ALIPHATIC AND AROMATIC AMINES AND AMIDES

First draft prepared by

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1. EVALUATION

1.1 Introduction

The Committee evaluated the group of 37 aliphatic and aromatic amine and amide flavouring agents shown in Table 1. The group comprised 13 primary aliphatic and aromatic amines (Nos 1579–1591), five tertiary aliphatic and aromatic amines (Nos 1610–1614), four alicyclic amines (Nos 1607–1609 and 1615), four aliphatic and alicyclic imines (Nos 1603–1606) and 11 amides (Nos 1592–1602). The evaluations were conducted according to the Procedure for the Safety Evaluation of

Table 1. Summary of results of safety evaluations of aliphatic and aromatic amines and amides used or proposed for use as flavouring agents

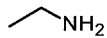
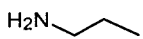
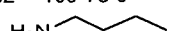
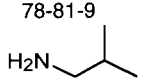
Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
Structural class I							
Ethylamine	1579	75-04-7 	No Europe: 0.1[b] USA: 0.2 [b]	N/R	N/R	See note 1	No safety concern (conditional)
Propylamine	1580	107-10-8 	No Europe: 0.01[b] USA: 0.02[b]	N/R	N/R	See note 1	No safety concern (conditional)
Isopropylamine	1581	75-31-0 	No Europe: 0.0[b] USA: 0.02[b]	N/R	N/R	See note 1	No safety concern (conditional)
Butylamine	1582	109-73-9 	No Europe: 104 USA: 0.01	N/R	N/R	See note 1	No safety concern
Isobutylamine	1583	78-81-9 	No Europe: 0.07[b] USA: 0.09[b]	N/R	N/R	See note 1	No safety concern (conditional)

Table 1 (contd)

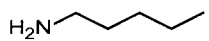
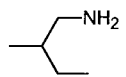
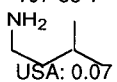
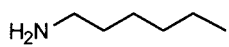
Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
sec-Butylamine	1584	13952-84-6 	No Europe: 2[b] USA: 2[b]	N/R	N/R	See note 1	No safety concern (conditional)
Pentylamine	1585	110-58-7 	No Europe: 0.1[b] USA: 0.2[b]	N/R	N/R	See note 1	No safety concern (conditional)
2-Methylbutylamine	1586	96-15-1 	No Europe: 0.01[b] USA: 0.02[b]	N/R	N/R	See note 1	No safety concern (conditional)
Isopentylamine	1587	107-85-7 	No Europe: 28 USA: 0.07	N/R	N/R	See note 1	No safety concern
Hexylamine	1588	111-26-2 	No Europe: 0.006[b] USA: 0.007[b]	N/R	N/R	See note 1	No safety concern (conditional)

Table 1 (contd)

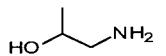
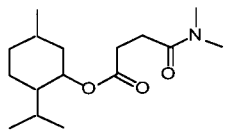
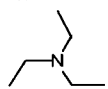
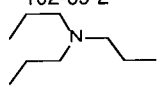
Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
1-Amino-2-propanol	1591	78-96-6 	No Europe: ND USA: 16[b]	N/R	N/R	See note 1	No safety concern (conditional)
(+/–)-*N*,*N*-Dimethyl-menthyl succinamide	1602	544714-08-1 	No Europe: 71[b] USA: 88[b]	N/R	N/R	See notes 2 and 3	No safety concern (conditional)
Trimethylamine	1610	75-50-3 	No Europe: 153 USA: 70	N/R	N/R	See note 4	No safety concern
Triethylamine	1611	121-44-8 	No Europe: 0.7[b] USA: 0.9[b]	N/R	N/R	See note 4	No safety concern (conditional)
Tripropylamine	1612	102-69-2 	No Europe: 0.01[b] USA: 0.02[b]	N/R	N/R	See note 4	No safety concern (conditional)

Table 1 (contd)

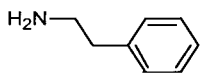
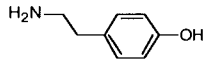
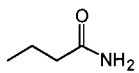
Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
Trimethylamine oxide	1614	1184-78-7 	No Europe: 0.07[b] USA: 0.09[b]	N/R	N/R	See note 5	No safety concern (conditional)
Structural class II							
Phenethylamine	1589	64-04-0 	No Europe: ND USA: 0.05	N/R	N/R	See note 6	No safety concern
2-(4-Hydroxyphenyl)-ethylamine	1590	51-67-2 	No Europe: 0.01[b] USA: 0.02[b]	N/R	N/R	See note 7	No safety concern (conditional)
Butyramide	1593	541-35-5 	No Europe: 0.001[b] USA: 0.002[b]	N/R	N/R	See notes 2 and 8	No safety concern (conditional)
1-Pyrroline	1603	5724-81-2 	No Europe: ND USA: 0.4[b]	N/R	N/R	See note 9	No safety concern (conditional)

Table 1 (contd)

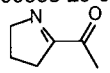
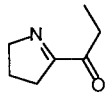
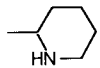
Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
2-Acetyl-1-pyrroline	1604	99583-29-6 	No Europe: 0.09[b] USA: 0.1[b]	N/R	N/R	See note 10	No safety concern (conditional)
2-Propionylpyrroline	1605	133447-37-7 	No Europe: 0.1[b] USA: 0.2[b]	N/R	N/R	See note 10	No safety concern (conditional)
Piperidine	1607	110-89-4 	No Europe: 103 USA: 96	N/R	N/R	See note 11	No safety concern
2-Methylpiperidine	1608	109-05-7 	No Europe: 0.001[b] USA: 0.002[b]	N/R	N/R	See note 11	No safety concern (conditional)
Pyrrolidine	1609	123-75-1 	No Europe: 0.2 USA: 2	N/R	N/R	See note 11	No safety concern

Table 1 (contd)

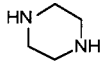
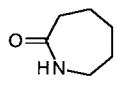
Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
Piperazine	1615	110-85-0 	No Europe: 0.001[b] USA: 0.002[b]	N/R	N/R	See note 11	No safety concern (conditional)
Structural class III							
1,6-Hexalactam	1594	105-60-2 	No Europe: 0.001[b] USA: 0.002[b]	Yes. The NOEL of 750 mg/kg bw per day (National Toxicology Program, 1982) is at least 2.5 x 1010 times the estimated daily intake of 0.00002 µg/kg bw in Europe and 0.00003 µg/kg bw in the USA) from its proposed use as a flavouring agent.		See notes 2 and 8	No safety concern (conditional)

Table 1 (contd)

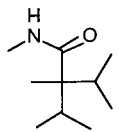
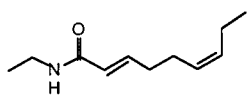
Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
2-Isopropyl-*N*,2,3-trimethylbutyramide	1595	51115-67-4 	Yes Europe: ND USA: 1054[b]	Yes. There is a 14-day study in rats (Nixon & Alden, 1978) and two 14-week studies in rats (Pence, 1980a; Cheng, 1982), as well as a study of reproduction and teratogenicity in rats (Pence, 1980b). The NOEL of 5 mg/kg bw per day in these studies is 280 times the estimated daily intake of 18 µg/kg bw from its proposed use as a flavouring agent in the USA.		See notes 2 and 8	No safety concern (conditional)
N-Ethyl (E)-2,(Z)-6-nonadienamide	1596	608514-56-3 	No Europe: ND USA: 88[b]	Yes. The NOEL of 572 mg/kg bw per day for the structurally related substance *N*-isobutyl-2,6,8-decatrienamide Moore, 2002) is 600 000 times the estimated daily intake of N-ethyl(E)-2,(Z)-6-nonadienamide of 1 µg/kg bw from its proposed use as a flavouring agent in the USA.		See notes 2 and 8	No safety concern (conditional)

Table 1 (contd)

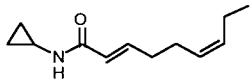
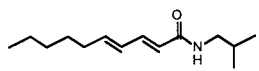
Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
N-Cyclopropyl (E)-2, (Z)-6-nonadienamide	1597	608514-55-2 	No Europe: ND USA: 40[b]	Yes. The NOEL of 572 mg/kg bw per day for the structurally related substance *N*-isobutyl-2,6,8-decatrienamide (Moore, 2002) is > 800 000 times the estimated daily intake of *N*-cyclopropyl-(E)-2,(Z)-6-nonadienamide of 0.7 µg/kg bw from its proposed use as a flavouring agent in the USA.		See notes 2 and 8	No safety concern (conditional)
N-Isobutyl (E,E)-2,4-decadienamide	1598	18836-52-7 	No Europe: 67[b] USA: 83[b]	Yes. The NOEL of 572 mg/kg bw per day for the structurally related substance *N*-isobutyl-2,6,8-decatrienamide (Moore, 2002) is at least 600 000 times the estimated daily intake of *N*-isobutyl-(E,E)-2,4-decadienamide of 1 µg/kg bw from its proposed use as a flavouring agent in Europe and the USA.		See notes 2 and 8	No safety concern (conditional)

Table 1 (contd)

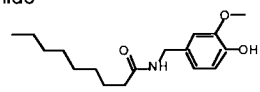
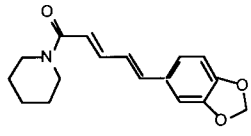
Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
Nonanoyl 4-hydroxy-3-methoxybenzylamide	1599	2444-46-4 	No Europe: 7 USA: 0.07[b]	Yes. The NOEL of 8.4 mg/kg bw per day (Posternak et al., 1969) is at least 70 000 times the estimated daily intake from its reported use as a flavouring agent in Europe (0.12 µg/kg bw) and 8 400 000 times that in the USA (0.001 µg/kg bw).		See note 12	No safety concern
Piperine	1600	94-62-2 	No Europe: 23 USA: 0.07	Yes. The NOEL of 20 mg/kg bw per day (Bhat & Chandrasekhara, 1986b) is 50 000 times the estimated daily intake from its reported use as a flavouring agent in Europe (0.4 µg/kg bw) and 20 000 000 times that in the USA (0.001 µg/kg bw).		See note 13	No safety concern

Table 1 (contd)

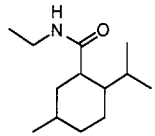
Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
N-Ethyl-2-isopropyl-5-methylcyclohexane-carboxamide	1601	39711-79-0 	Yes Europe: 0.5 USA: 127	Yes. There is a 28-day (Miyata, 1995) and a 22-week study in rats (Hunter et al., 1975) and a 28-day and a 52-week study in dogs (James, 1974). The NOEL of 8 mg/kg bw per day in the studies in rats (Miyata, 1995) is 1 000 000 times the estimated daily intake of *N*-ethyl 2-isopropyl-5-methylcyclohexanecar-boxamide from its reported use as a flavouring agent in Europe (0.008 μg/kg bw) and 4000 times that in the USA (2 μg/kg bw).	See notes 2 and 8	No safety concern

Table 1 (contd)

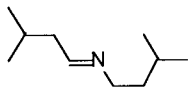
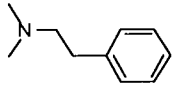
Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
Isopentylidene isopentylamine	1606	35448-31-8 	No Europe: 0.009[b] USA: 0.01[b]	Yes. The NOEL of 115 mg/kg bw per day for the related substance *sec*-butylamine (No. 1584) (Gage, 1970) is at least 5.75 x 10^8 times the estimated daily intake of isopentylidene isopentylamine from its proposed use as a flavouring agent in Europe (0.0001 µg/kg bw) and in the USA (0.0002 µg/kg bw).		See note 14	No safety concern (conditional)
N,N-Dimethylphenethylamine	1613	19342-01-9 	No Europe: 0.0[b] USA: 0.09[b]	Yes. The NOEL of 120 mg/kg bw per day) for the related substance phenethyl alcohol (No. 987) (Lynch et al., 1990) is at least 1 x 108 times the estimated daily intake of *N,N*-dimethylphenethylamine from its proposed use as a flavouring agent in Europe and the USA (0.001 µg/kg bw)		See note 4	No safety concern (conditional)

Table 1 (contd)

CAS, Chemical Abstracts Service; ND, no intake data reported; N/R, not required for evaluation because consumption of the substance was determined to be of no safety concern at Step A3 of the Procedure.
Step 1: Sixteen flavouring agents in this group are in structural class I, 11 are in structural class II and 10 are in structural class III (Cramer et al., 1978).
Step 2: Twenty-seven of the agents in this group (Nos 1579–1593, 1602–1605, 1607–1612, 1614 and 1615) are expected to be metabolized to innocuous products. The remaining 10 agents (Nos 1594-1601, 1606 and 1613) are not expected to be metabolized to innocuous agents.

[a] The thresholds for human intake of structural classes I, II and III are 1800, 540 and 90 μg/person per day, respectively. All intake values are expressed in μg/person per day. The combined per capita intakes of the flavouring agents in structural class I is 359 μg/person per day in Europe and 178 μg/person per day in the USA, that of the flavouring agents in structural class II is 103 μg/person per day in Europe and 99 μg/person per day in the USA, and that of the flavouring agents in structural class III is 98 μg/person per day in Europe and 1392 μg per day in the USA.
[b] Intake estimate based on anticipated annual volume of production

Notes:
1. Aliphatic primary amines readily undergo oxidative deamination, and the resulting aldehydes and ketones enter existing pathways of metabolism and excretion.
2. Amides undergo limited hydrolysis with the corresponding ammonium ion or amines and enter known pathways of metabolism and excretion.
3. Anticipated to undergo hydrolysis at the ester moiety, followed by conjugate formation and subsequent elimination in the urine.
4. Tertiary amines primarily undergo *N*-oxidation to form the corresponding N-oxide, which is readily excreted in the urine.
5. Trimethylamine oxide is expected to be readily excreted in the urine.
6. Phenethylamine undergoes oxidative deamination and further oxidation to form phenylacetic acid, which is readily excreted in the urine in conjugate form.
7. Tyramine undergoes rapid deamination by monoamine oxidase and is excreted as acidic metabolites.
8. Amides are expected to undergo oxidation and enter known pathways of metabolism.
9. Pyrroline, an imine, is anticipated to undergo hydrolysis to the corresponding iminoketone, which will be reduced to the corresponding alcohol.
10. The ketone moiety can be anticipated to be reduced to the corresponding alcohol, which will form glucuronic acid conjugates, which are excreted in the urine.
11. Alicyclic amines undergo both *N*- and *C*-oxidation, followed by excretion of the polar metabolites in the urine.
12. This phenolic substance is anticipated readily to form glucuronic acid conjugates, which are excreted in the urine.
13. Hydrolysis of the amide group of piperine and subsequent oxidation of metabolites to form conjugates of piperonylic acid and vanillic acid are expected.
14. This imine is expected to undergo hydrolysis to form isoamylamine and isoamyl aldehyde, which will enter known pathways of metabolism and excretion.

Flavouring Agents (see Figure 1, p. 170). None of these flavouring agents has been evaluated previously by the Committee.

The Committee noted that the available data on one of the compounds in the group, acetamide (No. 1592), indicated that it was clearly carcinogenic in both mice and rats; although the mechanism of tumour formation is unknown, the possibility of a genotoxic mechanism cannot be discounted. The Committee considered it inappropriate for such a compound to be used as a flavouring agent or for any other food additive purpose, and agreed that acetamide would not be evaluated according to the Procedure.

Twenty-eight of the 36 remaining flavouring agents (Nos 1579–1591, 1593, 1598, 1600, 1603, 1604 and 1607–1615) have been reported to occur naturally in various foods. They have been detected in apple, banana, cabbage, carrot, lettuce, rutabaga, tomato, radish, sweet corn, potato, kale, celery, cauliflower, beetroot, rhubarb, sauerkraut, jackfruit, truffle, pepper, laurel, garlic, blue cheeses, Cheddar, Swiss, Camembert, Limburger, Manchengo, provolone, Russian and Tilset cheeses, caviar, fatty fish (raw, smoked, tinned or salted), lean fish (raw, processed or cooked), clam, squid, shrimp, oyster, crab, scallop, beef, pork, chicken, mutton, beer, red and white wine, sherry, sake, cider, cocoa, coffee, black and green tea, barley, oats, popcorn, rice, and wheat and rye breads (Nijssen et al., 2003).

