yes. The NOEL of 10 mg/kg bw per day (Van Miller & Weaver, 1987) is > 333 300 times the estimated daily intake of 3-acetyl-2,5-dimethylfuran when used as a flavouring agent.	See note 3	No safety concern
Pentyl 2-furyl ketone	1512	14360-50-0 	No Europe: NR USA: 0.9	Yes. The NOEL of 25 mg/kg bw per day for the related substance 2-furyl methyl ketone (Lough et al., 1985) is 1 250 000 times the estimated daily intake of pentyl 2-furyl ketone when used as a flavouring agent.	See note 3	No safety concern
Propyl 2-furanacrylate	1518	623-22-3 	No Europe: NR USA: 1	Yes. The NOEL of 45 mg/kg bw per day for the related substance 3-(2-furyl)acrolein (Lough et al., 1985) is 2 250 000 times the estimated daily intake of propyl 2-furanacrylate when used as a flavouring agent.	See note 5	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
2,5-Dimethyl-3-oxo-(2*H*)-fur-4-yl butyrate	1519	114099-96-6 	No Europe: NR USA: 4	Yes. The NOEL of 200 mg/kg bw per day for the related substance 2,5-dimethyl-4-hydroxyl-3-(2H)-furanone (Kelly & Bolte, 2003) is > 2 857 100 times the estimated daily intake of 2,5-dimethyl-3-oxo-(2*H*)-fur-4-yl butyrate when used as a flavouring agent.	See note 5	No safety concern

CAS, Chemical Abstracts Service; NR, no data on intake reported

Step 2: None of the agents in this group can be predicted to be metabolized to innocuous products.

[a] The thresholds for human intake for structural classes II and III are 540 and 90 μg/day, respectively. All intake values are expressed in μg/day. The combined per capita intake of flavouring agents in structural class II is 65 μg/day in Europe and 50 μg/day in the USA. The combined per capita intake of flavouring agents in structural class III is 0.9 μg/day in Europe and 14 μg/day in the USA.

Notes:

1. Methylfuran can undergo oxidative ring opening to form acetylacrolein, which forms glutathione conjugates that are eliminated in the urine.
2. Alkyl-substituted furans are oxidized, conjugated with glucuronic acid and eliminated in the urine.
3. Carbonyl-substituted furans are expected to be reduced to the corresponding alcohols, conjugated and eliminated in the urine.
4. Furylpropanal and furylpropenal derivatives are expected to be oxidized to furylpropanoic acid and furylpropenoic acid derivatives, which are subsequently expected to form glycine conjugates and be eliminated in the urine.
5. Esters are expected to undergo hydrolysis to yield furylpropanoic acid and furylpropenoic acid derivatives, which are expected to form conjugates that are eliminated in the urine.
6. Ethers are anticipated to undergo hydrolysis and oxidation rapidly to furoic acid, which subsequently forms glycine

Table 2. Annual volumes of production of aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution 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		
2-Methylfuran (No. 1487)					
Europe[e]	1.7	0.2	0.004		
USA[e]	1.7	0.3	0.005	+	NA
2,5-Dimethylfuran (No. 1488)					
Europe[e]	0.1	0.01	0.0002		
USA[e]	0.1	0.02	0.0003	+	NA
2-Ethylfuran (No. 1489)					
Europe	0.5	0.07	0.001		
USA	4	0.5	0.009	883	221
2-Butylfuran (No. 1490)					
Europe[e]	2	0.3	0.005		
USA[e]	2	0.4	0.006	+	NA
2-Pentylfuran (No. 1491)					
Europe	1.5	0.2	0.004		
USA	0.2	0.03	0.0004	1370	6850
2-Heptylfuran (No. 1492)					
Europe	0.1	0.01	0.0002		
USA[e]	5	0.9	0.02	12	2
2-Decylfuran (No. 1493)					
Europe[e]	0.01	0.001	0.00002		
USA[e]	0.01	0.002	0.00003	+	NA
3-Methyl-2-(3-methylbut-2-enyl)furan (No. 1494)					
Europe[e]	1	0.1	0.002		
USA[e]	1	0.2	0.003	+	NA
2,3-Dimethylbenzofuran (No. 1495)					
Europe	4.3	0.6	0.01		
USA	0.1	0.01	0.0002	–	NA
2,4-Difurfurylfuran (No. 1496)					
Europe[e]	0.01	0.001	0.00002		
USA[e]	0.01	0.002	0.00003	–	NA
3-(2-Furyl)acrolein (No. 1497)					
Europe	0.3	0.04	0.0007		
USA	3	0.4	0.007	+	NA

Table 2 (contd)

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		
2-Methyl-3-(2-furyl)acrolein (No. 1498)					
Europe	2	0.3	0.005		
USA	45	6	0.10	–	NA
3-(5-Methyl-2-furyl)prop-2-enal (No. 1499)					
Europe[e]	0.01	0.001	0.00002		
USA[e]	0.01	0.002	0.00003	+	NA
3-(5-Methyl-2-furyl)-butanal (No. 1500)					
Europe	0.01	0.001	0.00002		
USA	4	0.5	0.009	–	NA
2-Furfurylidenebutyraldehyde (No. 1501)					
Europe	NR	NR	NR		
USA	0.05	0.007	0.0001	–	NA
2-Phenyl-3-(2-furyl)prop-2-enal (No. 1502)					
Europe	NR	NR	NR		
USA	0.05	0.007	0.0001	–	NA
2-Furyl methyl ketone (No. 1503)					
Europe	381	54	0.9		
USA	95	13	0.2	26 876	283
2-Acetyl-5-methylfuran (No. 1504)					
Europe	3	0.4	0.007		
USA	0.9	0.1	0.002	108	120
2-Acetyl-3,5-dimethylfuran (No. 1505)					
Europe[e]	0.01	0.001	0.00002		
USA[e]	0.01	0.002	0.00003	+	NA
3-Acetyl-2,5-dimethylfuran (No. 1506)					
Europe	NR	NR	NR		
USA	12	2	0.03	–	NA
2-Butyrylfuran (No. 1507)					
Europe[e]	1	0.1	0.002		
USA[e]	1	0.2	0.003	+	NA
(2-Furyl)-2-propanone (No. 1508)					
Europe	0.3	0.04	0.0007		
USA[f]	0.09	0.02	0.0003	+	NA
2-Pentanoylfuran (No. 1509)					
Europe[e]	0.5	0.07	0.001		
USA[e]	0.5	0.09	0.001	+	NA

Table 2 (contd)

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		
1-(2-Furyl)butan-3-one (No. 1510)					
Europe[e]	18	3	0.04		
USA[e]	18	3	0.05	+	NA
4-(2-Furyl)-3-buten-2-one (No. 1511)					
Europe	13	2	0.03		
USA	8	1	0.02	+	NA
Pentyl 2-furyl ketone (No. 1512)					
Europe	NR	NR	NR		
USA[e]	5	0.9	0.02	–	NA
Ethyl 3-(2-furyl)propanoate (No. 1513)					
Europe	0.1	0.01	0.0002		
USA	0.5	0.07	0.001	+	NA
Isobutyl 3-(2-furan)propionate (No. 1514)					
Europe	1	0.1	0.002		
USA	181	24	0.4	–	NA
Isoamyl 3-(2-furan)propionate (No. 1515)					
Europe	NR	NR	NR		
USA[f]	0.5	0.09	0.002	–	NA
Isoamyl 4-(2-furan)butyrate (No. 1516)					
Europe	NR	NR	NR		
USA[f]	0.5	0.09	0.002	–	NA
Phenethyl 2-furoate (No. 1517)					
Europe	NR	NR	NR		
USA[f]	1	0.2	0.003	–	NA
Propyl 2-furanacrylate (No. 1518)					
Europe	NR	NR	NR		
USA	10	1	0.02	–	NA
2,5-Dimethyl-3-oxo-(2*H*)-fur-4-yl butyrate (No. 1519)					
Europe	NR	NR	NR		
USA[e]	25	4	0.07	–	NA
Furfuryl methyl ether (No. 1520)					
Europe	NR	NR	NR		
USA[e]	0.5	0.09	0.001	3083	6,166
Ethyl furfuryl ether (No. 1521)					
Europe[e]	0.01	0.001	0.00002		
USA[e]	0.01	0.002	0.00003	+	NA

Table 2 (contd)

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		
Difurfuryl ether (No. 1522)					
Europe	NR	NR	NR		
USA[f]	0.5	0.09	0.002	2008	4,016
2,5-Dimethyl-3-furanthiol acetate (No. 1523)					
Europe[e]	25	4	0.06		
USA[e]	25	4	0.07	–	NA
Furfuryl 2-methyl-3-furyl disulfide (No. 1524)					
Europe	NR	NR	NR		
USA[e]	1	0.2	0.003	–	NA
3-[(2-Methyl-3-furyl)thio]-2-butanone (No. 1525)					
Europe[e]	0.1	0.01	0.0002		
USA[e]	0.1	0.02	0.0003	–	NA
O-Ethyl *S*-(2-furylmethyl)thiocarbonate (No. 1526)					
Europe[e]	5	0.7	0.01		
USA[e]	5	0.9	0.01	–	NA
Total					
Europe	462				
USA	457				

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)

in mammals, occur in most tissues of the body but predominate in hepatocytes (Heymann, 1980).

Figure 1. General ester hydrolysis

Hydrolysis can occur in the gastrointestinal tract before absorption. A concentration of 27 µl/l isoamyl furylpropanoate (No. 1515) or 40 µl/l ethyl furylpropionate (No. 1513) was reported to be completely hydrolysed within 2 h by pancreatin (Grundschober, 1977). A report that the glycine conjugate of furoic acid (furoylglycine) was the major metabolite in the urine of rats given an oral dose of 20 mg of furfural diacetate, a structurally related substance, provided evidence that the acetal ester of furfural was hydrolysed to acetic acid and furfural, which, in turn, was oxidized to furoic acid (Paul et al., 1949). At the same dosage, furfuryl propionate (No. 740) was hydrolysed to propionic acid and furfuryl alcohol, which was subsequently oxidized to furoic acid, while the methyl ester of 3-furylacrylic acid was hydrolysed to methanol and furylacrylic acid, which was subsequently oxidized and cleaved to yield furoic acid. It is anticipated that furfuryl and furoate esters would be hydrolysed to the parent alcohol and acid, respectively. The parent alcohol, aldehyde, and the acid are expected to participate in common pathways of metabolism and excretion.

Hydrolysis of isoamyl 3-(2-furyl)propionate (No. 1515) was determined in the duodenal lumen of male Dunkin-Hartley guinea-pigs. No free ester was detected in portal blood samples 2 or 5 min after injection of 30, 50 or 70 ppm of isoamyl-3-(2-furyl)propionate in saline into the intestinal lumen. In a study of ester stability in vitro, > 98% of isoamyl 3-(2-furyl)propionate was hydrolysed within 1 min of incubation of guinea-pig blood at 37 °C, and no free ester was detected after 5 min (Pelling et al., 1980).

The half-lives, as indicated by loss of parent ester by hydrolysis of furfuryl acetate (No. 739), furfuryl propionate (No. 740), furfuryl butyrate (No. 759) and fufuryl isopentanoate (No. 743) when incubated in artificial pancreatic fluid containing pancreatin, were < 0.01, < 0.01, < 0.01 and 5.1 ± 0.4 min, respectively. When 50 µmol/l furfuryl propionate were incubated with 5% rat liver homogenate, the rate of hydrolysis was reported to be > 70 nmol/min per mg protein. Furfuryl propionate was also readily hydrolysed when incubated with 5% rat blood homogenate, at a rate of 49 nmol/min per mg protein. At a single time, rat blood showed more hydrolysis of furfuryl propionate than human blood ($t_{1/2}$ = 0.0112 ± 0.0034 min, k = 0.0668 ± 0.0230 nmol/min per mg protein versus $t_{1/2}$ = 18.7 ± 1.9 min, k = 0.0373 ± 0.0038 nmol/min per mg protein, respectively). When furfuryl propionate was incubated with rat intestinal mucosa, rapid hydrolysis was observed ($t_{1/2}$ = 0.0130 ± 0.0005 min, k = 62.04 ± 0.061 nmol/min per mg protein). Rat intestinal homogenate showed more hydrolytic activity than the other tissue homogenates used in this study (Buck, 2000).