1.2 Estimated daily per capita exposure

Annual volumes of production were reported for nine of the 36 flavouring agents in this group (Nos 1582, 1587, 1589, 1599–1601, 1607, 1609 and 1610). For the remaining 27 substances, anticipated annual volumes were given for their proposed use as flavouring agents. The total reported and anticipated annual volume of the 36 aliphatic and aromatic amines and amides is about 3900 kg in Europe (International Organization of the Flavor Industry, 1995) and 9900 kg in the USA (Lucas et al., 1999). About 64% of the total reported and anticipated annual volume in Europe is accounted for by butylamine (No. 1582), piperidine (No. 1607) and trimethylamine (No. 1610), and about 78% in the USA is accounted for by 2-isopropyl-*N*-2,3-trimethylbutyramide (No. 1595), *N*-ethyl-2-isopropyl-5-methylcyclohexane carboxamide (No. 1601) and piperidine (No. 1607). The estimated per capita exposure in Europe to butylamine, piperidine and trimethylamine is about 100, 100 and 150 μg/person per day, respectively. The estimated per capita exposure in the USA to 2-isopropyl-*N*,2,3-trimethylbutyramide, *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide and piperidine is 1054, 127 and 96 μg/day, respectively. The estimated per capita exposure to all the other flavouring agents in the group is 0.001–71 μg/day in Europe and 0.002–88 μg/person per day in the USA (International Organization of the Flavor Industry, 1995; Lucas et al., 1999), most of the values being at the lower end of the ranges. The estimated per capita exposure to each agent is reported in Table 2.

1.3 Absorption, distribution, metabolism and elimination

A number of the amines in this group are endogenous and have been identified as normal constituents of urine from healthy individuals, as a result of the catabolism of sarcosine, creatine and choline. These include trimethylamine (No. 1610), ethylamine (No. 1579), isopentylamine (No. 1587), piperidine (No. 1607), pyrrolidine

Table 2. Annual volumes of production of aliphatic and aromatic amines and amides 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		
Ethylamine (1579)					
Europe[e]	1	0.1	0.002		
USA[e]	1	0.2	0.003	+	NA
Propylamine (1580)					
Europe[e]	0.1	000	0.0002		
USA[e]	0.1	000	0.0003	+	NA
Isopropylamine (1581)					
Europe[e]	0.1	000	0.0002		
USA[e]	0.1	000	0.0003	+	NA
Butylamine (1582)					
Europe	727	104	2		
USA	0.1	000	0.0002	+	NA
Isobutylamine (1583)					
Europe[e]	0.5	000	0.001		
USA[e]	0.5	000	0.001	+	NA
sec-Butylamine (1584)					
Europe[e]	14	2	000		
USA[e]	14	2	000	+	NA
Pentylamine (1585)					
Europe[e]	1	0.1	0.002		
USA[e]	1	0.2	0.003	+	NA
2-Methylbutylamine (1586)					
Europe[e]	0.1	000	0.0002		
USA[e]	0.1	000	0.0003	+	NA
Isopentylamine (1587)					
Europe	198	28	0.5		
USA	0.5	000	0.001	1 505.0	3 010
Hexylamine (1588)					
Europe[e]	0.04	0.006	0.0001		
USA[e]	0.04	0.007	0.0001	+	NA
Phenethylamine (1589)					
Europe	ND	ND	ND		
USA	0.4	000	0.0009	23 045	57 613
Tyramine (1590)					
Europe[e]	0.08	000	0.0002		
USA[e]	0.1	000	0.0002	+	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-Amino-2-propanol (1591)					
Europe	ND	ND	ND		
USA[e]	90	16	0.3	+	NA
Butyramide (1593)					
Europe[e]	0.01	0.001	0.00002		
USA[e]	0.01	0.002	0.00003	+	NA
1,6-Hexalactam (1594)					
Europe[e]	0.01	0.001	0.00002		
USA[e]	0.01	0.002	0.00003	–	NA
2-Isopropyl-*N*,2,3-trimethylbutyramide (1595)					
Europe	ND	ND	ND		
USA[e]	6000	1054	18	–	NA
N-Ethyl (E)-2,(Z)-6-nonadienamide (1596)					
Europe	ND	ND	ND		
USA[e]	500	88	1	–	NA
N-Cyclopropyl (E)-2,(Z)-6-nonadienamide (1597)					
Europe	ND	ND	ND		
USA[e]	225	40	0.7	–	NA
N-Isobutyl (E,E)-2,4-decadienamide (1598)					
Europe[e]	470	67	1		
USA[e]	470	83	1	+	NA
Nonanoyl 4-hydroxy-3-methoxybenzylamide (1599)					
Europe	49	7	0.12		
USA[e]	0.5	000	0.001	–	NA
Piperine (1600)					
Europe	162	23	0.4		
USA	0.5	000	0.001	1 762 030	3 524 060
N-Ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (1601)					
Europe	3.3	0.5	0.008		
USA	962	127	2	–	NA
(±)-*N*,*N*-Dimethyl menthyl succinamide (1602)					
Europe[e]	500	71	1		
USA[e]	500	88	1	–	NA
1-Pyrroline (1603)					
Europe	ND	ND	ND		
USA[e]	2	0.4	0.006	+	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-Acetyl-1-pyrroline (1604)					
Europe[e]	0.6	000	0.001		
USA[e]	0.6	0.1	0.002	+	NA
2-Propionylpyrroline (1605)					
Europe[e]	1	0.1	0.002		
USA[e]	1	0.2	0.003	–	NA
Isopentylidene isopentylamine (1606)					
Europe[e]	0.06	0.009	0.0001		
USA[e]	0.06	000	0.0002	–	NA
Piperidine (1607)					
Europe	720	103	2		
USA	730	96	2	1 115	2
2-Methylpiperidine (1608)					
Europe[e]	0.01	0.001	0.00002		
USA[e]	0.01	0.002	0.00003	+	NA
Pyrrolidine (1609)					
Europe	1	0.1	0.002		
USA	13	2	000	6 693	515
Trimethylamine (1610)					
Europe	1074	153	3		
USA	395	52	1	185	0.5
Triethylamine (1611)					
Europe[e]	5	0.7	000		
USA[e]	5	0.9	000	+	NA
Tripropylamine (1612)					
Europe[e]	0.1	000	0.0002		
USA[e]	0.1	000	0.0003	+	NA
N,N-Dimethylphenethylamine (1613)					
Europe[e]	0.5	000	0.001		
USA[e]	0.5	000	0.001	+	NA
Trimethylamine oxide (1614)					
Europe[e]	0.5	000	0.001		
USA[e]	0.5	000	0.001	+	NA
Piperazine (1615)					
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		
Total					
Europe	3 929				
USA	9 914				

NA, not available; ND, no intake data reported; +, reported to occur naturally in foods (Nijssen et al., 2003), but no quantitative data; –, not reported to occur naturally in foods

[a] From International Organization of the Flavour Industry (1995) and Lucas et al. (1999).

[b] Intake (μg/person per day) calculated as follows:
[[(annual volume, kg) × (1×10^9 μg/kg)]/[population x survey correction factor × 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 the USA (National Academy of Sciences surveys and anticipated annual volumes) and 0.8 for the survey of Lucas et al., representing the assumption that only 60% and 80% of the annual flavour volume, respectively, was reported in the poundage surveys (International Organization of the Flavor Industry, 1995; Lucas et al., 1999) or in the anticipated annual volume.
Intake (μg/kg bw per day) calculated as follows:
[(μg/person per day)/bw], 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)
The volume cited is the anticipated annual volume, which is the maximum amount of flavour estimated to be used annually by the manufacturer at the time the material was proposed for flavour use.

(No. 1609), phenethylamine (No. 1589) and trimethylamine oxide (No. 1614) (Rechenberger, 1940; Wranne, 1956; Williams, 1959; Ayesh et al., 1993; Zhang et al., 1993).

Aliphatic amines are metabolized primarily by flavin-containing monooxygenases, monoamine oxidases or amine oxidases by a process known as oxidative deamination. The initial step is hydroxylation of the carbon adjacent to the nitrogen (*C*-oxidation), followed by formation of an imine, with concomitant reduction of molecular oxygen to hydrogen peroxide. The resulting imine is rapidly hydrolysed to the corresponding aldehyde, which is oxidized to the corresponding carboxylic acid. Representative primary aliphatic and aromatic amines in this group are readily absorbed and rapidly metabolized to carboxylic acids, which are excreted in the urine.

Alicyclic secondary amines (Nos 1607–1609 and 1615) also undergo *C*-oxidation at the α-carbon, but oxidation can also occur at other carbons on the ring. The alicyclic imines in this group (Nos 1603–1606) are readily absorbed and rapidly hydrolysed in aqueous solution to yield the corresponding aminoaldehyde or iminoketone, both of which are further metabolized.

Primary, secondary and tertiary amines can also undergo *N*-oxidation by cytochrome P450 enzymes. Primary aliphatic amines with an accessible α-substituted carbon atom can be *N*-oxidized to nitroso groups and subsequently to oximes, which are labile and readily hydrolysed. Secondary amines can be *N*-oxidized to reactive hydroxylamines, which are further oxidized to form nitrones, which are readily hydrolysed. For tertiary amines, *N*-oxidation by flavin-dependent monooxygenases is the primary route of metabolism, resulting in the formation of stable *N*-oxides.

Tertiary aliphatic amines can also be metabolized by *C*-oxidation, leading to dealkylation and formation of the corresponding primary and secondary amines and an aliphatic aldehyde or ketone.

The aliphatic amides in this group are reported to undergo limited hydrolysis, the extent of which depends to some extent on the chain length. They are well absorbed and metabolized to polar metabolites, although there are limited data on the actual metabolic routes of the amides in this group; a variety of polar metabolites are detected in the urine of animals after an oral dose.

The available data on the aliphatic and aromatic amines in this group indicate that they are likely to be rapidly absorbed in the gastrointestinal tract and transformed by well-understood metabolic pathways to polar metabolites, which are rapidly eliminated in the urine. The information on the amides in this group is more limited.

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

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

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to these flavouring agents, the Committee assigned 16 agents (Nos 1579–1588, 1591, 1602, 1610–1612 and 1614) to structural class I, 10 flavouring agents (Nos 1589, 1590, 1593, 1603–1605, 1607–1609 and 1615) to structural class II and the remaining 10 flavouring agents (Nos 1594–1601, 1606 and 1613) to structural class III.

Step 2. Twenty-six flavouring agents in this group, namely all those in structural classes I and II (Nos 1579–1591, 1593, 1602–1605, 1607–1612, 1614 and 1615), are predicted to be metabolized to innocuous products. The evaluation of these agents therefore proceeded via the A-side of the procedure. For the 10 flavouring agents in structural class III, namely the medium chain saturated and unsaturated aliphatic and alicyclic amides (Nos 1594–1601 and 1606) and *N*,*N*-dimethylphenethylamine (No. 1613),

limited metabolic data were available, and evaluation of these agents therefore proceeded via the B-side of the procedure.

Step A3. The estimated daily per capita exposure to all 16 flavouring agents in structural class I is below the threshold of concern (1800 μg/day for class I). Three of these 16 substances (Nos 1582, 1587 and 1610) are reported to be used as flavouring agents, and, according to the Procedure, use of these three agents and their estimated current intakes raise no safety concern. The other 13 substances (Nos 1579–1581, 1583–1586, 1588–1602 and 1611–1614) are proposed for use as flavouring agents. Although, according to the Procedure, use of these 13 agents raises no safety concern at the exposure estimated from anticipated volumes of production, less uncertain estimates are needed. The estimated per capita exposure to all 10 flavouring agents in structural class II is below the threshold of concern (540 μg/day). Three of these 10 substances (Nos 1589, 1607 and 1609) are reported to be used as flavouring agents, and, according to the Procedure, their use raises no safety concern at the estimated current exposure. The other seven substances (Nos 1590, 1593, 1603–1605, 1608 and 1615) are proposed for use as flavouring agents. Although, according to the Procedure, use of these seven agents raises no safety concern at the intakes estimated from anticipated volumes of production, less uncertain exposure estimates are needed.

Step B3. The estimated per capita exposure to eight of the flavouring agents in structural class III (Nos 1594, 1596–1600, 1606 and 1613) is below the threshold of concern (90 μg/day). One of these substances (No. 1600) is reported to be used as a flavouring agent in Europe and the USA, one (No. 1599) is reported to be used in Europe and to be proposed for use in the USA, and six (Nos 1594, 1596–1598, 1606 and 1613) are proposed for use in both regions. For those seven substances proposed for use in one or more regions (Nos 1594, 1596–1599, 1606 and 1613), less uncertain exposure estimates are needed. In accordance with the Procedure, evaluation of these eight flavouring agents proceeded to Step B4. The per capita exposure in the USA of the two remaining flavouring agents in structural class III, 2-isopropyl-*N*-2,3-trimethyl-butyramide (No. 1595; intake, 1054 μg/day) and *N*-ethyl-2-isopropyl-5-methylcyclohexane carboxamide (No. 1601; intake, 127 μg/day), exceeds the threshold of concern for their class. In accordance with the Procedure, data must be available on these substances or closely related substances for an evaluation of safety. For No. 1595, which is proposed for use as a flavouring agent, a less uncertain exposure estimate is needed.

Step B4. The NOEL of 750 mg/kg bw per day for 1,6-hexalactam (No. 1594) (National Toxicology Program, 1982) is at least 2.5×10^{10} times higher than the estimated intake from its proposed use as a flavouring agent in Europe (0.00002 μg/kg bw per day) and in the USA (0.00003 μg/kg bw per day).

The NOEL of 572 mg/kg bw per day for the structurally related substance, *N*-isobutyl-2,6,8-decatrienamide (Moore, 2002), is applicable to *N*-ethyl-(E)-2,(Z)-6-nonadienamide (No. 1596), to *N*-cyclopropyl-(E)-2-(Z)-6-

nonadienamide (No. 1597) and to *N*-isobutyl-(E,E)-2,4-decadienamide (No. 1598), as they follow similar pathways of metabolism. This NOEL is 600 000 times the estimated intake of *N*-ethyl-(E)-2-(Z)-6-nonadienamide (No. 1596) from its proposed use as a flavouring agent in the USA (1 μg/kg bw per day) is also more than 800 000 times the estimated intake of *N*-cyclopropyl-(E)-2-(Z)-6-nonadienamide (No. 1597) from its proposed use as flavouring agent in the USA (0.7 μg/kg bw per day) and at least 600 000 times the estimated intake of *N*-isobutyl-(E,E)-2,4-decadienamide (No. 1598) from its proposed use as flavouring agent in Europe and in the USA (both 1 μg/kg bw per day).

The NOEL of 8.4 mg/kg bw per day for nonanoyl 4-hydroxy-3-methoxybenzylamide (No. 1599) (Posternak et al., 1969) is 70 000 times the estimated intake from its proposed use as a flavouring agent in Europe (0.12 μg/kg bw per day) and 8 400 000 times that in the USA (0.001 μg/kg bw per day).

The NOEL of 20 mg/kg bw per day for piperine (No. 1600) (Bhat & Chandrasekhara, 1986b) is 50 000 times the estimated intake of piperine from its reported use as a flavouring agent in Europe (0.4 μg/kg bw per day) and 20 000 000 times that in the USA (0.001 μg/kg bw per day).

The NOEL of 115 mg/kg bw per day for the structurally related substance *sec*-butylamine (No. 1584) (Gage, 1970) is applicable to isopentylidene isopentylamine (No. 1606) and is at least 5.75×10^8 times the estimated intake of isopentylidene isopentylamine from its proposed use as flavouring agent in Europe (0.0001 μg/kg bw per day) and in the USA (0.0002 μg/kg bw per day).

The NOEL of 120 mg/kg bw per day for the related substance phenethyl alcohol (No. 987) (Lynch et al., 1990) is applicable to *N,N*-dimethylphenethylamine (No. 1613) and is at least 1.2×10^8 times the estimated intake of *N,N*-dimethylphenethylamine from its proposed use as flavouring agent in Europe (0.001 μg/kg bw per day) and in the USA (0.001 μg/kg bw per day).

The Committee concluded that the margin between the estimated current intake of piperine (No. 1600), which is reported to be used as a flavouring agent, and the NOEL for this agent was adequate, and its use would not present a safety concern. The Committee also concluded that the margins between the estimated exposure to the other seven agents proposed for use as flavouring agents in one or more regions (Nos 1594, 1596–1599, 1606 and 1613) based on the anticipated annual volumes of production, and the NOELs for these agents were adequate. Although their use would raise no safety concern at the estimated exposure, less uncertain estimates are needed.

1.5 Consideration of flavouring agents with high exposure evaluated on the B-side of the Procedure

In accordance with the Procedure, more data on toxicity were considered to evaluate the safety of 2-isopropyl-*N*-2,3-trimethylbutyramide (No. 1595) and *N*-ethyl-

2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601), as the estimated intake levels from proposed use (No. 1595) and reported use (No. 1601) as flavouring agents were determined to exceed the threshold of concern for structural class III (90 μg/person per day).

The results of three studies in Sprague-Dawley (CD®) rats treated by gavage were available on 2-isopropyl-*N*-2,3-trimethylbutyramide: a 14-day study in groups of six rats of each sex at a dose of 0, 5, 25 or 50 mg/kg bw in corn oil twice daily (Nixon & Alden, 1978); a 14-week study in groups of 30 rats of each sex at a dose of 0, 10, 50 or 100 mg/kg bw in corn oil once daily (Pence, 1980a); and a 14-week study in groups of 30 rats of each sex at a dose of 0, 1, 2, 5, 10 or 50 mg/kg bw in corn oil once daily (Cheng, 1982). The studies showed treatment-related hepatic and renal toxicity at doses of 10 mg/kg bw and higher. The NOEL was 5 mg/kg bw per day, on the basis of histopathological lesions in the kidneys of male rats in the 14-week study (Cheng, 1982). A study of reproductive and teratogenic toxicity in rats at a dose of 0, 10, 50 or 100 mg/kg bw showed no reproductive effects or fetal abnormalities. The NOEL of 5 mg/kg bw per day is 280 times the estimated daily intake of 2-isopropyl-*N*-2,3-trimethylbutyramide when used as a flavouring agent in the USA (18 μg/kg bw per day).

Two studies were conducted on *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide in rats treated by gavage: a 28-day study in groups of six Crj:CD(SD) rats of each sex at a dose of 0, 8, 40, 200 or 1000 mg/kg bw per day (Miyata, 1995) and a 22-week study in groups of 15 Sprague-Dawley (CFY) rats of each sex at a dose of 0, 100, 300 or 725 mg/kg bw per day (Hunter et al., 1974). Mild toxicity in the liver and kidneys was observed at doses of 40 mg/kg bw and above. Two further studies were conducted in beagle dogs given gelatine capsules: a 28-day study in groups of one male and one female given a dose of 0, 600, 1000 or 1500 mg/kg bw per day and a 52-week study in groups of three animals of each sex given a dose of 0, 100, 300 or 1000 mg/kg bw per day (James, 1974). These studies showed mild toxic effects in the liver at all doses. The NOEL of 8 mg/kg bw per day in these studies is 1 000 000 times the estimated daily intake of *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide when used as a flavouring agent in Europe (0.008 μg/kg bw per day) and 4000 times that in the USA (2 μg/kg bw per day).

The additional toxicity data indicate that 2-isopropyl-*N*-2,3-trimethylbutyramide (No. 1595) and *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601) would not be expected to raise safety concerns at their estimated levels of intake when used as flavouring agents. For one of these agents (No. 1595), however, less uncertain exposure estimates are needed, as the existing estimate was based on anticipated pundage.

The stepwise evaluation of the 36 aliphatic and aromatic amines and amides evaluated according to the Procedure is summarized in Table 1.

1.6 Consideration of secondary components

One member of this group of flavouring agents, isopentylidene isopentylamine (No. 1606), has an assay value of < 95%. One of its secondary components, 3-methylbutyraldehyde (No. 258), was evaluated by the Committee at its forty-ninth meeting (Annex 1, reference 131) and considered to be of no concern at estimated levels of intake. The other secondary component, diisopentylamine, has not been evaluated by the Committee; however, it is structurally related to the primary and secondary amines that were evaluated in this group of flavouring agents and is

expected to have the same metabolic fate. These amines are primarily oxidized to imines by flavin-containing monooxygenases, monoamine oxidases or amine oxidases, and the resulting imine can be further oxidized to produce the corresponding aldehyde and ammonia (Kearney et al., 1971). Moreover, the NOELs for the structurally related compounds piperidine (No. 1607) and trimethylamine (No. 1610) are 80 and 160 mg/kg bw per day, respectively (Amoore et al., 1978). On this basis, diisopentylamine was considered not to present a safety concern at estimated levels of intake. The Committee also concluded that the flavouring agent as specified would not present a saftey concern at the estimated levels of exposure.

1.7 Consideration of combined exposure from use as flavouring agents

In the unlikely event that all 16 agents in structural class I were to be consumed concurrently on a daily basis, the estimated combined exposure would not exceed the human threshold for class I (1800 μg/person per day). Likewise, in the unlikely event that all 10 agents in structural class II were to be consumed concurrently on a daily basis, the estimated combined exposure would not exceed the human threshold for class II (540 μg/person per day). In the unlikely event that all 10 agents in structural class III were to be consumed concurrently on a daily basis, the estimated combined exposure would exceed the human threshold for class III (90 μg/person per day); however, the toxicity data for these substances adequately support their safety at the exposure levels estimated from their use as flavouring agents. Overall evaluation of the data indicates that combined exposure would not raise safety concerns.

1.7 Conclusions

On the basis of the available data on the toxicity of acetamide (No. 1592), the Committee concluded that its use as a flavouring agent or for any other food additive purpose would be inappropriate, and it was therefore not evaluated by the Procedure.

The Committee concluded that use of the remaining 36 flavouring agents in this group of aliphatic and aromatic amines and amides would not present a safety concern at the estimated intakes. For 27 flavouring agents (Nos 1579–1581, 1583–1586, 1588, 1590–1591, 1593–1598, 1602–1606, 1608 and 1611–1615), the evaluation was conditional because the intake was estimated on the basis of an anticipated annual volume of production. The conclusions of the safety evaluations of these 27 flavouring agents will be revoked if use levels or poundage data are not provided before December 2007. The Committee noted that the available data on the toxicity and metabolism of these aliphatic and aromatic amines and amides were consistent with the results of the safety evaluations.

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

This monograph summarizes the key background data relevant to evaluation of the 37 flavouring agents in this group. The group comprises 13 primary aliphatic and aromatic amines (Nos 1579–1591), five tertiary aliphatic amines (Nos 1610–1614), four alicyclic amines (Nos 1607–1609 and 1615), four aliphatic and alicyclic imines (Nos 1603–1606) and 11 amides (Nos 1592–1602).

2.2 Additional considerations on exposure

The 13 primary aliphatic and aromatic amines (Nos 1579–1591) and three tertiary aliphatic and aromatic amines (Nos 1610–1612) in this group occur naturally, mainly in varieties of cheese, red and white wine, coffee and tea, fish, and selected vegetables, mainly cabbage and radishes (Nijssen et al., 2003). The concentrations of these amines in cheeses are usually in the range 0.1–1 ppm but can be as high as 169 ppm (phenethylamine). In white and red wine, the concentrations range from 2 to 10 ppm for ethylamine to < 0.01 ppm for butylamine; the highest levels have been recorded for phenethylamine (10.4 ppm in white wine and 72 ppm in red wine). Another biogenic amine, tyramine (No. 1590), and methylated derivatives are present in cheese, yeast products, fermented foods, beer, wine, pickled herring, snails, chicken liver, broad beans, chocolate and cream products (Lovenberg, 1973).

The tertiary amines (Nos 1610–1614) triethylamine (No. 1611) and tripropylamine (No. 1612) are distributed in cheese, fish, wine, coffee and tea, while the highest concentrations of trimethylamine (No. 1610) (680 ppm) and trimethyl-amine oxide (No. 1614) (10 770 ppm) are found in fish, mainly fatty fish. *N,N*-Dimethylphenethylamine (No. 1613) has been measured in shrimp.

Of the alicylic amines in this group (Nos 1607–1609 and 1615), piperidine (No. 1607) and pyrrolidine (No. 1609) are found in cheese, fish, wine and coffee at levels up to 20 ppm. 2-Methylpiperidine (No. 1608) and piperazine (No. 1615) have been detected in fish and sherry, respectively.

The aliphatic and alicylic imines (Nos 1603–1606) also occur naturally in food but to a lesser extent. 1-Pyrroline (No. 1603) occurs in clams and mussels, and 2-acetyl-1-pyrroline (No. 1604) has been detected in rice, beef, chicken and bread. Of the 11 amides in the group (Nos 1592–1602), acetamide (No. 1592) and butyramide (No. 1593) have been detected in various cheeses and in cocoa, while two other amides, *N*-isobutyl-(E,E)-decadienamide (No. 1598) and piperine (No. 1600), occur in black pepper, the levels of piperine reaching 62 000 ppm.

Quantitative data on natural occurrence and consumption ratios have been reported for six agents in this group. They indicate that exposure occurs predominantly from consumption of traditional foods (i.e. consumption ratio > 1) (Stofberg & Kirschman, 1985; Stofberg & Grundschober, 1987). Production volumes and intake values for each flavouring agent in this group are shown in Table 2.

Most aliphatic and alicyclic amines are distinctly malodorous, usually with a fishy odour. As they are weak bases (*pKa* 9.8–11), they rapidly saturate receptors and cause olfactory fatigue. Although most amines are readily detectable at < 10 ppm, the aroma becomes stronger at 10–100 ppm and becomes intolerable at > 100 ppm. The bite in red wine, cheeses and radishes is partly due to the presence of amines at concentrations of 5–70 ppm (Schweizer et al., 1978).

2.3 Biological data

2.3.1 Biochemical data

(a) Absorption, distribution, and excretion

Aliphatic and aromatic primary amines

Many amines are endogenous and have been identified as normal constituents of urine from healthy persons as a result of the catabolism of sarcosine, creatine

and choline. The amines include methylamine, dimethylamine, trimethylamine, ethylamine, isoamlyamine, piperidine, pyrrolidine, phenethylamine and trimethylamine oxide (Rechenberger, 1940; Wranne, 1956; Williams, 1959; Ayesh et al., 1993; Zhang et al., 1993). Up to eight aliphatic and ring-substituted amines were recovered, at levels of up to 100 μg/day, in the urine of healthy and hyperlipidaemic persons eating a normal diet (Davies et al., 1954).

After a dose of 2000 mg ethylamine hydrochloride, humans excreted about 32% unchanged in the urine, while the remaining 68% was converted to urea and acetic acid. Almost all of a dose of 6000 mg of propylamine was metabolized, only 9.5% of the administered dose being recovered unchanged in the urine (Williams et al., 1959).

Phenethylamine (No. 1589) metabolites appear rapidly in the urine of healthy volunteers, 62% of an oral dose of 300 mg being accounted for within 2–4.5 h (Richter, 1937). More than 60% of a dose of 300 mg (about 5 mg/kg bw) of phenethylamine was excreted in human urine within 2 h (Seakins, 1971). Phenethylamine metabolites were identified in the urine of white mice and male white rats given 2 mg of ^{14}C-phenethylamine hydrochloride by subcutaneous injection within 2 h, and 80% of the administered dose was recovered in the urine, primarily as phenyl acetic acid, within 40 h (Block, 1953). Phenethylamine is produced endogenously in humans as a result of the decarboxylation of phenylalanine in tissues (Mosnaim et al., 1973) and bacterial degradation of amino acids in the gastrointestinal tract (Simenhoff, 1975). The average endogenous levels of free and conjugated phenethylamine excreted in the urine of healthy humans over 24 h were 453 ± 50 and 433 ± 50 μg, respectively (Mosnaim et al., 1973).

The aromatic amine tyramine (No. 1590) is also rapidly absorbed and excreted in humans. A group of eight male volunteers (seven completed the study) were given 200 mg of tyramine in capsules to be taken after an overnight fast. After a 1-week washout period, the men were asked to take the same dose midway through a standard breakfast. A serum sample was collected 30 min before tyramine was taken and at various times up to 6 h after each tyramine dose. Examination of the time-dependent plasma concentrations indicated that tyramine is rapidly absorbed and eliminated from the plasma. The peak plasma concentration occurred at 0.5 h and 1.25 h in fasted and unfasted men, respectively, and the half-life of tyramine in plasma was 0.53 and 0.92 h for fasted and unfasted men, respectively. Administration of tyramine with a meal decreased its bioavailability by an average of 53% (32–81%), which is consistent with the values for tyramine concentrations. The mean residence time of tyramine in the body was 0.85 and 2.2 h in fasted and unfasted men, respectively (Van Den Berg et al., 2003).

Alicyclic amines

The alicyclic amines piperidine (No. 1607) and pyrrolidine (No. 1609) are basic substances (pKa 11.1 and 11.3, respectively), and both have properties characteristic of typical aliphatic secondary amines (von Euler, 1945; Damani & Crooks, 1982). Piperidine and pyrrolidine are rapidly absorbed and excreted mainly in urine as oxidation products.

Piperidine is endogenous in humans (Kase et al., 1969a; Ishitoya et al., 1973). It is derived from lysine and cadaverine and is excreted by humans at levels of 3–30 mg/day (von Euler, 1945; Kase et al., 1969b). It has also been detected in

human blood (von Euler, 1945; Audunsson, 1988) and in the urine of cows, horses, dogs, pigs, cats, rabbits and rats at 0.5–5 mg/100 ml of urine (von Euler, 1945). Urine obtained from healthy male and female students contained 8.5 and 7.6 mg piperidine per day on average, respectively (von Euler, 1945). In a later study, the urine of healthy Japanese students contained an average of 1.2 ± 0.17 μg/day of piperidine (Kase et al., 1969b).

Urine was collected from Wistar rats for 72 h after an intraperitoneal injection of 1.0 mg piperidine hydrochloride. The urinary metabolites, detected by gas chromatography with mass spectrometry, comprised the parent compound, conjugated metabolites of piperidine and several unknown metabolites. Metabolites were considered to be conjugated if they disappeared after treatment with β-glucuronidase. 3-Hydroxypiperidine and 4-hydroxypiperidine were also detected in the urine of control animals given saline but at levels that were approximately one-half and one-third, respectively, of those seen in piperidine-exposed animals (Okano et al., 1978).

Pyrrolidine (azacyclopentane), a homologue of piperidine (azacyclohexane), is also basic (pK_a 11.3) and has been detected in normal adult human blood and urine (Ishitoya et al., 1973). It is presumed that pyrrolidine follows the same absorption and excretion pathways as the related compound. After a single intravenous dose of [$^{14}CH_3$]-*N*-methylpyrrolidine to rats and dogs, 81% and 91%, respectively, was excreted in the urine within 24 h (Forgue et al., 1987).

Oral administration of [2-^{14}C]pyrrolidone to Sprague-Dawley CD albino rats at a dose of 75 mg/kg bw resulted in peak levels of radioactivity in the plasma of male and female rats after 2 h (23.3 and 29.3 μg/ml, respectively). Five days later, only 0.0006% of the initial dose remained in plasma. Eight hours after dosing, 80% of the radioactivity detected in the plasma was found to have originated from the parent amine, indicating that little first-pass metabolism had occurred. Within the first 24 h, 85–88% of the initial dose of radioactivity was excreted, mainly in the urine, with 6–7% in expired CO_2 (Midgley et al., 1992).

Piperazine (1,4-diazocyclohexane) as the hydrochloride salt is widely used as an anthelmintic agent for humans, at therapeutic doses of 75–3500 mg/kg (Cork et al., 1990). In a study of endogenous nitrosation of piperazine in humans, about 25% of an oral dose of 1.9 mg was excreted unchanged within 24 h (Kumar et al., 1992). Mononitrosopiperazine but not dinitrosopiperazine was detected in the urine and gastric juices of four volunteers given 480 mg of piperazine (Bellander et al., 1985)

These observations indicate that the alicyclic amines, piperidine, pyrrolidine, *N*-methylpyrrolidine and piperazine, are rapidly absorbed, metabolized and excreted, primarily in the urine.

Aliphatic tertiary amines

Mainly on the basis of the available data for trimethylamine (No. 1610) and trimethylamine oxide (No. 1614), tertiary amines and their *N*-oxide metabolites are anticipated to be rapidly absorbed and excreted in the urine by most mammals.

When male Wistar rats were given 1 mmol/kg bw of trimethylamine or trimethylamine oxide orally, metabolites were found in the urine within 24 h (Zhang et al., 1998).

In cats given 500–1000 mg/kg bw of trimethylamine by intraperitoneal injection, the compound was distributed to the liver lungs, spleen and kidneys within 20 min (Iwamoto, 1957).

In a study designed to investigate the pharmacokinetics of intravenously administered trimethylamine, male Wistar rats were given 10, 20 or 40 mg/kg bw of trimethylamine by intravenous injection. The compound was rapidly eliminated from the blood, with reported half-lives of 2.0, 2.1 and 2.5 h, depending on the dose. The volume of distribution and systemic clearance values indicated that it is rapidly absorbed, distributed and eliminated in rats. The area under the curve of plasma concentration with time increased in a dose-dependent manner. These findings suggest that, in rats, the metabolic processes leading to elimination do not become saturated after intravenous administration. Peak plasma concentrations of trimethylamine metabolites were measured 0.75 h after injection. After oral administration of 20 mg/kg bw, the pharmacokinetics were comparable to those seen after intravenous injection. The half-life of trimethylamine in the blood of rats after oral administration was 1.6 h. These findings indicate rapid absorption, distribution and excretion of trimethylamine in rats (Nnane & Damani, 2001).

Trimethylamine and trimethylamine oxide have been detected in the urine of healthy humans as products of choline catabolism. In the 11-h urine of seven healthy male and female volunteers receiving 50 mg/kg bw choline, 12–29% of the total nitrogen ingested was excreted as trimethylamine and trimethylamine oxide. After ingestion of 0.2 mmol/kg bw of trimethylamine by three healthy female and four male volunteers, 40–70% of the total trimethylamine nitrogen was excreted in the urine within 4 h (Wranne, 1956).