Studies in vitro and in vivo thus show that the esters in this group of aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, and related esters,

sulfides, disulfides and ethers containing furan substitution are hydrolysed to the corresponding alcohols and carboxylic acids.

(b) Absorption, distribution and excretion

The furyl derivatives in this group are rapidly absorbed, metabolized and excreted from animals. Furfuryl alcohol and furfural are rapidly absorbed by rodents when administered by common routes, including orally (Nomeir et al., 1992), dermally and by inhalation (Castellino et al., 1963; National Institute for Occupational Safety and Health, 1979). After administration at doses ranging from 0.1 mg/kg bw to 200 mg/kg bw, furfuryl alcohol and furfural were absorbed from the gastrointestinal tract, metabolized and excreted, primarily in the urine (Rice, 1972; Nomeir et al., 1992; Parkash & Caldwell, 1994).

More than 86% of the radiolabel from 0.275, 2.75 or 27.5 mg/kg bw of ^{14}C-furfuryl alcohol or 0.127, 1.15 or 12.5 mg/kg bw of ^{14}C-furfural given to groups of four male Fischer 344 rats by gavage in corn oil was rapidly absorbed from the gastrointestinal tract, 83–88% of the radiolabel being excreted in the urine and 2–5% in the faeces. Most of the radiolabel was excreted within the first 24 h after dosing. About 7% of the dose of 12.5 mg/kg bw of furfural was exhaled as $^{14}CO_2$. By 72 h after administration, residual radioactivity was distributed primarily in the liver and kidney, the tissue radiolabel generally being proportional to the dose (Nomeir et al., 1992).

More than 90% of a single oral dose of 1, 10 or 60 mg/kg bw ^{14}C-furfural given to male and female Fischer 344 rats, or 1, 20 or 200 mg/kg bw ^{14}C-furfural given to male and female CD-1 mice was recovered within 72 h. The main route of elimination was the urine (> 76% in rats and > 61% in mice within 24 h). Elimination in faeces (1–6% within 72 h at all doses in rats and mice) and expired CO_2 (5% in male mice at the highest dose and 4% in female mice at the lowest dose after 24 h [no other CO_2 measurements taken]) constituted minor routes of excretion (Parkash & Caldwell, 1994).

A similar pattern of absorption, distribution and excretion was reported for alkyl-substituted furfural derivatives. Groups of male Fischer 344 rats and male B6C3F$_1$ mice were given 5, 10, 100 or 500 mg/kg bw of ^{14}C-5-hydroxymethyl-2-furfural intragastrically. In both species, 5-hydroxymethyl-2-furfural-derived radioactivity was rapidly cleared from all main tissues, with no evidence of accumulation. The tissue concentrations varied with dose in both species at most times. Within 48 h, 70–82% of the administered dose had been excreted in the urine of rats, while 8–12% was excreted in the faeces. In mice, 61–77% was excreted in the urine and 15–26% in the faeces within the same period (Godfrey et al., 1999).

Alkylfuran derivatives are also rapidly taken up, metabolized and excreted. Male Sprague-Dawley rats that received an intraperitoneal injection of 100 mg/kg bw of 2-^{14}C-methylfuran in sesame oil had radiolabelled 2-methylfuran metabolites in 12-h urine samples. Pre-treatment of the rats with buthionine sulfoximine, an inhibitor of γ-glutamyl transferase, a key enzyme in glutathione (GSH) synthesis, at least 1.5 h before administration of 2-^{14}C-2-methylfuran caused a 20% increase in urinary metabolites, indicating a decrease in covalently bound metabolites. These results indicate that the urinary metabolites include GSH conjugates. Maximal hepatic radioactivity was detected 4 h after administration (Ravindranath & Boyd, 1991).

In rats, the tissue distribution of radiolabel from 50–200 mg/kg bw of 2-^{14}C-methylfuran over 24 h was greatest in liver > kidney > lung > blood, the maximal amount being detected in liver 8 h after administration, followed by a steady decline up to 24 h (Ravindranath et al., 1986).

On the basis of these data and the lipid solubility of the members of this group of aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, and related esters, sulfides, disulfides and ethers containing furan substitution, it is predicted that they would all be rapidly absorbed, distributed to key organs involved in metabolic processes and then eliminated, primarily in the urine.

(c) Metabolism

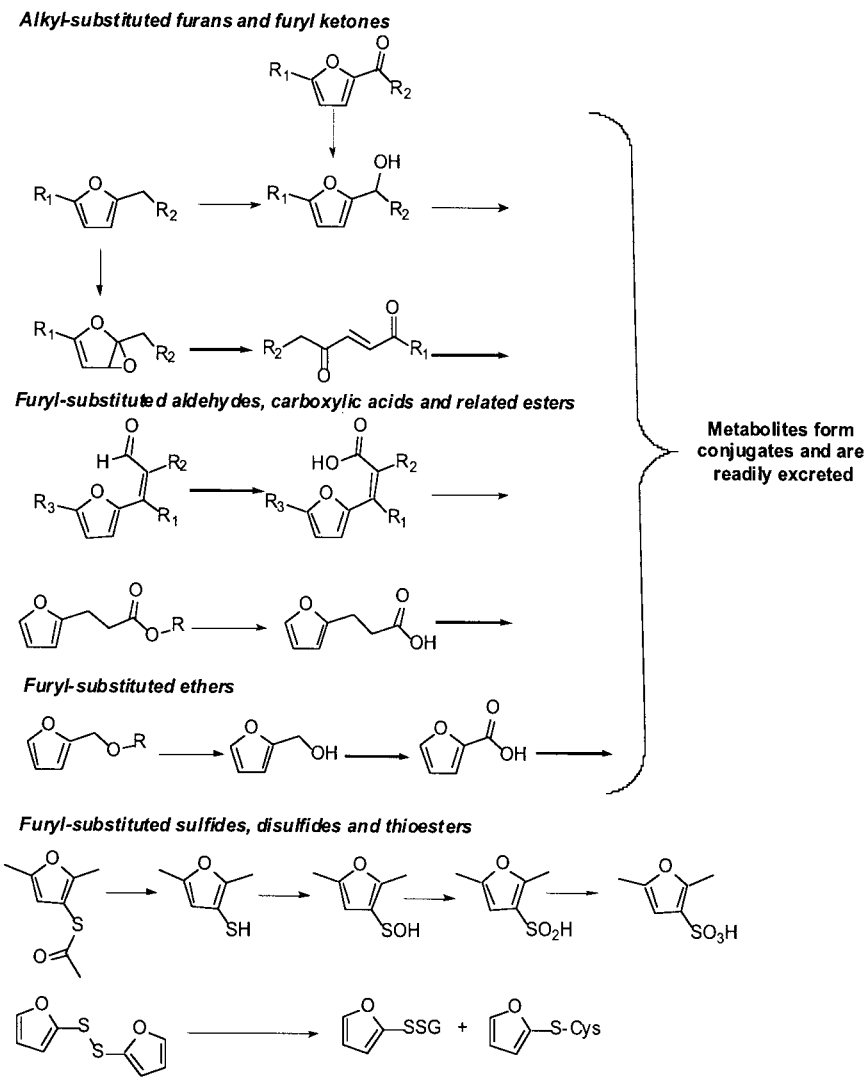
The metabolic options available to this group of furyl-substituted substances depend, in large part, on the presence or absence of specific functional groups on the aliphatic side-chain. The substances in this group are metabolized to polar products, which are mainly conjugated and excreted in the urine (see Figure 2).

Alky-substituted furan (Nos 1487–1496) and furyl ketone (Nos 1503–1512) derivatives

Alkyl-substituted furan and benzofuran derivatives undergo cytochrome P450 (CYP450)-mediated side-chain oxidation to yield an alcohol functional group at the position bonded directly to the furan ring. The resulting alcohol can be excreted in the urine, primarily as the glucuronic acid or sulfate conjugate, or it can be converted to the corresponding ketone, which can also be excreted in the urine. CYP450-induced side-chain oxidation, preferably at the C1′ position of furan, is similar to that observed with other alkyl-substituted heterocyclic derivatives (e.g. pyridine derivatives) (Hawksworth & Scheline, 1975; Ruangyuttikarn et al., 1992; Thornton-Manning et al., 1993; Gillam et al., 2000). In addition to side-chain oxidation, the furan ring can undergo CYP450-induced oxidation (epoxidation) to yield unstable epoxides, the rings of which can open to yield reactive 2-enedial intermediates. These types of intermediates have been shown to conjugate readily with GSH, depleting free GSH and, subsequently at high levels, forming protein and DNA adducts (Ravindranath et al., 1983, 1984; Ravindranath & Boyd, 1985; Ravindranath et al., 1986).

The metabolic fate of a 2-ethylbenzofuran derivative was investigated in humans, rats and dogs. Two healthy men were each given an oral dose of 100 mg of [3-^{14}C]benzarone [2-ethylbenzofuran, 3-(4-hydroxybenzoyl)] in two gelatin capsules. About 73% of the radioactivity was excreted in the urine over 5 days, more than 59% being excreted within the first 24 h. About 19% of the radiolabel was excreted in the faeces over 5 days. The principal metabolites included benzarone hydroxylated at the C1′ position of the furan ring and a glucuronic acid conjugate, either of the C1′ hydroxyl or the phenolic hydroxyl group. In dogs and rats, more than 80% of a dose of 0.5 or 2 mg/kg bw of [3-^{14}C]benzarone was excreted in the faeces during the first 48 h. In rats and dogs, most (> 70%) of the absorbed dose was eliminated by direct conjugation of the administered substance, whereas in humans > 70% was hydroxylated before conjugation. The authors speculated that the benzarone glucuronic acid conjugate was more readily excreted directly into the bile of rats and dogs than in humans, thereby minimizing further hydroxylation in the liver (Wood et al., 1987).

Figure 2. Summary of metabolic options for furyl derivatives



Two healthy male volunteers were given a single oral daily dose of 100 mg of benzbromarone [(3,5-dibromo-4-hydroxyphenyl) (2-ethyl-3-benzofuranyl) methanone] for 8 consecutive days. The main metabolites were formed by C1′ hydroxylation to yield the corresponding 1′-hydroxybenzbromarone and by hydroxylation of the benzene side-chain to yield 6-hydroxybenzbromarone. The corresponding C1′ ketone formed by oxidation of the 1′-hydroxy group was also identified in the urine. The ratio of C1′ enantiomers of 1′-hydroxybenzbromarone

was 2.12 in the plasma and 7.32 in the urine. These metabolic data support the conclusion that alkyl-substituted furan and benzofuran derivatives undergo side-chain oxidation to yield the corresponding alcohol metabolite, which can be excreted as the glucuronic acid conjugate or oxidized to the corresponding ketone, followed by excretion in the urine (DeVries et al., 1993).

Unsubstituted and short-chain alkyl-substituted furans were also shown to undergo ring epoxidation in the liver by mixed-function oxidases. Epoxy metabolites of furans have been reported to undergo ring opening to yield reactive 2-ene-1,4-dicarbonyl intermediates (see example in Figure 2), which can be conjugated with GSH and readily eliminated in the urine or, at relatively high concentrations, react with proteins and DNA to form adducts.

Initial experiments with rat microsomal preparations in vitro suggested that high concentrations of alkyl-substituted furans are partly metabolized to reactive acetylacrolein-type intermediates (Ravindranath et al., 1983, 1984). Acetylacrolein is a potent microsomal mixed-function oxidase inhibitor which has been reported to bind covalently and irreversibly to the oxidizing enzyme, thus deactivating it (Ravindranath & Boyd, 1985).

Significant protein binding (> 55 nmol/mg protein) was reported when 10 mmol/l of [2-^{14}C]methylfuran were incubated with rat hepatic microsomes in the presence of NADPH and oxygen. In the absence of oxygen or NADPH, little binding was observed (< 2 nmol/mg protein). These findings suggest that NADPH-dependent oxidation of 2-methylfuran is a prerequisite for protein binding. Increased protein binding (> 80 nmol/mg protein) was also reported when Sprague-Dawley rats were pre-treated with phenobarbital, a CYP450 inducer, while decreased or no protein binding was observed in the presence of piperonyl butoxide or *N*-octyl imidazole, both of which inhibit CYP450. The V_{max} and K_m for 2-methylfuran metabolism in phenobarbital pre-treated rats were 0.81 μmol/2 mg microsomal protein per min and 0.463 mmol/l, respectively, and those in rats without phenobarbital pre-treatment were 0.53 μmol/2 mg microsomal protein per min and 1.417 mmol/l, respectively. These values suggest that 2-methylfuran undergoes CYP450-mediated oxidation to yield a reactive metabolite (i.e. acetylacrolein) which binds covalently to protein (Ravindranath & Boyd, 1985).

In the same study, when 0.25 mmol/l (24.5 μg/ml) acetylacrolein was added to the incubation mixture, microsomal metabolism of 2-methylfuran was almost completely inhibited (covalent binding was 1.5% of that in the control incubation). At a concentration of 0.5 mmol/l (49.1 μg/ml) acetylacrolein, no metabolism of 2-methylfuran was detected, suggesting that acetylacrolein inhibits CYP450-mediated oxidation, probably by direct covalent bonding with the enzyme through free thiol. Acrolein has been shown to conjugate directly with free thiols in vitro (Esterbauer et al., 1975, 1976). Thus, 2-methylfuran is a suicide substrate for CYP450. Conjugation of the reactive metabolite with sulfhydryl trapping agents, including cysteine (10 mmol/l) or GSH (10 mmol/l), resulted in a marked decrease in microsomal protein binding, suggesting that sulfhydryl conjugation plays a role in the detoxication of acetylacrolein. Cysteine was a better trapping agent for the prevention of microsomal protein binding than GSH, semi-carbazide, lysine or *N*-acetylcysteine. The authors postulated that cysteine forms a stable cyclic conjugate with α,β-unsaturated aldehydes, while the ability of GSH to form stable conjugates with these compounds varies (Esterbauer et al., 1975, 1976; Ravindranath & Boyd, 1985).

Other experiments conducted in vitro support the conclusion that CYP450 oxidation of 2-methylfuran is directly related to its toxicity. Hepatocytes were isolated from adult male Wistar rats that had been treated with the CYP450 inducers phenobarbital (0.1% in drinking-water for 5 days) or β-naphthoflavone (80 mg/kg bw by intraperitoneal injection daily for 3 days). The cultured hepatocytes were incubated with 0, 100, 300, 600 or 1000 μmol/l (0, 8.2, 24.6, 49.3 and 82.1 μg/ml, respectively) of 2-methylfuran for 24 h. The median LC_{50} values for untreated, phenobarbital- and β-naphthoflavone-treated hepatocytes were 794, 34 and 57 μmol/l (65.2, 2.8 and 4.7 μg/ml), respectively, the LC_{50} values being lower with CYP450 induction (Hammond & Fry, 1991).

Free GSH levels in the liver, lungs and kidneys of rats 0.5–36 h after administration of 100 mg/kg bw of 2-methylfuran were initially decreased (67.5% of control in liver and 87% of control in kidneys at 0.5 h) but reached or exceeded control levels within 8–24 h (137% of control in kidneys and 130% of control in lungs at 12 h). The highest concentration of [2-^{14}C]methylfuran covalently bound to protein was detected in liver, followed by kidney, lung and blood. Liver and kidney DNA also showed covalent binding of radiolabel, with a twofold increase in binding in the liver after phenobarbital pre-treatment. Conversely, pre-treatment with *N*-octylimidazole decreased covalent binding of the radiolabel to proteins and DNA in liver, lung and kidney. Increased and decreased protein binding and hepatotoxicity [measured as serum alanine aminotransferase (ALT) activity] were observed in rats pre-treated with phenobarbital and *N*-octylimidazole, respectively. Pre-treatment with 3-methylcholanthrene or piperonyl butoxide did not affect covalent binding or hepatotoxicity. These results provide evidence that bioactivation of 2-methylfuran by a CYP450 system is a prerequisite for tissue necrosis in rats (Ravindranath et al., 1986).

In a study of the effects of GSH and cysteine conjugation on the toxic potential of 2-methylfuran, male Sprague-Dawley rats were treated with buthionine sulfoximine (an inhibitor of γ-glutamyl cysteine synthetase, which is a key enzyme in GSH synthesis) subcutaneously at a dose of 900 mg/kg bw 1.5 h before intraperitoneal administration of 100 mg/kg bw of [2-^{14}C]methylfuran in sesame oil. Marked decreases in covalent DNA and protein binding in the liver and reduced hepatotoxicity, as indicated by lower serum ALT activity, were observed. Buthionine sulfoximine caused a transient increase in plasma cysteine levels, concurrently with a decrease in GSH. Administration of 100 mg/kg bw of 2-methylfuran 1.5 h after buthionine sulfoximine, however, significantly reduced plasma cysteine levels and increased (20%) urinary elimination of 2-methylfuran-labelled metabolites when compared with a group receiving [2-^{14}C]methylfuran only. Subcutaneous pre-treatment with 0.4 ml/kg bw of diethylmaleate, which depletes liver GSH, increased binding to liver proteins and increased hepatotoxicity, as indicated by a higher concentration of serum ALT activity than in rats that received only 2-methylfuran. Subcutaneous pre-treatment of rats with the GSH synthesis promoter L-2-oxothiazolidine-4-carboxylate at a dose of 1000 mg/kg bw resulted in a marked increase in covalent protein binding in the liver and potentiated hepatotoxicity (more ALT activity than in rats that received only 2-methylfuran). When rats were pre-treated with both buthionine sulfoximine and L-2-oxothiazolidine-4-carboxylate, covalent protein binding in the liver and hepatotoxicity were markedly increased, as indicated by a reduction in serum ALT activity. No unchanged 2-methylfuran was found in urine, indicating that pre-treatment did not inhibit metabolic processes (Ravindranath & Boyd, 1991). The authors proposed

that buthionine sulfoximine pre-treatment indirectly aided the detoxication of 2-methylfuran by reducing the GSH supply and increasing the availability of cysteine, which forms a more stable conjugate with acetylacrolein (Esterbauer et al., 1976; Ravindranath & Boyd, 1991).

Groups of 10–15 adult male Swiss albino mice were given 200 mg/kg bw of 2-ethylfuran in sesame oil by intraperitoneal injection with or without pre-treatment with phenobarbital, piperonyl butoxide or cobaltous chloride. The mortality rates were 1/10, 2/10, 3/15 and 2/11 in the untreated and phenobarbital, piperonyl butoxide and cobaltous chloride pre-treated groups, respectively. Ethylfuran caused moderate necrosis of the liver and mild-to-moderate necrosis of the kidneys. The kidney necrosis was described as a coagulative lesion of the proximal convoluted tubules of the outer cortex, without damage to glomerular or medullary cells. Piperonyl butoxide and cobaltous chloride decreased the severity of necrosis in the liver and kidney (McMurtry & Mitchell, 1977).

In the same study, mice were given an intraperitoneal injection of 70 mg/kg bw of 2-acetylfuran in 0.9% NaCl, with or without pre-treatment with phenobarbital, or 80 mg/kg bw of 2-acetylfuran, with or pre-treatment without cobaltous chloride. The mortality rates were 1/12 in the group given 70 mg/kg bw 2-acetylfuran, 0/12 in those pre-treated with phenobarbital, 0/12 in mice given 80 mg/kg bw 2-acetylfuran and 0/12 in those pre-treated with cobaltous chloride. Mice given 2-acetylfuran showed no evidence of renal toxicity. Hepatic necrosis, described as midzonal centrilobular necrosis of the parenchymal hepatocytes, was markedly decreased in incidence and severity after pre-treatment with cobaltous chloride (McMurtry & Mitchell, 1977).

Male ICR mice were given 2.6 mmol/kg bw (250 mg/kg bw) of 2-ethylfuran in sesame oil by intraperitoneal injection. Histopathological examination of tissues collected 24 h later revealed extensive proximal tubular necrosis of the kidneys and focal hydroptic degeneration of the liver. Significant increases in plasma urea nitrogen (approximately five times control level) and ALT activity were reported (Wiley et al., 1984).

Severe bronchiolar necrosis was reported in male ICR mice given 2-ethylfuran at 2.6 mmol/kg bw (250 mg/kg bw) in sesame oil by intraperitoneal injection. Administration of 1.56 mmol/kg bw (150 mg/kg bw) of 2-ethylfuran to five male ICR mice approximately doubled the amount of ^{14}C-thymidine incorporation into pulmonary DNA over control values (Gammal et al., 1984).

In a study of the tumour-inhibiting properties of 2-heptylfuran, increased cytosolic glutathione transferase activity was observed in tissue preparations of liver, forestomach and small-bowel mucosa isolated from groups of five 7-week-old female A/J mice that had received a dose of 12, 25, 50 or 80 μmol/day of 2-heptylfuran dissolved in cottonseed oil by gavage every other day for a total of three doses. The dose of 50 μmol caused a significant increase in acid-soluble sulfhydryl concentration, which is a good measure of the GSH content of tissues, in all four tissue types when compared with controls (Lam & Zheng, 1992).

The neurotoxic potential of 2,5-dimethylfuran and a series of hexane derivatives was evaluated in freshly prepared Schwann cells isolated from the sciatic nerves of neonatal Sprague-Dawley rats. The cells were incubated with 0.17, 0.33, 0.67, 1.33, 2.66, 5.33, 10.7 or 21.3 mmol/l (16.3, 31.7, 64.4, 127.9, 255.7, 512.4, 1028.6 and 2047.6 μg/ml, respectively) of 2,5-dimethylfuran. Dimethylfuran caused

greater inhibition of the incorporation of ^{3}H-thymidine into Schwann cell DNA than other hexane derivatives, as indicated by its low EC_{50} value. Concentrations ≥ 5.33 mmol/l (512.4 μg/ml) induced cytotoxic changes in Schwann cell morphology, including loss of cell processes, rounding of cells and detachment from the substratum. At concentrations ≥ 5.33 mmol/l (512.4 μg/ml), 2,5-dimethylfuran completely inhibited the Schwann cells' ability to incorporate ^{3}H-thymidine. The cytotoxicity of 2,5-dimethylfuran was not mediated by dibutyryl cAMP, a known Schwann cell mitogen. The authors proposed that cytotoxicity occurred by suppression of DNA synthesis, which is related to the oxidative stress induced by 2,5-dimethylfuran (Kamijima et al., 1996).

Thus, alkyl-substituted furans can be metabolized by side-chain oxidation to yield, initially, the 1′-alcohol derivative, which can be conjugated and excreted or oxidized to the corresponding ketone. The conversion to the ketone is anticipated to be reversible, in which case the ketones can be reduced to the corresponding alcohols and excreted mainly in the urine. In a second pathway, the furan ring can be oxidized to form an unstable epoxide which can undergo rapid ring opening to yield reactive 2-ene-1,4-dicarbonyl intermediates such as acetylacrolein. The reactive intermediate can be conjugated with available sulfhydryl trapping agents such as GSH and cysteine or, at high concentrations in vivo, can be covalently bound to proteins and DNA.