In a study of 'fish malodour syndrome', 156 persons suspected of having this condition were challenged with 600 mg of trimethylamine; 145 persons excreted 89 ± 9% of the administered dose as oxidized trimethylamine in the urine within 8 h, while 11 of them excreted trimethylamine almost exclusively unchanged (Ayesh et al., 1993).

A series of experiments was conducted to examine the metabolism and excretion of trimethylamine and trimethylamine *N*-oxide in rats. After a bolus intraperitoneal injection of radiolabelled trimethylamine (dose not specified), 96% was recovered in the urine within 24 h. Recovery in faeces and exhaled air was negligible, as was accumulation in tissues. When a dose of < 5, 100 or 1000 μmol of radiolabelled trimethylamine was administered, no dose-dependent recovery of urinary metabolites was observed (Smith et al., 1994).

Amides

Studies on selected members of the group indicate that amides per se are rapidly absorbed and metabolized.

In a study in which male Fischer 344 rats were given 1500 mg/kg bw of radiolabelled 1,6-hexalactam (No. 1594) by gavage, the main substances detected by high-performance liquid chromatography in urine after 6 h were the unchanged compound, 1,6-hexalactam and an unidentified metabolite (Unger & Friedman, 1980).

Male Sprague-Dawley rats given a single oral dose of 4 mg/kg of *N*-(vanillyl)-[1-^{14}C]nonanamide [nonanoyl 4-hydroxy-3-methoxybenzylamide (No. 1599)] excreted 17.9%, 45.9% and 22.7% of the radiolabel in the urine, faeces and expired

CO_2, respectively, within 72 h, although most of the radiolabel was excreted within the first 24 h. Bile duct-cannulated rats excreted 11.4%, 3.7%, 11.7% and 65.1% of the radiolabel in the urine, faeces, expired CO_2 and bile, respectively. In fasted rats, peak blood levels of radiolabel occurred 10 min after administration. By 72 h after dosing, the highest concentrations of radiolabel were found in fat, liver and adrenal gland. These results indicate that nonanyl 4-hydroxy-3-methoxybenzylamide is rapidly absorbed and that appreciable quantities undergo enterohepatic circulation and partial conversion to CO_2 (Schwen, 1982).

Groups of male albino Wistar rats were given piperine (No. 1600) at a dose of 170 mg/kg bw by gavage or 85 mg/kg bw by intraperitoneal injection, and urine and faeces were collected every 24 h for 12 days. Urine and faeces from rats fed a control diet for 10 days were collected for 3 days before treatment and used as control samples. When given by either route, about 3% of the unchanged dose was detected in faeces over 5 days, indicating that 97% of the piperine was absorbed. Peak excretion in the faeces occurred on day 1 after intraperitoneal injection and on day 3 after gavage. No unchanged piperine was detected in urine after administration by either route; however, there was increased excretion of conjugated glucuronides, sulfates and phenols, with maxima on days 1–4. Overall, 91–97% of the administered dose was accounted for. After treatment, the animals were killed at various intervals, when blood was collected from the heart, and the liver, kidney, spleen and gut (stomach, small intestine, caecum and large intestine) were removed. By 30 min after ingestion of piperine, 29% was detected in the gut (22% in stomach and 6% in small intestine). By 48 h, 1% was detected in stomach, and 2–3% in the caecum and large intestine, indicating that 97% had been absorbed. A similar pattern was reported in rats intraperitoneally injected with piperine, although some of the values differed (data not reported). Between 1 and 10 h after treatment, only traces of piperine administered by either route were detected in blood. Between 0.5 and 24 h after treatment, intraperitoneally administered piperine was detected in the liver (2.12–0.4%) and kidney (0.04–0.2%). Similarly, orally administered piperine was detected in the liver (0.25–0.12%) and kidney (0.03–0.17%) up to 24 h after treatment. No piperine was detected after 48 h in any of the tissues examined (Bhat & Chandrasekhara, 1986a).

A group of male albino Wistar rats were given 170 mg/kg bw of piperine by gavage. After 1 h, some of the rats, including a group of untreated rats that served as controls, received a bile duct cannula, and bile was collected for 6 h. Urine was collected from the remaining rats for 4 days and pooled, while urine collected for 4 days before dosing served as control samples. No unchanged piperine was detected in urine. Piperic acid was detected in the bile (about 1% of the original dose) within 6 h, and various metabolites (piperonylic acid, piperonal, vanillic acid and piperonyl alcohol) were excreted in urine (about 15.5% of the original dose) within 96 h (Bhat & Chandrasekhara, 1987).

In rats given a single oral dose (not specified) of *N*-ethyl-*para*-menthane-[3-^{14}C]-carboxamide [*N*-ethyl 2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601)], 64.2% and 28.7% of the dose was excreted in urine and faeces, respectively, over 5 days. Almost 50% of the radioactivity was secreted into the bile within 2 days, most within 24 h, indicating enterohepatic circulation of the parent compound or its metabolites. The peak plasma concentration (0.3% of the total dose) was reached within 1 h. Subsequently, the compound was eliminated with a half-life of 11 h.

Whole-body autoradiography showed that most of the radioactivity was in the liver, kidneys and gastrointestinal tract. The results indicate that the substance was rapidly and extensively converted into more polar metabolites of unknown structure (James, 1974).

The metabolic fate of *N*-ethyl-*para*-menthane-[3-^{14}C]-carboxamide (No. 1601) was examined in one male and one female dog given a single oral dose of 10 mg/kg bw. The substance was readily absorbed and rapidly eliminated in the urine (72% of the dose within the first 24 h) and faeces (11% of the dose within 5 days). No parent compound was detected in urine. The main urinary metabolites were glucuronide or sulfate conjugates, whereas the faeces contained mainly unchanged compound. Radioactivity was detected (detection limit = 0.05 ppm) in the liver, adrenal glands (male only), testes and kidney (female only) 5 days after treatment. Peak plasma levels were reached within 4 h. Subsequently the compound was eliminated with a half-life of about 70 min. Plasma radioactivity was determined to consist mostly (> 90%) of metabolites of the test substance. About 70% was bound to plasma protein in vitro, but < 10% of the radioactivity was protein-bound in vivo. The author noted that rats metabolized the test substance to polar unconjugated metabolites, while dogs metabolized it to conjugates; however, both species metabolized it extensively and eliminated it rapidly (James, 1974).

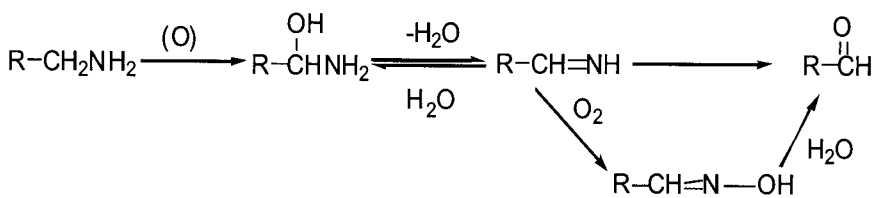
These studies indicate that the amides in this group of flavouring agents are quickly absorbed, metabolized and excreted, mainly in urine but also partly in the faeces.

(b) Metabolism

Aliphatic and aromatic primary amines

Aliphatic amines are metabolized primarily by flavin-containing monoxygenases, monoamine oxidases or amine oxidases, which catalyse the oxidation of primary, secondary and tertiary amines to imines with a concomitant reduction of molecular oxygen to hydrogen peroxide (see Figure 1). The resultant imine can be further converted to produce the corresponding aldehyde and ammonia. Monoamine oxidase A is responsible for oxidation of neurotransmitters, including serotonin, dopamine and norepinephrine (Moskvitina & Medvedev, 2001). Monoamine oxidase B is responsible for oxidation of exogenous and endogenous amines that might inhibit or interfere with neurotransmitter function. Both forms of the enzyme have a covalently bound flavin adenosine dinucleotide (FAD) co-factor (Kearney et al., 1971). Monoamine oxidase is present mainly as an integral membrane protein associated with

Figure 1. Oxidative deamination of aliphatic primary amines



the microsomal fraction of the endoplasmic reticulum (Moskvitina & Medvedev, 2001). Monoamine oxidase activity has been reported in the cytosolic fraction of rat liver (Medvedev et al., 1995), human placenta (Kirkel et al., 1991) and liver homogenate (Moskvitina & Medvedev, 2001).

The aldehydes produced by oxidative deamination of aliphatic primary amines are expected to oxidize to the corresponding carboxylic acid and enter existing pathways of metabolism. It is anticipated that the ammonia produced will be excreted in the form of urea.

Primary aliphatic amines with an accessible α-substituted carbon atom can also undergo *N*-oxidation to nitroso groups and subsequently oximes, which are labile and readily hydrolysed.

Ethylamine (No. 1579) is readily metabolized, and its nitrogen is converted to urea in humans; similarly, *n*-propylamine (No. 158) is converted to urea and excreted in urine (Williams, 1959).

n-Butylamine (No. 1582) is readily metabolized to acetoacetic acid in guinea-pig liver slices (Pugh & Quastel, 1937), while *n*-amylamine (pentylamine, No. 1585) is converted to acetone, valeric acid and urea in guinea-pig slices, and isoamyl amine is converted to isoamyl alcohol and aldehyde under identical experimental conditions (Richter, 1937). When 100 mg of isoamylamine were administered orally to humans, the only metabolite detected in urine was unchanged amine (Richter, 1937).

When butylamine (No. 1582) and phenethylamine (No. 1589) were combined with monoamine oxidase isolated from human plasma, the production of ammonia indicated that both compounds were deaminated (McEwen, 1965).

Studies with rabbit liver homogenate in vitro indicated that alkylamines readily undergo oxidative metabolism. Isoamylamine (1 mg) was readily oxidized when incubated with rabbit liver homogenate, and the consumption of oxygen reached a steady state within 30 min. Incubation of 1 mg of phenethylamine (No. 1589) with rabbit liver homogenate resulted in oxidation of 80% of the test material within 30 min and complete oxidation within 4 h. Incubation of both isoamylamine and β-phenethylamine (No. 1589) with rabbit liver homogenates resulted in the production of ammonia (Bernheim & Bernheim, 1938).

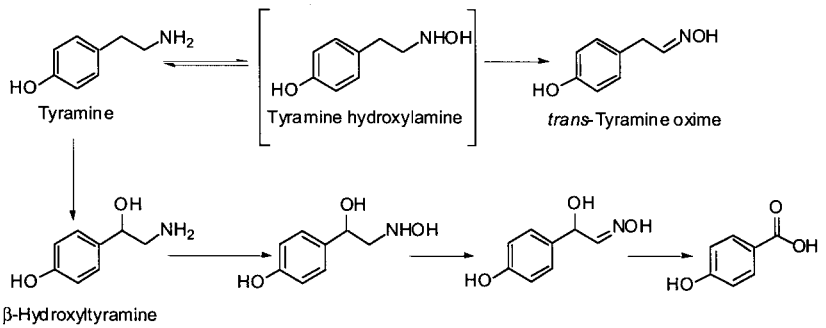
In a similar study, oxidative deamination was reported when amylamine (pentylamine, No. 1585), isoamylamine (isopentylamine, No. 1587) and β-phenthylamine (No. 1589) were incubated with isolated guinea-pig liver amine oxidase. Valeraldehyde, isovaleraldehyde and phenylacetaldehyde were identified as the primary metabolites (Richter, 1937).

Healthy volunteers given 1000 mg of phenethylamine orally excreted at least 50% in the form of phenylacetic acid in 24-h urine (Power & Sherwin, 1927). In a separate study, healthy volunteers given 300 mg of β-phenethylamine excreted about 60% within 2 h as phenylacetic acid (Seakins, 1971).

In studies in mouse heart and brain slices (Ross & Renyi, 1971) and in rabbit and guinea-pig liver homogenates (Snyder et al., 1946) in vitro, phenethylamine was converted to phenylacetic acid.

In guinea-pig liver slices incubated with 1 mmol/l of 2-phenethylamine in the presence or absence of enzyme inhibitors, phenylacetic acid was the primary metabolite (Panoutsopoulos et al., 2004).

Figure 2.* N-*Oxidation and* C-*oxidation of tyramine in mammals



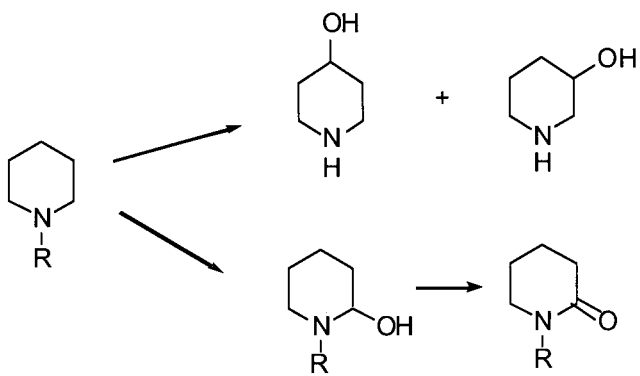
From Lin & Cashman (1997)

Tyramine (No. 1590) is formed as a result of normal metabolic processes in animals, plants and microbes. It can be biosynthesized by enzymatic decarboxylation of tyrosine by intestinal bacteria (ten Brink et al., 1990). Tyramine metabolism follows two pathways, *N*-oxidation or *C*-oxidation with subsequent *N*-oxidation (see Figure 2).

When tyramine was incubated in the presence of pig liver or human liver microsomes, the *trans*-oxime was detected as a metabolite, as well as the *C*-oxidation metabolite, 4-hydroxybenzaldehyde (Lin & Cashman, 1997).

After 2-(4-hydroxyphenyl)ethylamine (tyramine) was incubated with a crude mitochondrial fraction of rat intestine, the peroxidase-catalysed oxidation product, 2,2′-dihydroxy-5,5′-bis(ethylamino)diphenyl (dityramine), was identified. After further analysis with specific enzyme inhibitors, the authors suggested that the metabolism of tyramine in the gut is peroxidase-dependent and might be driven by H_2O_2 produced by monoamine oxidase (mainly monoamine oxidase A) activity and that monoamine oxidase A also is responsible for the oxidative deamination of tyramine (Valoti et al., 1998).

Figure 3. Metabolic fate of piperidine and its derivatives



Alicyclic amines

Piperidine (No. 1607) and pyrrolidine (No. 1609) are endogenous in mammals (von Euler, 1945; Kase et al., 1969a; Ishitoya et al., 1973; Audunsson, 1988). They are present in a variety of tissues, including normal serum, spinal fluid, neurons and intestine (Honegger & Honegger, 1960; Perry et al., 1965; Kase et al., 1969b). Gut microflora produce piperidine from lysine, influencing the amount of piperidine detected in urine (Kase et al., 1969b).

Piperidine and its derivatives are metabolized via *C*- and *N*-oxidation (Figure 3). Ring carbons undergo mainly *C*-oxidation (hydroxylation) at the carbon adjacent to the nitrogen, to yield the corresponding hydroxyl derivatives, which can form excretable conjugates or further oxidize to yield a lactam. *N*-Substituted piperidine derivatives can also form *N*-oxides, which are then metabolized similarly to *C*-oxides (Kaiser et al., 1972; Okano et al., 1978; Masumoto et al., 1991).

Urine collected from Wistar rats that had received an intraperitoneal dose of 0.167 mg (50 μCi) of ^{3}H-piperidine showed the presence of 4.15 μg/ml of 3-hydroxypiperidine and 3.15 μg/ml of 4-hydroxypiperidine (Okano et al., 1978).

The urine of healthy male volunteers who took tablets containing 250 mg of mepivacaine hydrochloride, an *N*-substituted piperidine derivative, contained the corresponding lactam (Meffin et al., 1973).

Other *N*-methyl piperidine derivatives, anabasine or methylanabasine [2-(3-pyridyl)piperidine or 1-methyl-2-(3-pyridyl)piperidine] were converted to the corresponding piperidine *N*-oxides when incubated with lung and liver microsomal fractions from rat, rabbit and guinea-pig (Beckett & Sheikh, 1973).

In dogs and healthy humans, diphenidol was oxidized at the α-position on the piperidine ring. The lactam subsequently underwent ring-opening hydrolysis to form a d-aminovaleric acid derivative (Kaiser et al., 1972).

α-, β- and γ-Hydroxy metabolites were identified when *N*-benzylpiperidine was incubated in the presence of rat liver microsomes and biomimetic chemical systems (Masumoto et al., 1991).

Pyrrolidine and its derivatives are most susceptible to a *C*-oxidation, which produces a lactam that undergoes ring-opening hydrolysis to the γ-amino acid derivative (Neurath et al., 1987; Fischer et al., 1990; Deal et al., 1992; McNulty et al., 1992; Midgley et al., 1992; Wu et al., 1992).