Furyl-substituted aldehydes, carboxylic acids and related esters (Nos 1497–1502 and 1513–1519)

The aldehydes in this group are alkyl- or aryl-substituted 3-furyl-2-propenal (Nos 1497–1499, 1501 and 1502) or 3-furyl-2-propanal (No. 1500) derivatives. As such, they are readily oxidized to the corresponding 3-furylpropenoic acid or 3-furylpropanoic acid derivatives. As noted above, the esters in the group are hydrolysed to yield 3-furylpropanoic acid (Nos 1513–1516) or 3-furylpropenoic acid (No. 1518), while one furoate ester is hydrolysed to furoic acid. The Committee has previously reviewed the metabolic fate of furoic acid and 3-furylpropenoic acid (Annex 1, references 150 and 160). Both are readily excreted by humans and experimental animals in the urine, primarily as glycine conjugates. In the main metabolic detoxication pathway for furfuryl alcohol, furfural and furoic acid, the coenzyme A (CoA) thioester of furoic acid is either conjugated with glycine and excreted in the urine or condensed with acetyl-CoA to form the CoA thioester of 2-furanacrylic acid (3-furylpropenoic acid). This compound, 2-furanacryloyl CoA, is also conjugated with glycine and excreted primarily in the urine (Nomeir et al., 1992; Parkash & Caldwell, 1994).

The condensation of furoic acid with acetyl-CoA to yield furanacrylic acid (3-furylpropenoic acid) appears to be a dynamic equilibrium favouring the CoA thioester of furoic acid (Parkash & Caldwell, 1994). The observation that furoic acid was excreted in the urine of dogs given furanacrylic acid is evidence for this equilibrium (Friedmann, 1911). An analogous equilibrium is established between other aromatic carboxylic acids (e.g. benzoic acid and cinnamic acid) (Nutley et al., 1994). Excretion of free furoic acid and furanacrylic acid in animals at higher doses suggests that glycine conjugation is capacity limited, probably by the supply of endogenous glycine (Gregus et al., 1993).

It is anticipated that the aldehydes in this group will be oxidized to the corresponding 3-furylpropenoic acid or 3-furylpropanoic acid derivatives. The esters

in this group will be hydrolysed to the same 3-furylpropenoic acid or 3-furylpropanoic derivatives. The acids will then be conjugated with glycine and excreted. Similarly, furoic acid formed by ester hydrolysis will be conjugated with glycine and excreted in the urine.

Furyl-substituted ethers (Nos 1520–1522)

The Committee previously reviewed the metabolic fate of alkyl-substituted aromatic ethers (Annex 1, references 166 and 167). If the substance is a methyl (No. 1520) or ethyl (No. 1521) furfuryl ether, *O*-dealkylation occurs in vivo to yield the furfuryl alcohol that subsequently undergoes oxidation to furoic acid. As discussed above, furoic acid conjugates with glycine and is excreted mainly in the urine. Difurfuryl ether (No. 1522) is anticipated to undergo CYP450-catalysed hydroxylation to yield the hemiacetal, which readily hydrolyses to yield furfuryl alcohol and furfural. Both these substances are then oxidized to furoic acid and excreted (Nomeir et al., 1992; Parkash & Caldwell, 1994).

Furyl-substituted sulfides, disulfides and thioesters (Nos 1523–1526)

The Committee previously reviewed the metabolic fate of furyl-substituted sulfides and disulfides (Annex 1, references 160 and 161).

The two thioesters in the group (Nos 1523 and 1526) are anticipated to undergo hydrolysis to the corresponding thiol (2,5-dimethyl-3-thiofuran, No. 1063), furfuryl mercaptan (No. 1072) and simple aliphatic carboxylic acid. The resulting thiols are highly reactive in vivo, mainly because most thiols are readily oxidized to unstable sulfenic acids, which are further oxidized to the corresponding sulfinic and sulfonic acids. Methylation of thiols, primarily by *S*-adenosyl methionine, yields methyl sulfides, which are then readily oxidized to sulfoxides and sulfones. Thiols can react with physiological thiols to form mixed disulfides or form conjugates with glucuronic acid. Oxidation of the α-carbon results in de-sulfuration and formation of an aldehyde, which oxidizes to the corresponding acid (McBain & Menn, 1969; Dutton & Illing, 1972; Maiorino et al., 1989; Richardson et al., 1991).

The labile nature of the S–S bond in furfuryl 2-methyl-3-furyl disulfide (No. 1524) also favours a variety of metabolic options for detoxication. The disulfide bond is rapidly reduced to the corresponding thiol (mercaptan) in a reversible reaction in vivo. Therefore, the metabolic options available to thiols are also available to disulfides. Thiol–disulfide exchange reactions are reversible, nucleophilic substitution reactions that occur in vivo between reduced or oxidized thiols of low relative molecular mass (e.g. GSH disulfide or GSH) and cysteinyl thiol components of proteins, resulting in the formation of mixed disulfides. The disulfide bonds in mixed disulfides can undergo reduction, releasing the thiol from the protein (Brigelius, 1985; Sies et al., 1987; Cotgreave et al., 1989).

The remaining substance is a sulfide (No. 1525). Monosulfides are expected to undergo oxidation, mainly to the corresponding sulfoxide and sulfone. Sulfoxides and sulfones are physiologically stable and are excreted unchanged in the urine (McBain & Menn, 1969; Nickson & Mitchell, 1994; Nickson et al., 1995; Nnane & Damani, 1995).

In summary, the metabolic options available to this group of furyl-substituted substances depend, in large part, on the presence or absence of specific functional

groups on the aliphatic side-chain. The substances in this group are metabolized to polar products, which are mainly conjugated and then excreted in the urine. At higher doses, alkyl furans of low relative molecular mass (e.g. 2-methylfuran) can undergo ring oxidation to yield reactive 2-ene-1,4-dicarbonyl intermediates, which can subsequently be conjugated with sulfhydryl trapping agents or react with protein and DNA.

2.3.2 Toxicological studies

(a) Acute toxicity

Oral LD_{50} values have been reported for 10 of the 40 substances in this group (Table 3). In rats, the LD_{50} values range from 138 to 4458 mg/kg bw, indicating little acute toxicity of aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, and related esters, sulfides, disulfides and ethers containing furan substitution when given orally (Long, 1977a,b; Moreno, 1977; Gabriel, 1979; Moran et al., 1980; Piccirillo et al., 1982, 1983a,b; Reagan & Becci, 1984a,b; Burdock & Ford, 1990a,b,c,d). In mice, the oral LD_{50} values ranged from 438 mg/kg bw for 2-acetyl-5-methylfuran to 1220 mg/kg bw for 2-pentylfuran (Shellenberger, 1971c; Griffiths & Babish, 1978; Moran et al., 1980).

Male Sprague-Dawley rats were given a single dose of 50, 100, 200 or 400 mg/kg bw of 2-methylfuran in sesame oil by intraperitoneal injection. The group at the lowest dose showed no signs of liver necrosis, but they had endothelial injury, with blebbing of the endothelium into the vascular lumen of the central veins. Animals given 100, 200 or 400 mg/kg bw showed a dose-dependent increase in the severity of hepatocellular injury (e.g. eosinophilic cytoplasm and vacuolation), centrilobular necrosis and necrosis of the bronchiolar epithelium, accompanied by increasing sloughing of the epithelium and, at the highest dose, complete obliteration of numerous respiratory and terminal bronchioles. Dose-related increases in serum ALT activity were observed at doses up to 200 mg/kg bw; however, the serum ALT activity in animals given 50 mg/kg bw was not significantly greater than that in control rats (Ravindranath et al., 1986).

When 2,5-dimethylfuran was administered in the drinking-water at concentrations of 0.25–1.0% [approximately 250–1000 mg/kg bw (Food & Drug Administration, 1993)] or by gavage at dosages of 200–1200 mg/kg bw to male rats, no neurotoxic effects were observed. No further details or discussion were given in this abstract (Krasavage et al., 1978).

(b) Short-term studies of toxicity

The results of short-term studies with 11 representative aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, and related esters, sulfides, disulfides and ethers containing furan substitution are summarized in Table 4 and described below. One study was performed with an alkyl-substituted furan derivative (No. 1491), four studies with three furyl-substituted ketones (Nos 1495, 1503, 1506 and 1511), two studies with two furyl-substituted aldehydes (Nos 1497 and 1502), one study with a furyl-substituted aliphatic ester (No. 1514) and one study with a furyl-substituted ether (No. 1520) and a furyl-substituted thioester (No. 1526). In addition, two studies were available on the products formed when thioesters (Nos 1523 and 1526) are hydrolysed in vivo.

Table 3. Acute toxicity of aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing a furan substitution used as flavouring agents

No.	Flavouring agent	Species; sex	LD_{50} (mg/kg bw)	Reference
1491	2-Pentylfuran	Mice; M, F	M: 1185 F: 1220	Shellenberger (1971c)
1491	2-Pentylfuran	Mice; M, F	1200	Moran et al. (1980)
1495	2,3-Dimethylbenzo-furan	Rats; M, F	1952	Long (1977a)
1494	3-Methyl-2-(3-methyl-2-butenyl)furan	Rats; M	660	Gabriel (1979)
1497	3-(2-Furyl)acrolein	Rats; M, F	M: > 900 F: > 857	Piccirillo et al. (1983a)
1497	3-(2-Furyl)acrolein	Rats; M, F	M: > 900 F: > 860	Burdock & Ford (1990a)
1498	2-Methyl-3-(2-furyl)-acrolein	Rats; M, F	1400	Reagan & Becci (1984a)
1498	2-Methyl-3-(2-furyl)-acrolein	Rats; M, F	1400	Burdock & Ford (1990d)
1502	2-Phenyl-3-(2-furyl)-prop-2-enal	Rats; M, F	717	Long (1977b)
1503	2-Furyl methyl ketone	Rats; M, F	138	Piccirillo et al. (1982)
1504	2-Acetyl-5-methylfuran	Mice; M, F	438	Griffiths & Babish (1978)
1504	2-Acetyl-5-methylfuran	Mice; M, F	438	Moran et al. (1980)
1514	Isobutyl 3-(2-furan)-propionate	Rats; M, F	3294	Piccirillo et al. (1983b)
1514	Isobutyl 3-(2-furan)-propionate	Rats; M, F	4458	Reagan & Becci (1984b)
1514	Isobutyl 3-(2-furan)-propionate	Rats; M, F	3300	Burdock & Ford (1990c)
1514	Isobutyl 3-(2-furan)-propionate	Rats; NR	1950	Moreno (1977)
1522	Difurfuryl ether	Rats; M, F	250	Burdock & Ford (1990b)
1522	Difurfuryl ether	Rats; M, F	249	Reagan & Becci (1984c)

M, male; F, female; NR, not reported

2-Pentylfuran (No. 1491)

Groups of 23 Sprague-Dawley albino rats of each sex were maintained on a diet calculated to provide an average daily intake of 25.6 and 26.0 mg/kg bw of 2-pentylfuran to male and female rats, respectively, for 13 weeks. Food and water were provided ad libitum. Weekly measurements of body weights, food consumption and food use showed no differences between test and control groups. Animals were observed daily for clinical signs of toxicity and behaviour, and, during weeks 6 and 13, the urine of eight rats of each sex was analysed. At week 6, eight rats of each sex were killed by exsanguination for haematological examination; the remaining 15 males and 15 females were killed at the end of week 13. All animals were

Table 4. Results of short-term studies of toxicity on aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution

No.	Substance	Species; sex	No. test groups[a]/ no. per group[b]	Route	Duration (days)	NOEL mg/kg bw per day)	Reference
1491	2-Pentylfuran	Rats; M, F	1/46	Diet	91	M: 25.6[c] F: 26.0[c]	Shellenberger (1971a,b)
1495	2,3-Dimethylbenzofuran	Rats; M, F	1/30	Gavage	91	0.6[c]	Long (1977a)
1497	3-(2-Furyl)acrolein	Rats; M, F	3/10	Gavage	28	100	Faber & Hosenfeld (1992)
1497	3-(2-Furyl)acrolein	Rats; M, F	3/20-64	Diet	90	45[d]	Lough et al. (1985)
1502	2-Phenyl-3-(2-furyl)prop-2-enal	Rats; M, F	1/30	Gavage	91	0.87[c]	Long (1977b)
1503	2-Furyl methyl ketone	Rats; M, F	3/20-64	Diet	90	25[d]	Lough et al. (1985)
1506	3-Acetyl-2,5-dimethylfuran	Rats; M, F	1/10	Diet	14	10[c]	Van Miller & Weaver (1987)
1511	4-(2-Furyl)-3-buten-2-one	Rats; M, F	1/10	Diet	14	30[c]	Gill & Van Miller (1987)
1514	Isobutyl 3-(2-furan)propionate	Rats; M, F	3/20-64	Diet	90	875[c]	Lough et al. (1985)
1520	Furfuryl methyl ether	Rats; M, F	1/10	Diet	14	27[c]	Van Miller & Weaver (1987)
1526	*O*-Ethyl *S*-(2-furylmethyl)thio-carbonate	Rats; M, F	3/6	Gavage	28	8	van Otterdijk & Frieling (2001)

[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 that had no adverse effect; the value is therefore not a true NOEL but is the highest dose tested that had no adverse effects. The actual NOEL might be higher.

[d] After 28 days, all the rats at the highest dose and 16 of each sex at the lowest dose were killed; however, the remaining rats at the lowest dose and all those at the intermediate dose continued on their dietary protocol for an additional 62 days, for a total treatment period of 90 days. The NOEL given here therefore represents the highest dose tested at the end of 90 days.

necropsied and tissues examined for gross lesions. The kidneys, liver, spleen, heart and testes or ovaries were weighed. The brain, pituitary, thyroid and salivary glands, lymph nodes (cervical and mesenteric), lung, diaphragm, heart, liver, stomach, duodeum, pancreas, femur with marrow, small intestine, large intestine, spleen, adrenals, kidney (transverse and longitudinal sections), testes and anexa, ovaries, uterus, bladder, spinal cord (thoracic), skin and any lesions were preserved in 10% buffered formalin and embedded in paraffin blocks for histological evaluation. Haematological examination and urine analysis revealed no differences between test and control rats. Treated rats had statistically significantly greater serum alkaline phosphatase activity than controls at week 13; however, the activity in control males was 333.6 IU at week 6 and inexplicably dropped to 116.0 IU at week 13 and that in control females fell from 346.3 to 120.3 IU; the activity in treated animals remained within the normal range. Some animals had mildly hyperaemic lung tissue, a chronic pulmonary condition common in this strain of rats. The average liver weight in treated males was significantly greater than that of control animals. Female rats had significantly greater liver and kidney weights than the control group, but examination of these organs revealed no histopathological abnormality. The author stated that the organ weights of the control animals were significantly lower than those of animals of the same strain and age used as controls in other studies under the same conditions (Shellenberger, 1971c). On the basis of the lower organ weights in controls and the absence of histopathological changes, the Committee concluded that no adverse effects could be attributed to administration of 2-pentylfuran to rats (Shellenberger, 1971a,b).

2,3-Dimethylbenzofuran (No. 1495) and 2-phenyl-3-(2-furyl)-prop-2-enal (No. 1502)

Groups of 16 male and female Sprague-Dawley-derived OFA rats weighing 100–120 g at the beginning of the study were given 0.6 mg/kg bw per day of 2,3-dimethylbenzofuran (Long, 1977a) or 0.87 mg/kg bw per day of 2-phenyl-3-(2-furyl)-prop-2-enal (Long, 1977b) intragastrically in olive oil 7 days per week for 13 weeks. A concurrently maintained control group was given the vehicle. Water and food were provided ad libitum. Animals were examined daily and their behaviour observed. The body weight of each rat and the food consumption in each cage of four animals were measured weekly. Haematology and serum biochemistry were examined in eight rats of each sex at weeks 4 (serum biochemistry limited to blood urea nitrogen) and 13. At 13 weeks, 16 male and 16 female control and treated rats were necropsied. The liver, kidneys, spleen, heart, adrenal glands, testes and ovaries were weighed, and major tissues were preserved for histopathological examination. Clinical examination revealed no differences in mortality, behaviour, body-weight gain or food consumption for either test group in comparison with the corresponding group of control animals.

Administration of 2,3-dimethylbenzofuran had no effect on haematological parameters, but a slight increase in alkaline phosphatase activity was found in males and a slight increase in bilirubin levels in males and females. Additionally, treated females had a decreased serum glucose level. All organ weights were comparable in test and control animals, and histological examination revealed no morphological changes that could be attributed to administration of 2,3-dimethylbenzofuran (Long, 1977a).

Administration of 2-phenyl-3-(2-furyl)-prop-2-enal for 13 weeks had no effect on haematological parameters. At 13 weeks, a decrease in blood urea was found in male rats and a decrease in cholesterol concentrations in treated animals of each sex; however, the values remained within the normal range of variation. Gross examination showed no lesions attributable to administration of the test substance. Organ weights were comparable in test and control groups, and histological examination revealed no alterations that could be related to administration of 2-phenyl-3-(2-furyl)-prop-2-enal (Long, 1977b).

3-(2-Furyl)acrolein (No. 1497)

Groups of five CD®(SD)BR/VAF Plus™ rats of each sex were given 100 or 400 mg/kg bw per day of 3-(2-furyl)acrolein in corn oil intragastrically. Another group of five male and five female rats were given 800 mg/kg bw, which resulted in the deaths of two females by day 1. These animals were replaced and dosing was continued at 600 mg/kg bw per day for the remainder of the study. Food and water were provided ad libitum throughout the study. Body weights were determined on days 0, 4, 7, 14, 21 and 28, and food consumption was measured on days 4, 7, 14, 21 and 28. The body weights of fasted animals were measured before necropsy. Rats were monitored daily for signs of toxicity and behavioural changes. Haematology and clinical chemistry examinations were conducted on blood drawn from the posterior vena cava before necropsy. At the end of the study, rats were fasted overnight, anaesthetized with CO_2 and exsanguinated via the posterior vena cava. The liver, kidneys, adrenal glands, testes, spleen and thymus were weighed, and all major organs, tissues and lesions from all animals were fixed in 10% buffered formalin. All tissues from animals at the highest dose and from controls and the thymus, stomach, liver, kidneys and gross lesions from animals at the two lower doses were examined microscopically.

As noted above, two female rats given 800 mg/kg bw per day of 3-(2-furyl)acrolein were found dead or moribund on day 1 of the study, and one female rat at 400 mg/kg bw per day was found dead on day 2. The cause of death of the latter could not be determined owing to autolysis, which had taken place by the time the animal was found. All other animals survived to completion of the study. The clinical signs observed included dehydration, decreased faeces, depressed general activity and sialorrhoea in animals at 400 and 600 mg/kg bw per day. The authors proposed that the sialorrhoea was due to the taste of the test material.

The mean body weights of males at the highest dose were significantly lower than those of controls on days 4, 7, 14 and 21; although they were also lower on day 28, the difference was not statistically significant. The mean body weights of females at the highest dose were slightly lower than those of controls on days 1 and 4 but were higher from day 7 to termination of the study. The mean body weight of females at 400 mg/kg be per day was also higher than that of controls from day 14 onwards. The mean body weights of males at 400 mg/kg bw per day and of males and females at the lowest dose were comparable to those of controls. The feed consumption of male and female animals that received 800 mg/kg bw per day was significantly lower (74%) than that of controls at day 4 and was significantly decreased in males at this dose on days 4 and 7. Females at the highest dose consumed more feed than controls on day 7, and the difference was statistically significant on days 21–28.

Males at the highest dose had a significantly lower mean erythrocyte volume fraction and greater mean corpuscular haemoglobin concentration; they also had nonsignificantly lower mean red blood cell counts. Male rats at the two lower doses also had nonsignificantly lower mean erythrocyte volume fraction, erythrocyte count and haemoglobin concentration. Females at the two higher doses had significant reductions in mean red blood cell counts, mean haemoglobin concentration and mean erythrocyte volume fraction, while females at the lowest dose had reduced values for these parameters, without statistical significance. The reduction in haematological values indicates loss of red blood cells, which was probably due to gastric irritation as no other sites of possible haemorrhage were identified. Minimal poikilocytosis (a common variant of erythrocyte morphology) was observed in two male rats in each test group and, among females, in one control, one at the lowest dose, one at the intermediate dose and two at the highest dose. Mean glucose levels were significantly lower and mean sorbitol dehydrogenase levels higher in males at the highest dose. One male at the intermediate dose and females at the highest dose had significantly greater sorbitol dehydrogenase activity than controls. The mean total protein level was significantly lower in male rats at the highest and lowest doses. Mean albumin levels and albumin:globulin ratios were significantly reduced in males at the lowest dose.

The mean relative kidney weights in males at the two higher doses were higher than those of controls, but were statistcially significant only for rats at the intermediate dose. The mean absolute and relative kidney weights of female rats at the highest dose were significantly greater than those of controls. Clinical chemistry did not indicate nephrotoxicity. The mean absolute and relative liver weights were significantly increased in males and females at the two higher doses. The relative thymus weights of males at the highest dose were significantly increased; however, this effect was not considered to be related to treatment but to reflect the lower terminal body weights of this group. No gross pathological changes related to treatment were reported.

Males at the two higher doses had hyperkeratosis (5/5) and acanthosis (5/5) of the non-glandular gastric mucosa, hypertrophy of the hepatocytes (4/5 at the intermediate dose, 4/5 at the highest dose) and an increased number of hepatocytes with enlarged nuclei (4/5 at the intermediate dose, 5/5 at the highest dose). Females had hyperkeratosis and acanthosis of the non-glandular stomach mucosa, hypertrophic hepatocytes and more hepatocytes with enlarged nuclei (4/4 at the intermediate dose, 5/5 at the highest dose). Hypertrophy of hepatocytes (1/5) and more hepatocytes with enlarged nuclei (1/5) were observed in female rats at the lowest dose. The test material was a strong gastric irritant, which accounts for the hyperkeratosis and acanthosis of the non-glandular stomach mucosa observed in rats at the two higher doses. The study pathologist concluded that hypertrophy of hepatocytes and hepatocytes with enlarged nuclei were adaptive responses to the influx of large amounts of 3-(2-furyl)acrolein by gavage to compensate for increased metabolic activity. The NOEL was 100 mg/kg bw per day (Faber & Hosenfeld, 1992).

3-(2-Furyl)acrolein (No. 1497), 2-furyl methyl ketone (No. 1503) and isobutyl 3-(2-furyl)propionate (No. 1514)

Groups of 32 Sprague-Dawley rats of each sex were assigned to groups of controls and low dietary dose, 12 of each sex to the intermediate dose and 10 of

each sex to the highest dose. The rats were maintained on diets calculated to provide 0, 5, 45 or 405 mg/kg bw per day of 3-(2-furyl)acrolein, 0, 5, 25 or 100 mg/kg bw per day of 2-furyl methyl ketone, or 0, 35, 175 or 875 mg/kg bw per day of isobutyl 3-(2-furyl)propionate for 28 days. The animals were given access to food and water ad libitum throughout the study. They were observed daily for clinical manifestations of toxicity and changes in behaviour. Body weights and food consumption were recorded weekly. Haematology, blood chemistry and urine analyses were conducted at 4 weeks. At that time, all rats at the highest dose and 16 of each sex in the control and lowest dose groups were killed by ether anaesthesia and subsequent exsanguination. The remaining animals continued their dietary protocol to 90 days.