αC-Oxidation of the pyrrolidine ring occurs in a variety of substituted pyrrolidine derivatives. Labelled bepridil {1-[2-(*N*-benzylanilino)-1-(isobutoxymethyl)ethyl]-pyrrolidine} was given orally to mice (150 mg/kg bw), rats (100 mg/kg bw), rabbits (100 mg/kg bw), rhesus monkeys (50 mg/kg bw) and humans (400 mg/kg bw). Radioactive metabolites were detected in the urine of all species, representing 48%, 28%, 53% and 41% of the administered radioactivity in the urine of mice, rats, rabbits and rhesus monkeys, respectively, within 168 h of dosing; 67% of the administered dose was detected in the urine of a volunteer 24 h after dosing, and most of the remaining dose was detected in faeces. One of the main metabolic pathways involved αC-oxidation of the pyrrolidine ring. α-Oxidation metabolites were identified in the plasma of rats and rhesus monkeys at 38 and 56 ng/ml, respectively, at concentrations of 14 and 22 ng/ml in the plasma of mice and rabbits, respectively, and at a concentration of 9 ng/ml in human plasma (Wu et al., 1992).

Radiolabelled triprolidine [*trans*-1-(4-methylphenyl)-1-(2-pyridyl)-3-pyrrolidino-prop-1-ene] was given orally to mice at a dose of 50 mg/kg bw. About 80% of the

administered dose was detected in urine and the remaining 20% in faeces. Four metabolites of triprolidine were identified, although one of them accounted for almost 58% of the administered dose (Deal et al., 1992).

When a healthy volunteer was given 50 mg of flecainide [*N*-(2-piperidylmethyl)-2,5-bis-(2,2,2-trifluoroethoxy)benzamide monoacetate], a metabolite in which the piperidine ring was oxidized to an amide at the α-carbon was detected in urine (Fischer et al., 1990).

These results indicate that piperidine and pyrrolidine undergo ring *C*-hydroxylation, yielding the corresponding hydroxy-substituted piperidine and pyrrolidine derivatives. *N*-Substituted derivatives favour α-oxidation to yield 2-hydroxy derivatives, which undergo further oxidation to yield lactams and subsequent ring-opened hydrolysis products.

Alicyclic imines

Pyrroline (No. 1603) and its derivatives (Nos 1604 and 1605) undergo rapid hydrolysis in aqueous solution in vivo to yield the resulting aminoaldehyde in the case of pyrroline and iminoketone in the case of 2-acetyl-1-pyrroline (No. 1604) and 2-propionylpyrroline (No. 1605). The primary amine produced is further oxidized, similarly to other aliphatic primary amines. The aldehyde is oxidized to the corresponding carboxylic acid, while the ketone is reduced to the corresponding alcohol and excreted either unchanged or as the glucuronic acid conjugate.

Imines are labile in aqueous conditions, and they readily hydrolyse in the presence of water (March, 1992). In the aqueous environment of the stomach, rapid hydrolysis occurs before absorption. The urine of 10 healthy adults given an oral dose of 800 mg of the imine H_2 receptor agonist ebrotidine contained metabolites derived from hydrolysis of the imine and those formed from oxidation of the sulfite functional group (Rozman et al., 1994).

Aliphatic tertiary amines

Trimethylamine (No. 1610) is oxidized predominantly by monoamine oxidase to form trimethylamine oxide (No. 1614). When four men, three women and one boy aged 8 years were given 0.2 mmol/kg bw of trimethylamine, 70–90% of the test material was excreted as trimethylamine oxide in 1-h urine. In all cases, > 90% of the original dose was eliminated as trimethylamine oxide in the 4-h urine (Wranne, 1956).

In a similar study, healthy volunteers excreted 88% of trimethylamine from a regular diet and 89% of a dose of 600 mg of trimethylamine as the *N*-oxidized metabolite in 24-h urine. Eleven volunteers with clinically diagnosed monoamine oxidase 5 deficiency (fish odour syndrome) excreted 11–54% of their dietary intake of trimethylamine and 5–23% of a bolus dose of 600 mg of trimethylamine as trimethylamine oxide in 24-h urine (Ayesh et al., 1993).

Animals can convert trimethylamine to trimethylamine oxide, but not to the extent that has been reported in humans. After administration of 59 and 74 mg of trimethylamine to guinea-pigs and rats by gavage, respectively, 41% and 33% of the dose was converted to trimethylamine oxide. When the compound was given by subcutaneous injection, 33% and 11% of the dose given to guinea-pigs and rats, respectively, was excreted as trimethylamine oxide (Müller & Immendorfer, 1942).

Male Wistar rats given 1 mmol/kg bw of trimethylamine (59.1 mg/kg bw) or trimethylamine oxide (75.1 mg/kg bw) excreted 2.1 and 7.5 mg/kg per day, respectively, of dimethylamine in urine. *C*-Oxidation of trimethylamine by the gut microflora was a minor route of metabolism (Zhang et al., 1998).

After stable isotopes of trimethylamine or trimethylamine *N*-oxides were administered to rats by intraperitoneal injection, about 60% of the radioactivity from trimethylamine and 74% of that from trimethylamine *N*-oxide was recovered in the urine. The urinary metabolites identified were a mixture of trimethylamine and trimethylamine *N*-oxide (Smith et al., 1994).

More than 90% of triethylamine *N*-oxide was isolated from the urine of male volunteers given 25 mg of triethylamine and 15 mg triethylamine oxide orally (Akesson et al., 1988, 1989).

Amides

Aliphatic amides have been reported to undergo limited hydrolysis. Extensive hydrolysis of aliphatic amides of various lengths was observed after incubation with rabbit liver extracts; however, hydrolysis was significantly slower for aliphatic amides with fewer than five or more than 10 carbons (Bray et al., 1949).

After administration of 1.5–5.0 g of acetamide (No. 1592) or butyramide (No. 1593) to rabbits, 62% of the dose of acetamide was recovered unchanged in the urine within 24 h, while only 13% of the butyramide dose was recovered unchanged.

Studies in which rats were given an oral dose (170 mg/kg bw) of piperine (No. 1600) or dogs were given an oral dose (10 mg/kg bw) of *N*-ethyl 2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601) indicated that amide hydrolysis products are not major metabolites of these compounds (James, 1974; Bhat & Chandrasekhara, 1986a).

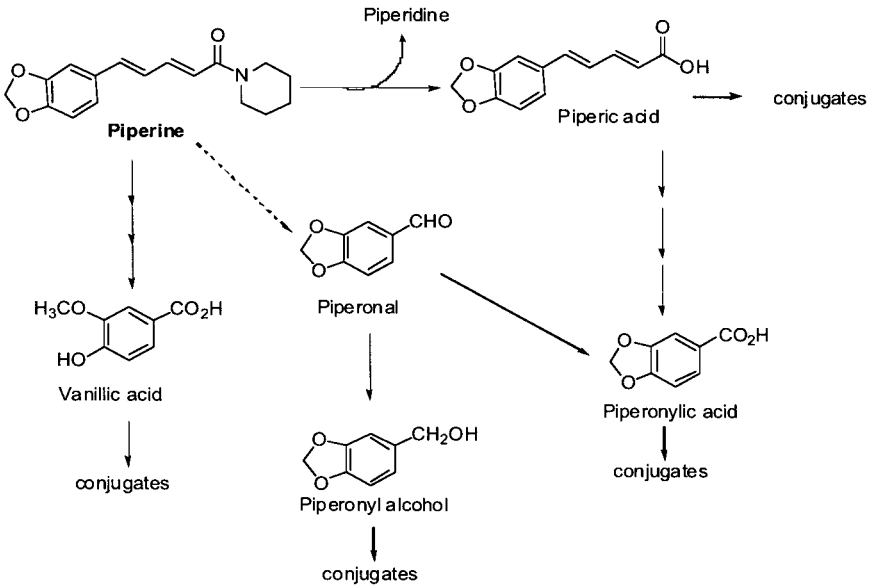
The metabolism of piperine (No. 1600) was studied in groups of male albino Wistar rats given a dose of 170 mg/kg bw by gavage or 85 mg/kg bw by intraperitoneal injection. Urine and faeces were collected every 24 h for 12 days, while control urine and faeces samples were collected for 3 days from rats fed a control diet before dosing. No unchanged piperine was detected in urine after exposure by either route; however, there was increased excretion of conjugated glucuronides, sulfates and phenols, with maximum excretion of all three on days 1–4. Demethylation of piperine was suggested by an increase in conjugated phenols. Over 8 days, about 36% of the gavage dose was excreted in urine as conjugated phenols and 62% as methylenedioxyphenyl metabolites. About 19% of the intraperitoneal dose was excreted as phenolics and about 72% as methylenedioxyphenyl derivatives (Bhat & Chandrasekhara, 1986a). Figure 4 shows the proposed pathway for the metabolism of piperine in rats. In addition to amide hydrolysis leading to piperic acid, metabolic oxidative cleavage of the benzylic alkene function results in a series of vanilloyl and piperonyl derivatives, which are excreted free or in conjugated form, mainly in the urine (Bhat & Chandrasekhara, 1987).

2.3.2 Toxicological studies

(a) Acute toxicity

Oral LD_{50} values have been reported for 17 of the 37 agents in this group (see Table 3). In rats, the values ranged from 59.6 to 2900 mg/kg bw, most values

Figure 4. Proposed pathway for the metabolism of piperine in rats



From Bhat & Chandrasekhara (1987)

falling between 300 and 500 mg/kg bw (Smyth & Carpenter, 1944; Smyth et al., 1962, 1969; Singh et al., 1973; James, 1974; Zaeva et al., 1974; Bond & Nixon, 1980; Cheever et al., 1982; National Toxicology Program, 1982; Piyachaturawat et al., 1983; Ciugudeanu et al., 1985; Van den Heuvel et al., 1990; Yam et al., 1991; Myers & Ballantyne, 1997; Til et al., 1997). In mice, the LD_{50} values ranged from 56.2 to 5300 mg/kg bw (Suter & Weston, 1941; Loit & Filov, 1964; Singh et al., 1973; James, 1974; National Toxicology Program, 1982; Piyachaturawat et al., 1983). The oral LD_{50} of piperine (No. 1600) in dogs was 40.3 mg/kg bw (Singh et al., 1973). These studies indicate that the acute toxicity of aliphatic and aromatic amines and amides given orally is low to moderate.

(b) Short-term studies of toxicity

The results of short-term studies with certain aliphatic and aromatic amines and amides are summarized in Table 4 and are described below.

sec-*Butylamine (No. 1584)*

In a short-term study designed to evaluate the toxicity of 109 industrial chemicals, seven male Alderly Park rats were exposed by inhalation to 233 ppm *sec*-butylamine for 6.5 h/day for 13 days, corresponding to an estimated daily intake of approximately 115 mg/kg bw (Fassett, 1978). Food and water were provided ad libitum between exposure periods. The animals were weighed daily, and their condition and behaviour were monitored during exposure. Urine was collected

Table 3. Results of studies of acute toxicity with aliphatic and aromatic amines and amides administered orally

No.	Flavouring agent	Species; sex	LD_{50} (mg/kg bw)	Reference
1580	Propylamine	Rats; M	570	Smyth et al. (1962)
1581	Isopropylamine	Rats; M	< 172.0[a]	Myers & Ballantyne (1997)
1582	Butylamine	Rats; M	365.7	Cheever et al. (1982)
1582	Butylamine	Rats; F	382.4	
1582	Butylamine	Rats; M	500	Smyth & Carpenter (1944)
1582	Butylamine	Rats; M	557	Ciugudeanu et al. (1985)
1582	Butylamine	Rats; F	470	
1582	Butylamine	Mice; M, F	575	Loit & Filov (1964)
1583	Isobutylamine	Rats; M	224.4	Cheever et al. (1982)
1583	Isobutylamine	Rats; F	231.8	
1584	*sec*-Butylamine	Rats; M	157.5	
1584	*sec*-Butylamine	Rats; F	146.8	
1585	Pentylamine	Mice; M, F	375	Loit & Filov (1964)
1589	Phenethylamine	Mice; F	400	Suter & Weston (1941)
1590	Tyramine	Rats; M, F	> 2000	Til et al. (1997)
1594	1,6-Hexalactam	Rats; M	1650	National Toxicology
1594	1,6-Hexalactam	Rats; F	1210	Program (1982)
1594	1,6-Hexalactam	Mice; M	2070	
1594	1,6-Hexalactam	Mice; F	2490	
1595	2-Isopropyl-*N*,2,3-trimethylbutryamide	Rats; M, F	345	Bond & Nixon (1980)
1600	Piperine	Dogs; NR	40.3	Singh et al. (1973)
1600	Piperine	Rat; NR	59.6	
1600	Piperine	Mice; NR	56.2	
1600	Piperine	Rats (adult); F	514	Piyachaturawat et al. (1983)
1600	Piperine	Rats (weanling); F	> 585	
1600	Piperine	Mice; M	330	
1601	*N*-Ethyl 2-isopropyl-5-methylcyclohexanecarboxamide	Rats; M, F	2900	James (1974)
1601	*N*-Ethyl 2-isopropyl-5-methylcyclohexanecarboxamide	Mice; NR	5300	
1607	Piperidine	Rats; F	337	Yam et al. (1991)
1607	Piperidine	Rats; M, F	445	Van den Heuvel et al. (1990)
1607	Piperidine	Rats; M	405	
1607	Piperidine	Rats; F	488	
1607	Piperidine	Rats; M	520	Smyth et al. (1962)
1609	Pyrrolidine	Rats; M	300	Zaeva et al. (1974)
1611	Triethylamine	Mice; M, F	500	Loit & Filov (1964)
1611	Triethylamine	Rats; M	< 182.3[b]	Myers & Ballantyne (1997)
1612	Tripropylamine	Rats; NR	96	Smyth et al. (1969)
1615	Piperazine	Rats; M	2830	Myers & Ballantyne (1997)

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

[a] LD_{50} value reported as < 0.25 ml/kg; calculation per kilogram body weight based on specific gravity of isopropylamine = 0.6881

[b] LD_{50} value reported as < 0.25 ml/kg; calculation per kilogram body weight based on specific gravity of triethylamine = 0.7293

Table 4. Results of short-term studies of toxicity and long-term studies of toxicity and carcinogenicity with aliphatic and aromatic amines and amides

No.	Substance	Species; sex	No. test groups[a]/ no. per group[b]	Route	Duration (days)	NOEL mg/kg bw per day)	Reference
Short-term studies							
1584	*sec*-Butylamine	Rat; M	1/7	Inhalation	13 days	115[c]	Gage (1970)
1590	Tyramine	Rat; M,F	3/20	Feed	5–6 weeks	180	Til et al. (1997)
1592	Acetamide	Mouse; M, F	2/100	Feed	1 year	< 1770[n]	Fleischman et al. (1980)
1592	Acetamide	Rat; M, F	1/100	Feed	1 year	< 1180[n]	Fleischman et al. (1980)
1594	1,6-Hexalactam	Mouse; M,F	5/10	Feed	14 days	4500[c]	National Toxicology Program (1982)
1594	1,6-Hexalactam	Mouse; M,F	5/20	Feed	13 weeks	< 750[d]	National Toxicology Program (1982)
1594	1,6-Hexalactam	Rat; M,F	5/10	Feed	14 days	< 500 (M)[e] 3000 (F)[c]	National Toxicology Program (1982)
1594	1,6-Hexalactam	Rat; M,F	5/24	Feed	13 weeks	750[f]	National Toxicology Program (1982)
1595	2-Isopropyl-*N*-2,3-trimethyl-butyramide	Rat; M,F	3/12	Gavage	14 days	100[c,g]	Nixon & Alden (1978)
1595	2-Isopropyl-*N*-2,3-trimethyl-butyramide	Rat; M,F	3/60	Gavage	14 weeks	< 10 (M) 10 (F)	Pence (1980a)
1595	2-Isopropyl-*N*-2,3-trimethyl-butyramide	Rat; M,F	5/60	Gavage	14 weeks	5 (M) 10 (F)	Cheng (1982)
1599	Nonanoyl 4-hydroxy-3-methoxy-benzylamide	Rat; M, F	1/20–32	Feed	90 days	8.4 (M)[c] 10.3 (F)[c]	Posternak et al. (1969)
1600	Piperine	Rat; F	4/8	Gavage	7 days	100	Piyachaturawat et al. (1983)
1600	Piperine	Rat; M	1/6	Feed	8 weeks	10[c]	Bhat & Chandrasekhara (1986b)
1600	Piperinei	Rat; M	3/6	Feed	8 weeks	20[c]	Bhat & Chandrasekhara (1986b)

Table 4 (contd)

No.	Substance	Species; sex	No. test groups[a]/ no. per group[b]	Route	Duration (days)	NOEL mg/kg bw per day)	Reference
1601	*N*-Ethyl 2-isopropyl-5-methyl-cyclohexanecarboxamide	Rat; M,F	4/12	Gavage	28 days	8	Miyata (1995)
1601	*N*-Ethyl 2-isopropyl-5-methyl-cyclohexanecarboxamide	Rat; M,F	3/30	Gavage	22 weeks	300 (M) < 100 (F)[i]	Hunter et al. (1974)
1601	*N*-Ethyl 2-isopropyl-5-methyl-cyclohexanecarboxamide	Dog; M,F	3/2	Capsules	28 days	< 600[k]	James (1974)
1601	*N*-Ethyl 2-isopropyl-5-methyl-cyclohexanecarboxamide	Dog; M,F	3/6	Capsules	52 weeks	100	James (1974)
1603	1-Pyrroline	Rat; M,F	1/10	Feed	14 days	12.9 (M)[c] 12.2 (F)[c]	Wnorowski (1995)
1607	Piperidine	Rat; M	3/5–6	Feed	98 days	80	Amoore et al. (1978)
1609	Pyrrolidine[l]	Mouse; F	1/10	Drinking-water	12 months	16.5[c]	Pearson et al. (1982)
1610	Trimethylamine	Rat; M	4/5-6	Feed	98 days	160	Amoore et al. (1978)
1615	Piperazine	Mouse; M,F	1/80	Feed	28 weeks[m]	938[c]	Greenblatt et al. (1971)
Long-term studies							
1592	Acetamide	Rat; M	1/60	Feed	17 months	< 2500[n]	Flaks et al. (1983)
1594	1,6-Hexalactam	Mouse; M,F	2/100	Feed	103 weeks	2250[c]	National Toxicology Program (1982)
1594	1,6-Hexalactam	Rat; M,F	2/100	Feed	103 weeks	375[c]	National Toxicology Program (1982)

[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 effect. The actual NOEL might be higher.
[d] Based on significant reduction in body weights (27% to 63%) in comparison with controls; dose-related in females but not males.