In the study with 3-(2-furyl)acrolein, a significant decrease in body-weight gain was seen in males and females at the highest dose when compared with controls at week 4. Males at 45 mg/kg bw per day had decreased body-weight gain at week 12. The decreases in body-weight gain were accompanied by decreased food intake, which might have been due to the unpalatability of the test material. Animals at the two lower doses had inconsistent intervals of low food consumption. The results of haematology and urine analyses were comparable in test groups and control animals at 28 and 90 days. Blood chemistry analysis revealed significant decreases in alkaline phosphatase activity and glucose levels in rats at 405 mg/kg bw per day at 28 days. Necropsy revealed no significant gross alterations. At 28 days, the mean relative liver weight of females at the highest dose was significantly increased, and the mean relative weights of the right and left kidney were increased at 405 mg/kg bw per day. After 13 weeks, the mean relative right kidney weights were increased in males at 5 mg/kg bw per day, and the mean relative left kidney weights were increased in females at the lowest dose. No changes in the weights of the kidney or any other organ were observed in the group at 45 mg/kg bw per day. The increases in organ weights were not accompanied by gross or microscopic signs. The NOEL was 45 mg/kg bw per day.

In the study with 2-furyl methyl ketone, male and female rats at 100 mg/kg bw per day had decreased body-weight gain at day 28. Males at week 13 and females at week 9 given 25 mg/kg bw per day gained less body weight than controls. These body-weight changes corresponded in part to changes in food consumption: males and females at 100 mg/kg bw per day and females at 5 and 25 mg/kg bw per day had significantly lower food consumption than controls, although males at the two lower dose showed no decrease. At 4 weeks, male and female rats at 100 mg/kg bw per day had significantly increased blood urea nitrogen and significantly decreased glucose concentration and alkaline phosphatase activity when compared with controls. Gross pathological examinations gave comparable results for control and test animals. Male and female rats at 100 mg/kg bw per day had higher mean relative liver weights than controls. As the absolute liver weights were comparable to those of controls, the lower body weights of animals at the highest dose might have been partly responsible for the observed increase in relative liver weight. At the end of treatment, the mean absolute and relative liver weights were comparable in controls and rats at the highest dose. Rats at lower doses showed no significant difference in organ weights from controls. At 28 days, males receiving 100 mg/kg bw per day had increased right and left gonad weights after 90 days, but no abnormal histopathological changes were observed. The NOEL was 25 mg/kg bw per day. This agent was most active in assays for genotoxicity (see below). This NOEL,

however, indicates that furan-like compounds are unlikely to be hepatocarcinogenic, as the dose of furan that caused high incidences of hepatocellular and cholangiocellular carcinomas at 13 weeks was 8 mg/kg bw per day (National Toxicology Program, 1993).

In the study with isobutyl 3-(2-furyl)propionate, one female at the lowest dose died from causes reported to be unrelated to treatment. Males on diets designed to provide 175 or 875 mg/kg bw per day showed significant reductions in body-weight gain at 28 days, which persisted in the group at 175 mg/kg bw per day up to week 11. No such changes were seen in the corresponding females or in males or females at the lower dietary level. Males and females at the highest dose consumed significantly less food than controls. Haematology, clinical biochemistry and urine analyses revealed no differences between test groups and controls. Gross pathological examination revealed no significant lesions that could be associated with treatment. Measurement of relative organ weights revealed non-dose-related increases in the weight of the right kidney in males at 35 mg/kg bw per day and in females at 175 mg/kg bw per day, and in the right gonad of males at 175 mg/kg bw per day. The authors stated that most of the differences in organ weights were inconsistent with regard to occurrence, sex and unilateral involvement of bilateral organs and that it was difficult to ascertain whether they represented treatment-related effects. In the absence of a clear dose–response relation, the changes could not be associated with administration of the test substance. No abnormal histopathological changes were observedl. The NOEL was 875 mg/kg bw per day (Lough et al., 1985).

4-(2-Furyl)-3-buten-2-one (No. 1511)

Five male and five female Fischer 344 rats were maintained on diets estimated to provide 0 or 30 mg/kg bw per day of 4-(2-furyl)-3-buten-2-one for 14 days. The animals were examined for viability twice daily. Body weights were recorded on days –1, 6 and 14 of the study, and food consumption was measured on days 7 and 14. Gross necropsy was performed on each of the animals at the end of the study. No adverse clinical effects were found. Females showed a slight increase in food consumption, but there was no such increase for males, and there was no corresponding increase in weight gain. The absolute and relative liver weights of females and the relative liver weights of males were greater than those of controls by 13%, 15% and 8%, respectively, but no histological findings accompanied these increases. The authors stated that, in comparison with control animals of the same strain from the same vendor used in other studies under identical conditions, the absolute liver weights of the control animals in this study were lower than usual (Gill & Van Miller, 1987).

3-Acetyl-2,5-dimethylfuran (No. 1506) and furfuryl methyl ether (No. 1520)

Five Fischer 344 rats of each sex were maintained on diets estimated to provide 0, 10 mg/kg bw per day of 3-acetyl-2,5-dimethylfuran or 27 mg/kg bw per day of furfuryl methyl ether for 14 days. Animals were examined for viability twice daily. Body weights were recorded on days –1, 6 and 14 of the study, and food consumption was measured on days 7 and 14. Gross necropsy was performed on each of the animals at the end of the study. Kidney and liver weights were recorded

before fixation in 10% buffered formalin for histological examination, and all gross lesions were fixed for histological examination. No statistically significant differences in any of the parameters tested were seen between treated and control animals (Van Miller & Weaver, 1987).

O-Ethyl S-(furfurylmethyl)thiocarbonate (No. 1526)

Groups of three Wistar rats of each sex were given doses of 0, 2, 8 or 32 mg/kg bw per day of *O*-ethyl *S*-(2-furylmethyl)thiocarbonate by gavage for 28 days. The animals were observed twice daily, and clinical signs were recorded once daily. Body weights and food consumption were recorded weekly. All animals that survived to the end of the study and all those that became moribund were necropsied, and organs were weighed. No deaths occurred during the study. Simultaneous decreases in body weight and food consumption were reported in rats at the highest dose. The body weights of control males were slightly lower than those of previous control groups. The body weights and food consumption of animals at the two lower doses did not differ significantly from those of controls. Hunched posture, laboured respiration and diarrhoea were reported in animals at the highest dose, mainly during the first week of treatment. Females at this dose showed reduced motor activity at the end of the treatment period. Weekly functional observations showed no differences between treated and control animals in hearing, papillary reflex, static righting reflex or grip strength. At necropsy, organ weights and haematological and macroscopic parameters did not reveal any treatment-related effects. The mean urea concentration was increased in females at the highest dose, which was attributed to an increased value in one rat. A slight increase in ALT activity was reported in one male at the lower doses when compared with controls, but, in the absence of changes in liver weight, of abnormalities or a dose-related effect, this increase was considered not to be related to treatment. The NOEL was 8 mg/kg bw per day (van Otterdijk & Frieling, 2001).

Structurally related substance: 2,5-dimethyl-3-thioisovalerylfuran (No. 1070)

Hydrolysis of 2,5-dimethyl-3-furyl thioisovalerate (No. 1070) is expected to yield the structurally related compound 2,5-dimethyl-3-furylmercaptan. In a 90-day study, 0 or 0.73 mg/kg bw per day of 2,5-dimethyl-3-furyl thioisovalerate was added to the diet of groups of 15 male and 15 female Wistar rats. The survival, behaviour and general appearance of the animals was monitored daily, while body weights and food consumption were recorded weekly. Haematology, blood chemistry and urine analysis (eight animals of each sex per group) were performed during weeks 6 and 12 of the study. At the end of treatment, all surviving animals were necropsied, and the liver and kidneys were weighed; tissues from major organs were preserved for histopathological examination. No significant variations were observed in body weights, food consumption or calculated food use efficiency of treated animals in comparison with controls. Likewise, haematological and blood chemistry parameters were comparable in control and test animals, and the results of urine analysis were unremarkable. Moreover, neither gross nor histopathological examination revealed any significant compound-related difference between test and control animals (Morgareidge & Oser, 1974).

Structurally related substance: furfuryl mercaptan (No.1072)

Furfuryl mercaptan (No. 1072) is the principal hydrolysis product of *O*-ethyl *S*-(2-furylmethyl)thiocarbonate (No. 1526). In a 13-week study, groups of 15 weanling Wistar rats of each sex were given daily doses of 0 (vehicle control), 1, 3 or 30 mg/kg bw of fufuryl mercaptan in corn oil by stomach tube for 13 weeks. Additional groups of five rats per sex were given a daily dose of 0, 3 or 30 mg/kg bw fufuryl mercaptan for 2 or 6 weeks. Clinical observations were made daily, and body weights were measured on days 1, 6 and 9 and then weekly up to week 12. Food and water intakes were measured 1 day before the weight measurements. Males and females at the highest dose had significant decreases in body-weight gain, beginning on days 6–9 and continuing until study termination, when the reductions were 12–16%. These were associated with significantly reduced food intake. At the end of the study, animals at 30 mg/kg bw per day had significant reductions in absolute organ weights and increases in relative organ weights (i.e. brain, kidneys, stomach, small intestine, caecum, adrenals and gonads in males, and brain, heart, liver, kidneys, stomach, caecum, adrenals and thyroid in females). These were considered to be related to the lower body weights. In addition, isolated organ weight changes were observed in males and females at the highest dose in the group terminated at week 6, and increased relative heart weights in males and reduced relative kidney weights in females were reported at 3 mg/kg bw per day. No such changes were seen in the group at 3 mg/kg bw per day terminated at 13 weeks. They were therefore considered not to be related to treatment. Urine analysis, including concentration and dilution tests, performed on the last day of treatment revealed no significant differences between test and control groups. Likewise, no significant variations were observed in clinical chemistry values. Statistically significant variations in haematological parameters in males at the highest dose included increased packed cell volume and total leukocyte count at week 6 and increased haemoglobin concentration and packed cell volume at study termination; however, these changes were not considered to represent toxicologically significant adverse effects. At study termination, macroscopic and microscopic examinations showed no lesions related to treatment. The NOEL was 3 mg/kg bw per day (Phillips et al., 1977).

(c) Genotoxicity

The genotoxicity of eight representative aliphatic hydrocarbons (Nos 1487, 1488 and 1494), aldehydes and ketones (Nos 1497, 1503 and 1511), carboxylic acids and related esters (No. 1513) and sulfides (No. 1526) in this group has been tested. The results of these tests are summarized in Table 5.