Table 4 (contd)

[e] Pale mottled kidneys observed at every dose (60–100% incidence) in males
[f] Reductions in weight gain (4–14%) reported at every dose in both males and females and in food consumption in males (23%) and females (19%) at 7500 ppm (750 mg/kg bw per day)
[g] Significantly elevated absolute and relative kidney weights in females at 10 and 100 mg/kg bw per day in comparison with controls; not accompanied by histopathological variations Animals dosed twice daily.
[h] Test animals had significant increases in activities of several digestive enzymes and decreased food transit time in comparison with controls.
[i] Rats given black pepper oleoresin containing approximately 45% piperine
[j] Significant dose-related increases in absolute and relative liver weights at every dose
[k] Liver weights of most dogs (doses not specified) constituted > 4% of dogs' body weight; however, gross examination revealed no abnormalities (no histology)
[l] Pyrrolidine administered with 1000 mg/l nitrite in drinking-water
[m] 196-day treatment period followed by 12 weeks on basal diet before final necropsy
[n] Treament at this dose resulted in hepatocellular carcinomas in rats and lymphomas in mice

overnight after the last exposure, and on the following day animals were necropsied and blood samples collected for haematological testing. Gross and microscopic examinations were conducted on a range of major organs. During the exposure, clinical signs of discomfort and lethargy were observed. Treated animals gained less weight than controls; however, no remarkable changes were seen at gross necropsy, and no histopathological changes related to exposure to sec-butylamine were reported (Gage, 1970).

Tyramine (No. 1590)

Groups of 10 male and 10 female weanling Wistar rats (Bar:WISW) were fed diets containing 0 (control), 200, 2000 or 10 000 ppm tyramine (equivalent to 0, 18, 180 and 900 mg/kg bw per day) for 5–6 weeks. They were observed daily for any overt symptoms of toxicity, and body weights and food intake were assessed weekly; water consumption was monitored daily during the first week of the study. One male at 200 ppm tyramine died before the end of the study, but details of the death were not reported. At day 28, the body weights of males at the highest dose were statistically significantly lower than those of controls. There were no differences in systolic blood pressure, measured on several occasions throughout the study, in food or water consumption or in food use efficiency between control and treated groups. Likewise, various haematological and urinary parameters evaluated in week 5 were comparable to those of controls; however, clinical chemistry analysis of blood samples taken in week 5 showed a statistically significant decrease in alkaline phosphatase activity in males and females at the highest dose and a slight but significant decrease in males at 2000 ppm tyramine. At necropsy, a wide range of major organs were weighed, and samples of tissues from controls and rats at the highest dose were prepared for detailed microscopic examination. The only variation in organ weights was a statistically significant increase in the relative liver weights of males at the highest dose in comparison with controls. Histopathological examination showed a statistically significant increase in the combined incidences of reticulo-endothelial cells and necrotic hepatocytes in livers of males at the highest

dose. Although no other statistically significant histopathological findings were made, a slight increase in the incidence of basophilic tubules in the kidneys of these animals was noted (controls: 3/10; males at 10 000 ppm: 7/10). The NOEL was 2000 ppm, equivalent to 180 mg/kg bw per day (Til et al., 1997).

Acetamide (No. 1592)

Mice

In a 1-year study, groups of 50 C57Bl/6 mice of each sex were given diets containing 1.18% or 2.36% acetamide (equivalent to 1770 and 3540 mg/kg bw). Groups of 100 control animals of each sex were maintained concurrently on the basal diet. At day 365, five mice from each group were necropsied. A wide range of major organs (brain, lung, heart, thymus, pituitary, thyroids, parathyroids, adrenals, oesophogus, stomach, duodenum, jejunem, ileum, colon, liver, pancreas, kidney, urinary bladder, gonads, accessory sex organs, spleen, lymph nodes, bone with bone marrow, skin, salivary and mammary glands) were collected and fixed in 10% buffered formalin, embedded in paraffin, stained with haematoxylin–eosin and examined microscopically. All the remaining mice were placed on a control diet for recovery for 4 months, after which time they were necropsied and tissue samples preserved for histological examination. No compound-related effects were observed on body weights or survival rates. At necropsy, the gross changes included enlargement of multiple lymph nodes, including the submandibular, mesenteric, lumbar, inguinal and renal nodes, which were reported as white–grey, firm and sometimes nodular or irregular on the capsular surface. Enlargement of the spleen and thymus was also seen. Additionally, benign papillomas of the squamous portion of the stomach were reported in male mice at the lower dose (5/50, $p = 0.005$). Histopathological examination revealed a compound-related increase in the incidence of neoplastic lesions of the haematopoietic system in treated males, but not in females, with incidences of 0 in controls and 14% (7/50) and 15% (7/46) in males at the lower and lhigher intake levels, respectively. The most frequently observed neoplasm was a histiocytic type of malignant lymphoma; less commonly observed haematopoietic tumours included other malignant lymphomas and plasmacytomas (Fleischman et al., 1980).

Rats

In a 1-year study, groups of 50 Fischer 344 rats of each sex were given diets containing 0 or 2.36% acetamide (23 600 ppm; equivalent to 1180 mg/kg bw per day). At day 365, five rats from each group were killed and necropsied. Brain, lung, heart, thymus, pituitary, thyroids, parathyroids, adrenals, oesophagus, stomach, duodenum, jejunum, ileum, colon, liver, pancreas, kidney, urinary bladder, gonads, accessory sex organs, spleen, lymph nodes, bone with bone marrow, skin, salivary and mammary glands were collected and fixed in 10% buffered formalin, embedded in paraffin, stained with haematoxylin–eosin and examined microscopically. All the remaining rats were placed on a control diet to recover for 4 months, after which they were necropsied and tissue samples preserved for histological examination. Additionally, liver sections were stained and evaluated for the presence of fat. Body-weight gain and the survival of females were comparable in test and control groups, but, while control males had a survival rate of 86% at the end of the study, only 56%

of the acetamide-treated rats survived until that time. The mean absolute liver weight of male rats was twice that of controls, and that of females was 1.4 times that of controls. The relative liver weight of treated rats that did not have liver tumours was comparable to that of controls, while that of rats with liver tumours was two to three times greater than that of controls. Pathological examination of organs revealed neoplastic lesions of the liver. Neoplastic nodules and carcinomas of the liver occurred in 1/47 and 41/47 acetamide-treated males and 3/48 and 33/48 females, respectively, but males showed faster and greater onset of metastases than females. None of the control animals developed liver tumours. Metastases occurred in 32% (15/47) of treated males and 10% (5/48) of treated females. In males, metastases were reported in the lung (15), kidneys (4), peritoneal cavity (2), pancreas, diaphragm, heart and mediastinum (1 each), whereas in females metastases was confined to the lungs. Hepatocytes from the neoplastic nodules showed varying degrees of cytoplasmic change, including clear-cell eosinophilic and basophilic alterations. Mitotic figures and nuclear atypia, including multi-nucleation and hyperchromasia, indicative of apoptosis, were characteristic of the nodules. In comparison with the nodules, the carcinomas were large and in some cases extended over the entire lobe of the liver. Cytoplasmic vacuolization, eosinophilia and basophilia were frequent, as were high nuclear:cytoplasmic ratios, unusual mitotic figures and single and multiple hyperchromatic nucleoli. Areas of degeneration, necrosis and haemorrhage were seen in about half the tumours. Two testicular adenomas were observed in treated rats, while tumours of the testes were reported in 21 of 50 controls ($p < 0.001$) (Fleischman et al., 1980).

1,6-Hexalactam (No. 1594)

Mice

Groups of five male and five female $B6C3F_1$ mice were given diets containing 0, 5000, 10 000, 15 000, 20 000 or 30 000 ppm 1,6-hexalactam (equivalent to 0, 750, 1500, 2250, 3000 and 4500 mg/kg bw per day) for 14 days. No early deaths or any other compound-related adverse effects were reported in treated animals (National Toxicology Program, 1982).

In a 13-week study conducted to determine the dietary concentrations for a longer feeding study, groups of 10 $B6C3F_1$ mice of each sex were fed diets containing 0, 5000, 10 000, 15 000, 20 000 or 30 000 ppm 1,6-hexalactam (equivalent to 0, 750, 1500, 2250, 3000 and 4500 mg/kg bw). Animals were monitored twice daily for clinical signs of toxicity and weighed weekly. At necropsy at the end of the study, tissue samples were examined histopathologically. One female at 20 000 ppm died accidentally, and two females at 30 000 ppm died of unspecified causes. No males died before scheduled termination of the study. All treated mice gained less weight than controls (27–63%), and the decrease was dose-related in female mice. No compound-related histopathological changes were reported. There was no NOEL (National Toxicology Program, 1982).

Rats

Groups of five male and five female Fischer 344 rats were given diets containing 0, 5000, 10 000, 15 000, 20 000 or 30 000 ppm 1,6-hexalactam (equivalent to 0, 500, 1000, 1500, 2000 and 3000 mg/kg bw per day) for 14 days. All animals

survived the treatment period. Treated males at all doses showed pale mottled kidneys, affecting 60–100% of rats (National Toxicology Program, 1982).

In a 13-week study, groups of 12 Fischer 344 rats of each sex were fed diets containing 0, 625, 1250, 2500, 5000 or 7500 ppm 1,6-hexalactam (equivalent to 0, 62.5, 125, 250, 500 and 750 mg/kg bw). The animals were monitored twice daily for clinical signs of toxicity and weighed weekly. At necropsy at the end of the study, tissue samples were examined histopathologically. One male rat at 5000 ppm became moribund and was killed. Treated rats at each dose gained less weight than controls (4–14%), but the decrease was not dose-related. Rats at the highest dose also showed decreased food consumption, by 23% for males and 19% for females, in comparison with controls (statistics not reported). No compound-related histopathological changes were reported (National Toxicology Program, 1982).

*2-Isopropyl-*N*-2,3-trimethylbutyramide (No. 1595)*

In a pilot study, groups of six male and six female Sprague-Dawley (CD®) rats were given 2-isopropyl-*N*-2,3-trimethylbutyramide in corn oil by gavage at a dose of 0, 5, 25 or 50 mg/kg bw twice daily for 14 days. No changes in overall condition, terminal body weights or food consumption and use efficiency were reported in comparison with controls. At necropsy, one male at 50 mg/kg bw per day showed bilateral multifocal white areas in the kidneys, cystic calculi in the bladder and splenomegaly. In treated females, the absolute and relative kidney weights were significantly increased at the lowest and highest doses compared with controls, and one female at the lowest dose had hepatic lipidosis, which was described as a non-specific variation occasionally observed spontaneously. Since the increases in absolute and relative kidney weights in females were not dose-related and were not accompanied by histopathological changes in the renal tissues, the Committee considered these findings not to be biologically relevant (Nixon & Alden, 1978).

Groups of 30 Sprague-Dawley CD® rats of each sex were given 2-isopropyl-*N*-2,3-trimethylbutyramide in corn oil by gavage at a dose of 0, 10, 50 or 100 mg/kg bw once daily for up to 14 weeks. Animals were necropsied at 6, 13 and 14 weeks. During the study, two control females, one female at the lowest dose, two at the intermediate dose and two at the highest dose were found dead of unspecified causes, and four control females, one female at the lowest dose and one at the highest dose died accidentally in week 13 after orbital sinus bleeding; however, statistical analysis did not show a significant difference in the survival rates of test and control animals. Likewise, with the exception of a statistically significant reduction in food use efficiency in week 9, no statistically significant differences in body weight, growth rate or food consumption were found between treated and control animals throughout the study. Occasional statistically significant variations were observed in haematological parameters (elevated leukocyte count in males at the highest dose at 13 weeks) and in clinical chemistry values (lower lactic acid dehydrogenase activity in males at the two lower doses at 6 and 13 weeks, higher globulin value in females at the lowest dose, higher cholinesterase activity in males at the lowest dose, lower glucose value in females at the intermediate dose and lower albumin:globulin ratios in females at the lowest dose and males at the highest dose at week 6); however, none of these findings was considered by the author to be treatment-related. Similarly, urine analysis revealed no significant variations at 6 and 13 weeks. At week 6, significant increases were observed in the absolute and relative liver weights of

females at the highest dose and in the relative liver weights of females at the two higher doses, in the absolute spleen weights of males at the lowest dose, and in the relative brain weights of females at the intermediate dose. At week 13, the absolute and relative liver weights were significantly increased in rats of each sex at the highest dose, and relative ovary weights were significantly lower in females at the lowest and the highest doses. Ophthalmoscopy and gross pathological examination revealed no compound-related adverse effects. The histopathological findings were limited to abnormalities of renal and hepatic tissues. Specifically, microscopic examination of 10 animals of each sex at week 6 showed compound-related hepatic fatty degeneration in males and renal tubular nephrosis in males and females at the two higher doses. Histopathological examination of controls and 10 animals of each sex at the highest dose at week 13 showed renal tubular nephrosis and hepatic fatty degeneration in treated males and minimal vacuolar changes in the livers of treated females. At the end of the study (14 weeks), hepatic fatty degeneration was reported in the remaining animals of each sex at the two higher doses. Renal tubular nephrosis was also reported in treated males at every dose and in females at the intermediate dose. The authors noted that all the hepatic and renal lesions were more severe in males than in females (Pence, 1980a).

In a replication of the above study with lower doses, groups of 30 male and 30 female Sprague-Dawley rats were given 2-isopropyl-*N*-2,3-trimethylbutyramide in corn oil by gavage at a dose of 0, 1, 2, 5, 10 or 50 mg/kg bw once daily. The study included interim necropsies on day 23 and days 30–31 and a necropsy at the end of the study on days 92–93. Additionally, one group of rats was given the compound for 112 days, were then allowed to recover for 30–31 days and were subsequently necropsied. All animals were examined microscopically for renal lesions. One rat of each sex at 50 mg/kg bw per day and one of each sex at 2 mg/kg bw per day died as a result of the gavage procedure. One control male and one at the highest dose died, with no abnormal changes, and one male at 2 mg/kg bw per day was killed after a traumatic injury. One control of each sex and one male at 2 mg/kg bw per day had palpable tissue masses in the right axillary region during the study. All the masses grew, then decreased in size until they were no longer detectable at the end of the study. All the other clinical signs were incidental, occurred sporadically and were evenly distributed in all groups, and none of the clinical signs was considered to be related to administration of the test substance. Likewise, none of the isolated statistically significant changes in body weight observed throughout the study were considered to be compound-related, including a significant increase in the body-weight gain of males at 10 mg/kg bw per day at week 12. During the recovery period (weeks 17–20), the animals tended to have an increased incidence of weight loss and reduced weight gain in comparison with the last 4 weeks of treatment (weeks 13–16). The author considered this to be due to a loss of calories from the corn oil. No dose-dependent changes were observed in the food consumption or food use efficiency of test animals in comparison with controls. Ophthalmological examination revealed two isolated ocular lesions, which were considered to be of no toxicological significance. Although variations in haematological values were reported in treated males after the recovery period, the values were within biological limits and thus not toxicologically significant. Furthermore, no significant differences were observed in clinical chemistry and urine values, although an increase in urinary protein excretion was seen in males relative to females. Variations in the absolute, but not the relative, weights of the stomachs of males given 1 or 5 mg/kg bw per day observed at study

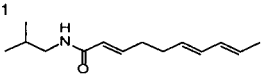
termination (days 92–93) were reported to fall within the normal limits of deviation. Females at 50 mg/kg bw per day had significant increases in absolute and relative liver weights and in absolute kidney weights at the 92- and 93-day necropsies. The treatment-related histopathological findings were limited to changes in the kidneys of males, consisting of tubular dilatation with casts and hyaline droplet formation, the latter being reported at both interim necropsies and at the final necropsy but not after the recovery period. Tubular dilatation with granular casts, classified as mild to moderate, occurred in males treated not only at 50 mg/kg bw per day dose but also at 10 mg/kg bw per day. Although tubular dilatation was seen at all scheduled necropsies, the incidence was markedly increased only at the necropsies conducted on days 30–31 and 92–93 (Cheng, 1982).

Structurally related medium-chain unsaturated amide Spilanthol[1] (N-isobutyl-2,6,8-decatrienamide)

In a 28-day study, groups of five male and five female Sprague-Dawley Aai:N(SD)BR rats were maintained on a diet containing 0, 130, 1300 or 13 000 ppm gold root extract of unknown purity (equal to 0, 11, 115 and 1144 mg/kg bw per day for males and 0, 13, 127 and 1258 mg/kg bw per day for females). As *N*-isobutyl-2,6,8-decatrienamide comprises approximately 50% of the composition of gold root extract (private communication to the Flavor and Extract Manufacturers Association, 2004), the effective dietary concentration of *N*-isobutyl-2,6,8-decatrienamide was about 5.5, 57 and 572 mg/kg bw per day for males and 6.5, 64 and 629 mg/kg bw per day for females, respectively. The animals were observed daily for clinical signs and mortality. Individual body weights and food consumption were recorded weekly. On day 29 of the study, blood was sampled from all animals for haematological and clinical chemistry analyses, and gross necropsies were performed on all rats. The heart, thymus, adrenals, spleen, liver, kidneys and ovaries or testes were collected and weighed. Histopathological examinations were performed on the liver, kidneys, stomach, small intestine (duodenum, jejunum, ileum) and caecum of all control rats and those at the highest dose, as well as any gross lesions of potential toxicological significance in any of the test groups.

During the study, no deaths or clinical signs of toxicity were observed in any test group. At necropsy, no gross abnormalities were reported. Some minor alterations were observed during the histopathological examination (e.g. inflammation and vacuolation of the liver and slight mineralization in the kidney); however, these were considered to be typical background findings occurring commonly among rats of this strain and age. Body weight, body-weight gain, daily food consumption and food use efficiency were comparable in test and control animals. Likewise, no treatment-related changes in absolute or relative organ weights were observed between control and treated rats. In males, haematological examination revealed a statistically significant ($p < 0.01$) decrease in lymphocytes at all doses, and the mean lymphocyte count of males at the highest dose was 33% less than that of controls; this decrease resulted in a concurrent decrease in the total white blood cell count. Treated females also showed a decreased mean lymphocyte count (20% at

1



the highest dose) and total white blood cell count, but these differences were not statistically significant. Two of the five control animals had abnormally elevated lymphocyte counts (i.e. 11.53 and 10.39 $\times$ 10^{-3}/μl), which might have skewed the mean lymphocyte count of controls (Farber, 2003). With the exception of one female at the highest dose, the lymphocyte and total white blood cell counts of both male and female rats fell within the range of values for controls in previous studies, and the observed changes were not considered clinically relevant. Additionally, minimal but statistically significant ($p < 0.05$) decreases in monocyte counts were found in females at the two higher doses and in the basophil count among males at the two higher doses; decreased monocyte counts were also seen among treated males. Although these changes were dose-dependent, statistical significance was not attained. The occurrence of large unstained cells was significantly decreased among males at the intermediate ($p < 0.01$) and highest doses ($p < 0.05$) compared with controls. The minor shifts in these cell populations were not considered clinically relevant by the authors. Erythrocyte volume fractions were significantly decreased ($p < 0.05$) in males at 130 ppm, but not at the two higher doses, thereby casting doubt on the clinical significance of these results. Moreover, all erythrocyte volume fractions were within the range of values for controls in previous studies. A slight but statistically significant increase ($p < 0.01$) in serum sodium was observed in females at the highest dose, and serum chloride levels were significantly increased in males at the intermediate ($p < 0.05$) and highest doses ($p < 0.01$) and in females at the highest dose ($p < 0.05$). All the changes in electrolyte levels were of minimal magnitude and the values fell within the ranges of values for controls in previous studies. Calcium and phosphorus levels were significantly increased ($p < 0.01$) in females at the intermediate dose, but not in those at the highest dose, thereby casting doubt on the clinical significance of the observation. Total protein ($p < 0.01$) and albumin ($p < 0.05$) levels were significantly increased in males at the highest dose, but all the values fell within the ranges seen in controls in previous studies. The changes in haematological and serum chemistry parameters were not considered to be of biological significance. The NOEL for gold root extract was 13 000 ppm, or 1144 mg/kg bw per day. If it is assumed that *N*-isobutyl-2,6,8-decatrienamide is present at a concentration of 50% in gold root extract, the NOEL for this compound was 572 mg/kg bw per day (Moore, 2002).

To further evaluate the effect of exposure to gold root extract on haematological parameters in rats, a second 28-day study was conducted, in which groups of five Sprague-Dawley rats of each sex were fed diets containing 0, 50, 130 or 13 000 ppm gold root extract (equal to 0, 11 and 1144 mg/kg bw per day for males and 0, 13 and 1258 mg/kg bw per day for females). Lymphocyte counts were slightly decreased in males at 13 000 ppm and possibly decreased at 50 and 130 ppm, but these changes were not statistically significant. Of the animals receiving 50 or 130 ppm gold root extract, 4/10 and 5/10, respectively, had lymphocyte counts that were lower than the lowest value observed in control animals, and these changes were reflected in the total leukocyte count. Although the lymphocyte changes were subtle, they were considered by the authors as potentially related to treatment. Published composite reference intervals for Sprague-Dawley rats indicate, however, that the normal lymphocyte counts of young adult males range from 3000 to 12 000 lymphocytes per μl (Hall, 1992); the individual values for rats in this study were all within this range and therefore not biologically significant. The lymphocyte count

was slightly, not significantly decreased in females at 13 000 ppm, the values being concentrated mainly towards the lower end of the range of values for concurrent controls. The authors considered this change to be treatment-related. The change in lymphocyte count was reflected in the total leukocyte count. The lower limit of lymphocyte counts for all groups, including controls, in the current study were similar, 3.58, 3.43, 2.72 and 2.78 $\times$ 10^3/µl for females receiving 0, 50, 130 and 13 000 ppm, respectively, indicating little difference between control and treated groups. In addition, the published composite reference intervals for lymphocyte counts in young adult female Sprague-Dawley rats range from 1000 to 10 000 lymphocytes/µl (Hall, 1992); the individual values for rats in this study were all within this range. The NOEL was 13 000 ppm, equal to 1144 mg/kg bw per day (Product Safety Laboratories, 2004).

Nonanoyl 4-hydroxy-3-methoxybenzylamide (No. 1599)

Groups of 10–16 male and 10–16 female Charles River CD rats were fed diets containing nonanoyl 4-hydroxy-3-methoxybenzylamide for 90 days at concentrations resulting in average dietary intakes of 8.4 and 10.3 mg/kg bw per day for males and females, respectively. Throughout the study, body weights and food consumption were monitored weekly, and the food use efficiency was calculated. Blood samples were taken at weeks 7 and 13 for haematological and clinical chemistry analysis. At study completion, the animals were necropsied and examined grossly. The kidneys and liver were excised and weighed, and a wide range of major organs were examined microscopically. In comparison to the group given basal diet, no significant variations were observed in any of the toxicological parameters evaluated. Furthermore, no compound-related histopathological abnormalities were reported (Posternak et al., 1969).

Piperine (No. 1600)

Groups of eight female Fischer rats were given piperine intragastrically at a dose of 0 (solvent control), 100, 250, 350 or 500 mg/kg bw per day for 7 days. Body-weight gain decreased and the number of deaths increased with increasing doses. Although no deaths occurred at the two lower doses, a slight reduction in body-weight gain (statistical significance not reported) was reported at 250 mg/kg bw per day. Two rats at 350 mg/kg bw per day and five at 500 mg/kg bw per day died after 1–3 days of treatment. Necropsy of rats at 500 mg/kg bw per day revealed severe haemorrhage and oedema of the gastrointestinal tract (stomach, small and large intestine). Haemorrhage in the urinary bladder also was reported in some rats. At 250 mg/kg bw per day, three of eight rats had haemorrhage in the stomach. Histopathological examination showed changes in the stomach, urinary bladder, adrenal glands and small intestine. In the stomach, the main changes seen in rats fed 500 mg/kg bw per day were haemorrhagic erosion and ulceration in the mucosa of the glandular portio. Severe haemorrhagic oedema was also reported in the submucosa of the glandular and squamous portions. All animals at 500 mg/kg bw per day, three at 250 mg/kg bw per day and four at 350 mg/kg bw per day had gastric ulceration. Variations similar to those observed in the stomach were seen in the urinary bladder (doses not specified), consisting of degenerative lesions and haemorrhagic necrosis of the epithelial cell lining, accompanied by extremely severe haemorrhagic oedema in the submucosa and muscular layers. Mild-to-moderate

enteritis with epithelial cell necrosis and desquamation were reported in the small intestine of rats at the highest dose. Additionally, signs of severe haemorrhage and degenerative necrosis of the adrenal medulla, varying in degree of severity among rats, were reported at the highest dose. Mild fatty infiltration in the liver and cell necrosis in the corpora lutea were the only other significant histopathological changes reported (Piyachaturawat et al., 1983).

Groups of six male Wistar rats were fed diets containing black pepper oleoresin at a concentration of 11, 22 or 44 mg% (110, 220 and 440 ppm, respectively), pepper at 0.2% (2000 ppm) or piperine at 10 mg% (100 ppm) at the expense of corn starch for a period of 8 weeks. The dietary levels of pepper and oleoresin were intended to be approximately 5–20 times the normal daily intake of humans. Given that the piperine content of the black pepper oleoresin was approximately 45%, the dietary levels of piperine resulting from consumption of the oleoresin were approximately 50, 100 and 200 ppm. These levels of piperine in pepper oleoresin (45% piperine) were calculated to provide average daily intakes of approximately 5, 10 and 20 mg/kg bw of piperine. The 100 ppm dietary level of piperine was calculated to provide an average daily intake of approximately 10 mg/kg bw. A group of control animals was maintained on basal diet. Ingestion of black pepper oleoresin, pepper or piperine had no effect on food intake, feed use efficiency, organ weights (liver, kidney, spleen and adipose tissue), haematological parameters (haemoglobin, red blood cells, white blood cells, lymphocytes and neutrophils) or clinical chemistry values (total protein, albumin:globulin ratio, glucose, cholesterol and serum aspartate and alanine aminotransferase and alkaline phosphatase activities). Isolated, non-dose-dependent variations were observed in nitrogen and fat absorption and in the retention of nitrogen in some groups of treated rats (Bhat & Chandrasekhara, 1986b).

N-*Ethyl 2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601)*

In a 28-day study, groups of six Crj:CD(SD) rats of each sex were given *N*-ethyl 2-isopropyl-5-methylcyclohexanecarboxamide by gavage daily at a dose of 0 (vehicle control), 8, 40, 200 or 1000 mg/kg bw. Two additional groups were given the vehicle or 1000 mg/kg bw per day for 28 days, followed by a 14-day recovery period. No unscheduled deaths were reported and no changes in food consumption or urinary parameters were observed. Clinical signs (e.g. salivation, decreased spontaneous locomotion, tremor, staggering gait) indicative of general toxicity were reported in rats at the highest dose. Milder effects were reported at 200 mg/kg bw per day (e.g. salivation) in both sexes and at 40 mg/kg bw per day (e.g. salivation) in males. Body-weight gain was significantly reduced in males at the highest dose. Changes in haematological and clinical chemistry parameters were noted in rats given the highest dose and consisted of increased prothrombin time and reticulocyte count (both sexes), increased white blood cell count and activated partial thromboplastin time (males), increased γ-guanosine triphosphatase, total cholesterol, triglyceride and calcium values (both sexes), increased total protein (females) and decreased alkaline phosphatase activity and chloride (females). Increased liver weights were reported in males and females at 1000 mg/kg bw per day and in females at 200 mg/kg bw per day. In males at the highest dose, kidney and testis weights were also increased. At necropsy, the gross findings included a dose-dependent response consisting of spotty patterns on kidney surfaces and enlarged

livers in males at 200 and 1000 mg/kg bw per day, enlarged livers and blackish changes in the spleen in females at 1000 mg/kg bw per day, and enlarged kidneys in males at 1000 mg/kg bw per day. Histopathological examination revealed dose-dependent swelling of hepatocytes and increased haemosiderin-laden cells in the spleens of treated animals of each sex at 200 and 1000 mg/kg bw per day, congestion of the spleen in animals of each sex at 1000 mg/kg bw per day, and increased numbers of eosinophilic bodies in the kidneys of males at ≥ 40 mg/kg bw per day. Males at the highest dose continued to show reduced body-weight gain until day 15 of recovery, after which the body-weight gains began to normalize. In both sexes at the highest dose, increased liver weight, swelling of hepatocytes, increased haemosiderin-laden cells in the spleen, increased eosinophilic bodies in the kidneys, increased total protein levels and decreased chloride concentrations continued to be observed during the recovery period; however, the severity and frequency of the renal and hepatic changes were less marked than directly after treatment. The NOEL was 8 mg/kg bw per day (Miyata, 1995).

In a 22-week study, groups of 15 Sprague-Dawley (CFY) rats of each sex were given *N*-ethyl 2-isopropyl-5-methylcyclohexane carboxamide by gavage at a dose of 0, 100, 300 or 725 mg/kg bw. After initial dosing, but not thereafter, several males were lethargic and had mild hypothermia, ataxia and lachrymation, and two females at the highest dose were semi-comatose. Throughout the study, mild transient salivation was noted in rats at the highest dose and to a lesser extent in those at 300 mg/kg bw per day. At the end of the second week and for the remainder of the study, rats at the highest dose and a few at the intermediate dose showed reduced grooming activity. Several rats had mild sialodacryoadenitis during weeks 8–12. One male and one female at the highest dose died at week 4; however, the deaths were probably the result of an intubation error. Another male at the highest dose was killed because of ocular haemorrhage after orbital blood sampling. One male at the intermediate dose was found dead during week 12 with signs of lung congestion. An additional 11 treated rats died during the last 3 weeks of the study, probably due to blood sampling error.

No significant variations were found in food consumption between test and control rats; however, during the first 3 months, males at the two higher doses had a dose-related reduction in growth rate. Significantly reduced body-weight gain was also reported in females at the highest dose, and rats at the highest dose continued to show lower weight gain during the last 9 weeks. The reduced body-weight gain observed at the highest dose was accompanied by a reduction in feed use efficiency from week 5 of the study. No abnormalities of the eye were reported. At weeks 6 and 7, urine samples from controls and animals at the two higher doses contained leukocytes; however, none were seen at week 12. Epithelial cells were reported in urine samples taken at weeks 6 and 7, but not at week 12. Females at the highest dose had a dose-related increase in specific gravity with no change in urine volume at weeks 7 and 12, whereas males had an increase in volume output with no change in specific gravity. An increased urinary volume was observed in males at the intermediate dose at week 13. Additionally, reducing substances were found in the urine of females with increasing frequency over the test period. The only variations in haematological parameters reported were in males at weeks 12, 13, 15 and 22, which showed increased blood clotting times in thrombotests in comparison with controls; however, this effect was thought to be a result of tissue fluid contamination,

and therefore additional blood samples were collected by cardiac puncture at weeks 21 and 22. At week 21, the thrombotest values for rats at the two lower doses were below the upper normal limit; although the values for animals at the highest dose remained above the normal limits of variation, the values were markedly reduced from previous weeks. At week 22, a further improvement was noted in thrombotest values in blood collected by cardiac puncture in comparison with values in blood obtained from the orbital sinus. Statistically significant variations in clinical chemistry at week 6 were limited to a reduction in serum alkaline phosphatase activity in animals at the highest dose in comparison with controls; however, the values were within the normal limits of variation. Clinical chemistry parameters examined at week 12 revealed the following statistically significant changes: increased plasma glucose and serum sodium levels, decreased serum alkaline phosphatase activity in males at the highest dose and increased total serum protein levels in both sexes. In an extension of the study, serum protein was also increased in rats of each sex at the two lower doses. Dose-related variations in organ weights were limited to significant increases in the absolute and relative liver weights of females in all test groups in comparison with controls, and significantly elevated relative liver weights in males at the highest dose. All other variations in organ weights were considered to be incidental or to reflect body weight differences and, as such, were not deemed to be toxicologically significant. The results of gross examinations were unremarkable. Although histopathological examination revealed some changes in cardiac and pulmonary tissues and in the livers in all groups of animals, the changes were typical of this strain of laboratory rat and considered not to be related to treatment (Hunter et al., 1974).