In vitro

In standard assays for mutagenicity in *Salmonella typhimurium*, 2,5-dimethylfuran (No. 1488), 3-methyl-2-(3-methyl-2-butenyl)furan (No. 1494), 3-(2-furyl)acrolein (No. 1497), 4-(2-furyl)-3-buten-2-one (No. 1511), ethyl 3-(2-furyl)-propanoate (No. 1513), and *O*-ethyl *S*-furfurylthiocarbonate (No. 1526) were not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, TA102, TA1535, TA1537 and TA1538 when tested at concentrations up to10 000 µg/plate, either alone or with a rat liver-derived bioactivation system (Wild et al., 1983; Mortelmans et al.,

Table 5. Studies of genotoxicity with aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
In vitro						
1487	2-Methylfuran	Reverse mutation	*S. typhimurium* TA98, TA100	0.165, 0.330, 0.495 or 0.660 µmol/plate (13.5, 27.1, 40.6 or 54.2 µg/plate[a])	Negative[b]	Shinohara et al. (1986)
1487	2-Methylfuran	Reverse mutation	*S. typhimurium* TA98, TA102, TA1535, TA100	≤ 10 000 µg/plate	Negative[b,c,d]	Zeiger et al. (1992)
1487	2-Methylfuran	Reverse mutation	*S. typhimurium* TA97, TA104	≤ 10 000 µg/plate	Equivocal[b,c,d]	Zeiger et al. (1992)
1487	2-Methylfuran	Reverse mutation	*S. typhimurium* TA98, TA100, TA102	11 nmol/plate to 1.1 mmol/plate (0.9–90 310 µg/plate[a])	Negative[b]	Aeschbacher et al. (1989)
1487	2-Methylfuran	DNA damage	*Bacillus subtilis* H17 (rec$^+$) and M45 (rec$^-$)	0.16, 16 or 1600 µg/disc	Negative/positive[b,e]	Shinohara et al. (1986)
1487	2-Methylfuran	Chromosomal aberration	Chinese hamster ovary cells	0–150 mmol/l (0–12 315 µg/ml[a])	Positive[b,f]	Stich et al. (1981)
1488	2,5-Dimethylfuran	Reverse mutation	*S. typhimurium* TA98, TA100	0.165, 0.330, 0.495 or 0.660 µmol/plate (13.5, 27.1, 40.6 or 54.2 µg/plate[a])	Negative[b]	Shinohara et al. (1986)
1488	2,5-Dimethylfuran	Reverse mutation	*S. typhimurium* TA98, TA100	Not specified	Negative[b]	Lee et al. (1994)
1488	2,5-Dimethylfuran	Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535	≤ 3333 µg/plate	Negative[b,c,d]	Zeiger et al. (1992)
1488	2,5-Dimethylfuran	DNA damage	*Bacillus subtilis* H17 (rec$^+$) and M45 (rec$^-$)	190, 1900 or 9500 µg/disc	Negative/positive[b,h]	Shinohara et al. (1986)
1488	2,5-Dimethylfuran	Chromosomal aberration	Chinese hamster V79 cells	1 mmol/l (96.13 µg/ml)[g]	Negative	Ochi & Ohsawa (1985)
1488	2,5-Dimethylfuran	Chromosomal aberration	Chinese hamster ovary cells	0–20 mmol/l (1923 µg/ml)[g]	Positive[b,f]	Stich et al. (1981)
1494	3-Methyl-2-(3-methyl-2-butenyl)-furan	Reverse mutation	*S. typhimurium* TA1535, TA1537, TA98, TA100	3.2, 16, 80, 400 or 2000 µg/plate	Negative[b]	Asquith (1989)

Table 5 (contd)

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
1497	3-(2-Furyl)acrolein	Reverse mutation	*S. typhimurium* TA100	Not specified	Negative[b,c]	Eder et al. (1991)
1497	3-(2-Furyl)acrolein	DNA damage (SOS Chromotest)	*E. coli* PQ37	Not specified	Negative[j]	Eder et al. (1991)
1497	3-(2-Furyl)acrolein	DNA damage (SOS Chromotest)	*E. coli* PQ37	Not specified	Weakly positive[j]	Eder et al. (1993)
1503	2-Furyl methyl ketone	Reverse mutation	*S. typhimurium* TA98, TA100	0.165, 0.330, 0.495 or 0.660 µmol/plate (13.5, 27.1, 40.6 or 54.2 µg/plate[j])	Negative/ positive[b,k]	Shinohara et al. (1986)
1503	2-Furyl methyl ketone	DNA damage (SOS Chromotest)	*E. coli* PQ37	Not specified	Slightly positive[j]	Eder et al. (1993)
1503	2-Furyl methyl ketone	DNA damage	*Bacillus subtilis* H17 (rec^+) and M45 (rec^-)	550, 5500 or 55 000 µg/disc	Negative/ positive[b,l]	Shinohara et al. (1986)
1503	2-Furyl methyl ketone	Chromosomal aberration	Chinese hamster ovary cells	0–125 mmol/l (0–13 764 µg/ml)[j]	Positive[b,m,n]	Stich et al. (1981)
1511	4-(2-Furyl)-3-buten-2-one	Reverse mutation	*S. typhimurium* TA1535, TA98, TA100, TA1537	33, 100, 333, 1000, 2166 or 3333 µg/plate	Negative[b,c,o]	Mortelmans et al. (1986)
1513	Ethyl 3-(2-furyl)-propanoate	Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98	≤ 3600 µg/plate	Negative[b]	Wild et al. (1983)
1526	*O*-Ethyl *S*-furfuryl-thiocarbonate	Reverse mutation	*S. typhimurium* TA1535, TA1537, TA100, TA98	33, 100, 333, 1000 or 3330 µg/plate	Negative[b,p]	Verspeek-Rip (2000)
1526	*O*-Ethyl *S*-furfuryl-thiocarbonate	Reverse mutation	*E. coli* WP2uvrA	33, 100, 333, 1000 or 3330 µg/plate	Negative[b,q]	Verspeek-Rip (2000)
1526	*O*-Ethyl *S*-furfuryl-thiocarbonate	Chromosomal aberration	Human peripheral lymphocytes	150, 300 or 350 µg/ml	Negative[b,r]	Meerts (2000)
1526	*O*-Ethyl *S*-furfuryl-thiocarbonate	Chromosomal aberration	Human peripheral lymphocytes	130, 240 or 280 µg/ml	Positive[i,s]	Meerts (2000)
1526	*O*-Ethyl *S*-furfuryl-thiocarbonate	Chromosomal aberration	Human peripheral lymphocytes	100, 130 or 240 µg/ml	Positive[i,t]	Meerts (2000)
1526	*O*-Ethyl *S*-furfuryl-thiocarbonate	Chromosomal aberration	Human peripheral lymphocytes	150, 325 or 375 µg/ml	Negative/ positive[r,u,v]	Meerts (2000)

Table 5 (contd)

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
In vivo						
1487	2-Methylfuran	Chromosomal	Swiss albino mice (bone-	1000, 2000 or 4000 ppm	Negative	Subramanyam et al.
		aberration	marrow cells and spermatocytes)	(100, 200 or 400 mg/kg bw per day)[w]		(1989)
1503 (1993)	2-Furyl methyl	Chromosomal	Swiss albino mice (bone	1000, 2000 or 3000 ppm	Positive[y,z]	Sujatha et al.
	ketone	aberration	marrow)	(20, 40 or 60 mg/kg bw)[x]		
1503 (1993)	2-Furyl methyl	Chromosomal	Swiss albino mice	1000, 2000 or 3000 ppm	Negative[aa]	Sujatha et al.
	ketone	aberration	(spermatocytes)	(20, 40 or 60 mg/kg bw)[x]		
1526	*O*-Ethyl *S*-furfuryl-thiocarbonate	Micronucleus induction	NMRI BR mice (bone marrow)	100, 250,or 500 mg/kg bw[bb]	Negative	Verspeek-Rip (2001)

[a] Calculated from a relative molecular mass for 2-methylfuran = 82.1
[b] With and without metabolic activation
[c] Pre-incubation method
[d] Occasional incidences of slight-to-complete clearing of background lawn at higher concentrations
[e] Negative at all concentrations with metabolic activation; positive without metabolic activation
[f] Clastogenic activity decreased with metabolic activation (statistical significance of results not specified)
[g] Calculated from a relative molecular mass for 2,5-dimethylfuran = 96.13
[h] Positive at all concentrations without metabolic activation; with metabolic activation, negative at 190 µg/disc but positive at higher concentrations
[i] Without metabolic activation
[j] Calculated from a relative molecular mass for 2-furyl methyl ketone = 110.11
[k] Positive only in strain TA98; increased with metabolic activation
[l] Negative at 550 µg/disc; positive at 5500 and 55 000 µg/disc with and without metabolic activation
[m] Cytotoxicity at 12 398 µg/ml with metabolic activation
[n] Clastogenic activity increased with metabolic activation (statistical significance of results not specified)

Table 5 (contd)

[o] Cytotoxicity at 3333 μg/plate in all *S. typhimurium* strains and at 2166 μg/plate in *S. typhimurium* TA100 and TA1537
[p] Cytotoxicity at 3300 μg/plate in all *S. typhimurium* strains and at 1000 μg/plate in *S. typhimurium* TA100 and TA1535
[q] Cytotoxicity at 3300 μg/plate without metabolic activation
[r] 3-h continuous exposure
[s] 24-h continuous exposure
[t] 48-h continuous exposure
[u] With metabolic activation
[v] Statistically significant dose-dependent increases in chromosomal aberrations at two highest concentrations (325 and 375 μg/ml)
[w] Rats received 2-methylfuran in the diet for 5 consecutive days at 24-h intervals.
[x] Two experimental protocols were used: in one, animals received single oral doses of the test compound; in the other, the test compound was given orally once a day at the same concentrations as in the single-dose study for 5 consecutive days with 24-h intervals between doses.
[y] No effects observed at 1000 ppm and mild but significant ($p < 0.05$) effects at higher concentrations
[z] Chromosomal aberrations observed in the presence of significant mitodepression
[aa] Statistically significant increase in chromosomal aberrations 3 weeks after single dose of 3000 ppm; statistically significant increases in polyploidy and XY univalents at weeks 3 and 4 in rats given multiple doses of 3000 ppm
[bb] Single dose administered by gavage

1986; Shinohara et al., 1986; Asquith, 1989; Eder et al., 1991; Zeiger et al., 1992; Lee et al., 1994; Verspeek-Rip, 2000). Likewise, with the exception of a single assay in which equivocal results were reported in *S. typhimurium* strains TA97 and TA107 (Zeiger et al., 1992), 2-methylfuran (No. 1487) gave consistently negative results in several other strains of *S. typhimurium* (TA98, TA100, TA102 and TA1535) either alone or with bioactivation (Shinohara et al., 1986; Aeschbacher et al., 1989). 2-Furyl methyl ketone (No. 1503) evaluated with and without metabolic activation in *S. typhimurium* at concentrations up to 0.660 μmol/plate (54.2 μg/plate) had significant mutagenic potential only in strain TA98 with metabolic activation at the two lower concentrations (0.165 and 0.330 μmol/plate). At higher concentrations, significant cytotoxicity was observed, as reflected in a concentration-dependent decrease in the number of revertants (Shinohara et al., 1986).

Bacterial mutagenicity testing of furans that can be metabolically oxidized to reactive α,β-unsaturated dicarbonyl (2-ene-1,4-dicarbonyl) intermediates is problematic owing to their high bacterial toxicity. The cytotoxicity of these substances is believed to arise from their interactions with protein sulfhydryl and amino groups (Marnett et al., 1985; Eder et al., 1992). Owing to the nature of the GSH conjugation pathway, reactions in which high concentrations of α,β-unsaturated carbonyl compounds are formed are likely to promote oxidative stress. It is anticipated that cells exposed to high concentrations of these types of substances will rapidly deplete GSH, eventually leading to cellular damage and decreased cell viability, as indicated by the results described above.

O-Ethyl *S*-furfurylthiocarbonate (No. 1526) had no mutagenic potential when tested in *Escherichia coli* WP2uvrA at concentrations up to 3330 μg/plate, with or without metabolic activation (Verspeek-Rip, 2000). 3-(2-Furyl)acrolein (No. 1497) was not mutagenic in *E. coli* PQ37 under the conditions of the SOS Chromotest (Eder et al., 1991); in a subsequent evaluation, however, both 3-(2-furyl)acrolein (No. 1497) and 2-furyl methyl ketone (No. 1503) gave slightly positive results in the SOS Chromotest without metabolic activation, as evidenced by 1.7- and 1.8-fold increases in the SOS induction factor over a background value of 1, respectively. (Results are considered to be significant if the induction factor is at least 1.5.) (Eder et al., 1993).

In the rec assay, in which differences in growth inhibition zones of DNA repair-competent and DNA repair-impaired organisms are used to detect DNA-damaging activity, *Bacillus subtilis* strains H17 (rec^+) and M45 (rec^-) were incubated with 2-methylfuran (No. 1487), 2,5-dimethylfuran (No. 1488) or 2-furyl methyl ketone (No. 1503) at concentrations up to 55 000 μg/disc, with and without metabolic activation (Shinohara et al., 1986). 2-Furyl methyl ketone was not active at a concentration of 550 μg/disc but gave positive results at concentrations ≥ 5500 μg/disc with and without metabolic activation. Likewise, 2,5-dimethylfuran was not active at the lowest concentration tested (190 μg/disc) with metabolic activation, but gave positive results at every concentration tested in the absence of metabolic activation. In contrast, 2-methylfuran gave negative results with metabolic activation and induced significant differences in the zones of inhibition only without metabolic activation. Additionally, 2-methylfuran and 2-acetylfuran were reported to cleave the double strand of λ-phage DNA in the presence of Cu^{2+}; however, in the absence of a negative control, the statistical significance of these results could not be assessed. Furthermore, potential concomitant cytotoxicity was not monitored in this study.

In order to examine potential genotoxicity in mammalian cells, 2-methylfuran (No. 1487), 2,5-dimethylfuran (No. 1488), and 2-furyl methyl ketone (No. 1503) were incubated with Chinese hamster ovary cells, which were then evaluated for chromosomal aberrations. All three compounds increased the number of chromosomal aberrations (statistical significance not specified) in the absence of metabolic activation; however, in the presence of metabolic activation, only the clastogenicity of 2-furyl methyl ketone was increased, whereas the activities of 2-methylfuran and 2,5-dimethylfuran were reduced. Additionally, when NADP was not included in the activation system, the numbers of chromosomal aberrations observed with 2-methylfuran and 2,5-dimethylfuran were reduced and the increase in the clastogenic activity of 2-furyl methyl ketone in the presence of the activation system was abolished (Stich et al., 1981). These results suggest that mixed-function oxidases are integral in the metabolism of alkyl furan derivatives.

In the late 1980s, researchers began studying the test conditions (e.g. osmolality, ionic strength, low pH) that could increase the frequency of chromosomal aberrations and micronuclei in the absence of a direct effect on DNA (Zajac-Kaye & Ts'o, 1984; Brusick, 1986; Bradley et al., 1987; Galloway et al., 1987; Seeberg et al., 1988; Morita et al., 1989; Scott et al., 1991). More recent research indicates that extreme culture conditions (hypo- and hyperosmolality and high pH) induce apoptosis and necrosis, leading to DNA fragmentation, producing false-positive responses in assays for clastogenicity (Meintieres & Marzin, 2004).

Apoptosis is a type of cell death that occurs under physiological conditions or external stimuli, such as DNA-damaging agents, growth factor deprivation or receptor triggering. The mechanism of formation of apoptotic cells includes activation of cysteine proteases (caspases), leading to increased mitochondrial permeability, release of cytochrome cDNA, cleavage and redistribution of phosphatidyl serine to the outer layers of the cell membrane, which enhances binding of cells to phagocytes. DNA cleavage, due to irreversible activation of endonucleases, is followed by chromatin condensation and oligonucleosomal fragmentation resulting from double-strand cleavage of DNA in nucleosomal linker regions (Saraste & Pulkki, 2000). During chromatin condensation, the nucleus can split into a number of dense micronuclei. Fragmented DNA and chromatin condensation due to apoptotic events are not easily distinguished from a direct action of a specific chemical. In view of these observations, evidence for chromosomal aberrations must be evaluated in the context of the potential for apoptosis to occur under the test conditions. Relatively high concentrations (≤ 1923–12 315 μg/ml) were used in the study of Stich et al. (1981), and no information was available on the culture conditions. The results for chromosomal aberration and micronucleus induction are difficult to interpret in the absence of this information.

In a study of the effect of oxygen scavengers on cadmium chloride-induced chromosomal aberrations in Chinese hamster V79 cells, 96.13 μg/ml of 2,5-dimethylfuran did not increase the frequency of chromosomal aberrations in comparison with control values. When 2,5-dimethylfuran was incubated at the same dose with the V79 cells in the presence of cadmium chloride, no reduction in the clastogenic capacity of cadmium chloride was observed (Ochi & Ohsawa, 1985).

A series of assays was conducted to determine the clastogenicity of *O*-ethyl-*S*-furfurylthiocarbonate (No. 1526) in human peripheral lymphocytes. The doses used were based on a preliminary evaluation of the mitotic index in the cells. As,

generally, *O*-ethyl *S*-furfurylthiocarbonate had marked mitogenicity and cytotoxicity, only a relatively narrow range of concentrations was used. In the first set of tests, with an exposure time of 3 h, the compound did not induce an increase at concentrations of 150–350 μg/ml with or without metabolic activation. In a trial with a 3-h exposure period and with metabolic activation, significant, dose-dependent increases in the number of chromosomal aberrations were observed at concentrations of 325 and 375 μg/ml but not at 150 μg/ml. Moreover, after a 24- or 48-h exposure period, *O*-ethyl *S*-furfurylthiocarbonate (at up to 280 μg/ml) induced dose-dependent, statisti-cally significant increases in the number of chromosomal aberrations in the absence of metabolic activation in comparison with a negative control (Meerts, 2000).

In vivo

As reported in an abstract, no chromosomal aberrations were observed in bone-marrow cells or spermatocytes of Swiss albino mice given 2-methylfuran (No. 1487) in the diet at a concentration of 1000, 2000 or 4000 ppm (approximately 100, 200 and 400 mg/kg bw per day, respectively) at 24-h intervals for 5 days. Moreover, 2-methylfuran did not inhibit spindle protein synthesis or cell division in the somatic cells. In the germ cells, which were evaluated at weekly intervals for 5 weeks after the final dose to cover one full spermatogenetic cycle, no structural sperm-head abnormalities were found (Subramanyam et al., 1989).

In a comparison of the potential clastogenic activity of 2-furyl methyl ketone and 2-methylfuran in somatic and germ cells, groups of two Swiss albino mice per dose and per sampling time were given the compounds orally at concentrations of 0, 1000, 2000 or 3000 ppm in 0.5 ml water (approximately 0, 20, 40 and 60 mg/kg bw, respectively), either as a single dose or once daily for 5 consecutive days. Bone-marrow cells were collected periodically between 6 and 72 h after the last dose, while meiotic and sperm preparations from testes and epididymis, respectively, were assessed at 24 h and weekly for 5 weeks after treatment. In bone-marrow cells, 2-methylfuran inhibited mitosis beginning 18 h after single or multiple doses of the highest dose. By 24 h, mitodepression was observed with the single high dose and also with multiple intermediate and highest doses. In the multiple-dose protocol, the effect remained significant for up to 36 h after treatment. The mitodepression was accompanied by a significant increase in the incidence of chromosomal aberrations in the bone-marrow cells. Thus, at 3000 ppm, the incidence of structural chromosomal aberrations was significantly increased 18–24 h after administration of a single dose and 12 and 48 h after the final dose in multiple-dose groups. In animals receiving multiple doses of 2-furyl methyl ketone, significant increases in the number of chromosomal aberrations were observed at 2000 ppm 24–36 h after treatment. In contrast to the dose- and time-dependent increase in chromosomal aberrations in somatic cells, only a single statistically significant increase in structural chromosomal aberrations was observed in mouse spermatocytes 3 weeks after a single dose of 2-methylfuran and only at the highest dose. After administration of multiple doses, the abnormalities in germ cells were limited to significant increases in polyploidy and XY univalents, which occurred at weeks 3 and 4 at the highest dose. No sperm-head abnormalities were observed at any dose, irrespective of the treatment protocol. The absence of sperm-head abnormalities at all doses indicates that the substance has no spermatoxic activity. The Committee concluded that 2-furyl methyl ketone

has only mild clastogenic activity in mouse bone marrow and is not clastogenic in germ cells (Sujatha et al., 1993).

Groups of five NMRI BR mice of each sex per sampling time were given a single dose of 0 (vehicle control), 100, 250 or 500 mg/kg bw of *O*-ethyl *S*-(2-furylmethyl)thiocarbonate (No. 1526) in corn oil by gavage and were killed 24 h later. A second group of mice at 500 mg/kg bw and a positive control group were killed 48 h after dosing. Bone-marrow smears were prepared from the femurs. No increase in the incidence of micronucleated polychromatic erythrocytes was observed in bone-marrow cells when compared with controls. The authors noted that cells obtained from treated animals did not show a reduction in the ratio of polychromatic to normochromatic erythrocytes, indicating that the compound is not cytotoxic (Verspeek-Rip, 2001).

Conclusion

Four of the eight chemicals gave only negative results in tests for genotoxicity, and all of the four that gave positive results (Nos 1487, 1488, 1497 and 1503) also gave negative results. No. 1487 gave a negative result in vivo, whereas No. 1503 gave a positive result.

With a few exceptions, representative agents of this group gave consistently negative results in assays for mutation in various strains of *S. typhimurium* and *E. coli*. Equivocal results were obtained in the rec assay in *B. subtilis*. In assays for genotoxicity in Chinese hamster ovary and V79 cells and human peripheral lymphocytes, the results were inconsistent. Although positive results were reported in the assay for chromosomal aberrations in Chinese hamster ovary cells conducted by Stich et al. (1981), relatively high concentrations ($\leq$ 1923 µg/ml) were used, the statistical significance of the results was not specified and potential cytotoxicity was not monitored. Moreover, positive results in tests for chromosomal aberrations in vitro are difficult to interpret in the presence of concomitant cytotoxicity and cell cycle delay, which appear to be characteristic of the furan derivatives. Mammalian cells in culture might not have enough metabolic capacity to counter this toxicity. In fact, with the exception of one assay in which an increase in the clastogenic activity of 2-furyl methyl ketone was reported in the presence of metabolic activation (Stich et al., 1981; statistical significance not reported), the frequency of chromosomal aberrations caused by other representative furan derivatives was reduced in the presence of metabolic activation (Stich et al., 1981; Meerts, 2000). Furthermore, although positive results were obtained with 2,5-dimethylfuran in Chinese hamster ovary cells at high concentrations (Stich et al., 1981), the compound was not clastogenic when tested at lower concentrations in Chinese hamster V79 cells (Ochi & Ohsawa, 1985).

With regard to assays conducted in vivo, mild clastogenic activity was reported in mouse bone-marrow cells treated with 2-furyl methyl ketone at a dose of 40 or 60 mg/kg bw, accompanied by significant mitodepression after single and multiple doses; however, no increases in chromosomal aberration frequency were observed in spermatocytes obtained from the same mice (Sujatha et al., 1993), Furthermore, the chromosomal aberration frequency was not increased in somatic or germ cells in another study with single doses in mice (Subramanyam et al., 1989). The weak clastogenic effects achieved statistical significance only after repeated daily exposure to near lethal doses. These transient effects declined quickly, as shown by their

reduction or absence more than 24 h after exposure. The frequency of micronucleus formation in mouse bone-marrow cells was comparable to control values after administration of a single dose of *O*-ethyl *S*-furfurylthiocarbonate in another experiment (Verspeek-Rip, 2001).

Thus, the results of the tests for genotoxicity and mutagenicity in vitro were mixed, positive results being reported less frequently in the presence of metabolic activation. This finding argues against the effects being due to CYP450 oxidation to ring-opened metabolites. The one positive result for induction of chromosomal aberrations in mouse bone marrow in vivo occurred under conditions toxic to the bone marrow. The results of tests for chromosomal effects in the bone marrow with two other agents were negative. Overall, the data do not provide a clear indication that this group of furan-substituted derivatives would be genotoxic, particularly under their conditions of use as flavouring agents. Moreover, as noted above, the findings in studies of toxicity with the agents that were found to be genotoxic indicate the probable absence of carcinogenicity.

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