N-*Ethyl 2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601)*

Groups of three male and three female beagle dogs were given gelatine capsules containing *N*-ethyl 2-isopropyl-5-methylcyclohexanecarboxamide at a dose of 0, 100, 300 or 1000 mg/kg bw per day for 52 weeks. One female at 1000 mg/kg bw per day died on day 271. The clinical signs consisted of loose stools in all groups, but with greater frequency in treated animals, occasional vomiting at the highest dose and convulsive episodes in one dog at the highest dose. Decreased body-weight gain was reported in females during the first 6 weeks of the study, but no differences were seen subsequently. Midway through the study (weeks 10–13 and 19–22), dogs at the highest dose drank significantly more water than controls. Food consumption was not affected by treatment. No abnormalities of the eye were reported. From week 4 and continuing until study termination, clinical chemistry revealed serum alkaline phosphatase activity approximating or in excess of the normal upper limits in animals at the highest dose. Serum alanine aminotransferase activity exceeding the normal upper limits was observed at 24 weeks in 4/6 dogs at the highest dose and 1/6 at 300 mg/kg bw per day and, at week 50, in 3/6 dogs at the highest dose, 2/6 receiving 300 mg/kg bw per day and 1/6 receiving 100 mg/kg bw per day of the test substance. The gross findings consisted of enlarged, unusually dense, discoloured livers in 4/6 dogs at the highest dose and 1/6 dogs at 300 mg/kg bw per day. The absolute and relative spleen and liver weights of dogs at the highest dose were significantly higher than those of controls. No treatment-related histopathological changes were reported (James, 1974).

In a preliminary 28-day study, groups of one male and one female beagle dog were given gelatine capsules containing *N*-ethyl 2-isopropyl-5-ethylcyclohexanecarboxamide at a dose of 0, 600, 1000 or 1500 mg/kg bw per day for 4 weeks. Occasional vomiting and loose faeces were reported in treated animals at every dose. Additionally, an incident of convulsion was reported on the first day of treatment in a dog at 600 mg/kg bw per day. Although no dose-related effects were observed on body weight, most dogs gained little or no weight during the first week of treatment; however, appetite was not adversely affected. At necropsy, the liver weights of most dogs were higher than the normally accepted upper limit of 4% of body weight; however, no macroscopic abnormalities were found. No additional study details were provided (James, 1974).

1-Pyrroline (No. 1603)

For the first 7 days of a 14-day study, a group of five male and five female Sprague-Dawley rats was given a diet containing 150 ppm of 1-pyrroline, reduced to 115 ppm for the remainder of the study. A control group of animals was maintained for the duration of the study. The authors calculated the average intake of 1-pyrroline to be 12.9 and 12.2 mg/kg bw per day for males and females, respectively. No early deaths and no signs of gross toxicity, adverse pharmacological effects or abnormal behaviour were reported. The food consumption of test animals was comparable to that of controls, and all animals were reported to gain weight and to be active and healthy throughout the study. At necropsy, no significant variations were observed in liver or kidney weights of test animals in comparison with controls. Likewise, the gross findings were unremarkable, and there were no histopathological changes in the liver or kidney attributable to treatment (Wnorowski, 1995).

Piperidine (No. 1607)

Groups of five or six male Sprague-Dawley rats were given diets containing 0, 0.04%, 0.08%, 0.16%, 0.31% or 0.62% piperidine for 14 days (equivalent 0, 40, 80, 160, 310 and 620 mg/kg bw per day). Subsequently, rats fed 0.08%, 0.16% or 0.31% piperidine were continued on the same diets for an additional 84 days. Urine samples taken on day 70 and blood samples taken on day 80 were analysed. No significant differences in urinary, haematological or clinical chemistry parameters, body weights, organ weights or histopathological appearance were seen between rats fed the 0.08% diet and controls. Rats at 0.16% and 0.31% gained significantly less weight than controls. At necropsy, full pathological examination revealed significant alterations in the seminal vesicles (reductions in size, weight and number of secretory granules) and prostate (tubular collapse and a reduced amount of secretory materials) in rats at 0.31% piperidine. No effects were reported at dietary levels of 0.04% and 0.08% (40 and 80 mg/kg bw per day) (Amoore et al., 1978).

Pyrrolidine (No. 1609)

In a 12-month study, the effect of a secondary amine (pyrrolidine) ingested in combination with nitrite on the incidence of tumours in mice was examined. Four groups of 10 female Swiss-ICR mice were given basal diet and drinking-water ad libitum. The drinking-water of test groups contained 100 mg/l *N*-nitrosopyrrolidine,

1000 mg/l sodium nitrite or 1000 mg/l sodium nitrite plus 100 mg/l pyrrolidine. The control group received pure drinking-water. On the basis of water consumption, the authors estimated the intake of pyrrolidine to be 16.5 mg/kg bw per day. This study was repeated four times in four consecutive years. After each 12-month period, mice were killed and necropsied, and lung, liver, kidney, stomach and intestine sections were fixed for microscopic examination. There were no statistically significant differences in water or food consumption, survival or weight gain between pyrrolidine (plus nitrite)-treated and control mice; however, the water consumption of the mice given nitrite only was significantly lower than that of controls. Statistically significant increases in the total numbers and in the numbers of malignant liver and lung neoplastic lesions and in the average total and number of malignant tumours per mouse were observed in groups given *N*-nitrosopyrrolidine in their drinking-water. Ingestion of pyrrolidine (plus nitrite) did not increase the incidence of tumours (Pearson et al., 1982).

Trimethylamine (No. 1610)

Groups of five or six male Sprague-Dawley rats were given diets containing 0, 0.08, 0.16, 0.31 or 0.62% trimethylamine for 14 days (equivalent to 0, 80, 160, 310 and 620 mg/kg bw per day). Subsequently, rats fed 0.08%, 0.16%, 0.31% or 0.62% trimethylamine were continued on the same diets for an additional 84 days. Urine samples taken on day 70 and blood samples taken on day 80 were examined. No significant differences in urinary, haematological or clinical chemistry parameters, body weights, organ weights or histopathological appearance were reported between rats at 0.08% or 0.16% and controls. Rats at 0.31% and 0.62% grew significantly less than controls. At necropsy, full pathological examination revealed significant alterations in the seminal vesicles (reductions in size, weight and number of secretory granules) and prostate (tubular collapse and reduced amount of secretory materials) of rats fed 0.62% trimethylamine. No effects were reported at dietary levels of 0.08% or 0.16% (80 or 160 mg/kg bw per day) (Amoore et al., 1978).

Triethylamine (No. 1611)

Groups of 50 male and 50 female Fischer rats were exposed by inhalation daily to 0, 25 or 247 ppm triethylamine for 6 h/day, 5 days per week for up to 28 weeks, corresponding to an estimated daily oral intake of 16 or 157 mg/kg bw (Fassett, 1978). Food and water were provided ad libitum between exposures, and the animals were monitored daily for changes in behaviour and appearance. Body weights were recorded the day before the first exposure, every 2 weeks thereafter and immediately before sacrifice. Ten randomly selected animals from each group were killed after 32–34 and 58–61 days of exposure. No statistically significant effects on body weights, organ weights or haematological, clinical chemistry or electrocardiographic indices were reported. No gross or histopathological changes were observed that were related to exposure (Lynch et al., 1990).

Piperazine (No. 1615)

A study was conducted to examine the effect of nitrosation of ingested amines in vivo on the incidence of lung adenomas in mice. Groups of 40–80 Swiss mice of

each sex were given diets containing 0 or 0.625% piperazine (equivalent to 938 mg/kg bw per day) in combination with drinking-water containing 0 or 1 g/l sodium nitrite for 28 weeks (sodium nitrite was given 5 days/week, while test feed was provided continuously). The animals were subsequently maintained on basal diets and tap water for 12 weeks before necropsy at 40 weeks. No significant variations were observed in body-weight gain. In comparison with the control group, no significant differences in the incidence of lung adenomas were observed in mice given piperazine only; however, in mice receiving piperazine plus sodium nitrite, the incidence of lung adenomas was significantly increased over that in untreated controls. The incidence of malignant lymphomas and other tumours was comparable in both groups (Greenblatt et al., 1971).

(c) Long-term studies of toxicity and carcinogenicity

The results of long-term studies are summarized in Table 4 and are described below.

Acetamide (No. 1592)

Sixty inbred male Leeds rats were maintained on a diet containing 5% by weight acetamide (equivalent to 2500 mg/kg bw per day) for up to 35 weeks, while 40 rats of the same strain received a control diet. The test animals were returned to the control diet from the end of week 35 until termination of the study at 17 months. Four treated and four control animals were killed at day 9, in weeks 4, 10, 26 and 35 of treatment and 1, 4 and 6 months after treatment. All the surviving rats were necropsied at 17 months. At sacrifice, hepatic tissue was taken from each animal and preserved for electron microscopy, and samples from the liver, pancreas, kidney, spleen and lung plus any tumours or tissues with lesions were preserved for histological examination. Survival, food consumption and body-weight gain were comparable in test and control groups. After 26 weeks of treatment, all the necropsied animals showed neoplastic nodules of the liver, which increased in abundance during the remainder of the experiment. Hepatocellular carcinoma was initially reported in one of four rats necropsied 1 month after the end of treatment and, subsequently, in 5/10, 7/8 and all four rats necropsied at 4, 6 and 9 months after the end of treatment, respectively. Of the four hepatocellular carcinomas reported at 9 months, three metastasized to either the mesenteric tissues or lungs. Hepatic parenchymal cells had undergone fine structural changes, including early glycogen depletion, dispersal and dislocation of the rough endoplasmic reticulum, proliferation of smooth endoplasmic reticulum, nuclear irregularities and nucleolar abnormalities. Electron microscopy additionally revealed occasional enlargement of the bile canaliculi, with few microvilli. Although apparent recovery of glycogen stores was seen during the recovery period, the glycogen was usually interspersed with elements of proliferated smooth endoplasmic reticulum. Considerable morphological variation was seen in the cells of the neoplastic nodules; most of the hepatocellular carcinomas were of the trabecular type and were moderately well differentiatied. Nodules and carcinomas, and in some cases both, persisted after withdrawal of acetamide from the diet. The authors reported that, overall, the morphological changes observed in the liver were consistent with acquired resistance and adaptive response to carcinogens in new populations of initiated hepatocytes (Flaks et al., 1983).

1,6-Hexalactam (No. 1594)

Mice

Groups of 50 male and 50 female B6C3F$_1$ mice were fed diets containing 0, 7500 or 15 000 ppm 1,6-hexalactam (equivalent to 0, 1125 or 2250 mg/kg bw per day) for 103 weeks and observed for an additional 2 weeks (total observation period, 105 weeks). Throughout the study, animals were observed twice daily for any signs of toxicity, and clinical signs were recorded monthly. All moribund animals and those that survived until the end of the study were necropsied. The mean body weights, monitored every 2 weeks for the first 13 weeks of the study and monthly thereafter, of male and female mice were lower than those of controls throughout the study (statistics not reported); however, food consumption did not differ from that of controls. The survival of treated males was comparable to that of controls (80–96%), but control females had significantly poorer survival (76%) than females at the lower (82%) or higher dose (92%). The incidence of none of the neoplasms found at necropsy in treated animals (alveolar–bronchiolar adenomas of the lung, lymphomas and leukaemia of the haematopoietic system, haemangiosarcoma and hepatocellular adenomas or carcinomas) was significantly increased in comparison with controls. Likewise, the frequency and severity of non-neoplastic lesions in test animals were comparable with those in controls. The authors noted that the observed neoplasms were typical of those seen in this strain of mouse. Under the condition of this study, 1,6-hexalactam was not carcinogenic (National Toxicology Program, 1982).

Rats

Groups of 50 male and 50 female Fischer 344 rats were fed diets containing 0, 3750 or 7500 ppm 1,6-hexalactam (equivalent to 0, 187.5 and 375 mg/kg bw per day) for 103 weeks and observed for an additional 2 weeks for a total observation period of 105 weeks. Animals were observed twice daily for signs of toxicity, and clinical signs were recorded monthly. Body weights were recorded every 2 weeks for the first 13 weeks of the study and monthly thereafter. All moribund animals and those that survived until the end of the study were necropsied. The mean body weights of treated rats were lower than those of controls in a dose-dependent manner (statistics not reported). Food consumption decreased with dose, such that the food consumption of rats at the higher dose was 70–80% that of controls. Survival (64–84%) was comparable in all groups, including controls. The neoplastic (interstitial-cell tumours of the testis, carcinomas of the pituitary and liver, adenomas and carcinomas of the thyroid, adrenal tumours, papillary adenocarcinomas of the mammary gland, leukaemia and lymphoma, uterine polyps and fibromas or fibrosarcomas of the subcutaneous tissue) and non-neoplastic lesions found at necropsy were considered to be of the types and frequencies commonly seen in ageing Fischer 344 rats and none was considered related to treatment. Under the conditions of this study, 1,6-hexalactam was not carcinogenic (National Toxicology Program, 1982).

(e) Genotoxicity

Genotoxicity has been tested with 17 aliphatic and aromatic amines and amides. The results of these tests are summarized in Table 5 and described below. The conversions used are shown in the table.

Table 5. Studies of genotoxicity with aliphatic and aromatic amines and amides

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
In vitro						
1579	Ethylamine	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	100–10000 µg/plate	Negative[a]	Mortelmans et al. (1986)
1581	Isopropylamine	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	10–10 000 µg/plate	Negative[a]	Zeiger et al. (1987)
1582	Butylamine	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3.3–3333 µg/plate	Negative[a]	Zeiger et al. (1987)
1582	Butylamine	Reverse mutation	*S. typhimurium* TA100 (219 µg/plate)[b]	3 µmol/plate	Negative[a]	Florin et al. (1980)
1583	Isobutylamine	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33–10 000 µg/plate	Negative[a]	Mortelmans et al. (1986)
1584	*sec*-Butylamine	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	10–3333 µg/plate	Negative[a]	Zeiger et al. (1987)
1585	Pentylamine	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33–3333 µg/plate	Negative[a]	Mortelmans et al. (1986)
1590	Tyramine	Forward mutation	Mouse lymphoma L5178Y cells	500–3500 µg/ml	Negative	McGregor et al. (1988)
1590	Tyramine	Forward mutation	Mouse lymphoma L5178Y cells	0.08, 0.80, 2.0, 4.0 or 6.0 mmol/l (11, 109, 274, 548 and 823 µg/ml)[c,d]	Positive[e,f]	Wangenheim & Bolcsfoldi (1988)
1590	Tyramine	Forward mutation	Mouse lymphoma L5178Y cells	0.40, 0.80, 1.60, 2.39 or 3.2 mmol/l (55, 109, 220, 327 and 439 µg/ml)[c,d]	Positive[f,g]	Wangenheim & Bolcsfoldi (1988)
1592	Acetamide	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	100–10 000 µg/plate	Negative[a]	Haworth et al. (1983)
1592	Acetamide	DNA repair	*E. coli* 343/591 uvrB$^-$/recA$^-$/lac$^+$ and uvrB$^-$/recA$^-$/lac$^+$	≤ 1080 mmol/l (63 793 µg/ml)[i]	Negative[a]	Hellmér & Bolcsfoldi (1992)
1592	Acetamide	Single-strand DNA breaks	Rat hepatocytes	0.03, 0.3, 3, 10, 30, 100, 300 or 1000 mmol/l (2, 18, 177, 591, 1772, 5907, 17 720 and 59 068 µg/ml)[i]	Negative	Sina et al. (1983)

Table 5 (contd)

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
1595	2-Isopropyl-*N*-2,3-trimethylbutyramide	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	200, 1,000, 5,000, 10,000 or 20,000 µg/plate	Negative[a]	Haworth et al. (1978)
1595	2-Isopropyl-*N*-2,3-trimethylbutyramide	Forward mutation	Mouse lymphoma L5178Y cells	0.01–1000 µg/ml	Negative[a]	Kirby et al. (1978)
1595	2-Isopropyl-*N*-2,3-trimethylbutyramide	Unscheduled DNA synthesis	WI-38 cells (human)	125–2000 µg/ml[j]	Negative[a]	Skinner (1978)
1598	*N*-Isobutyl-(E,E)-2,4-decadienamide	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	5–1500 µg/plate[k]	Negative[e]	King (2003)
1598	*N*-Isobutyl-(E,E)-2,4-decadienamide	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	5–5000 µg/plate[l]	Negative[g]	King (2003)
1600	Piperine	Reverse mutation	*S. typhimurium* TA97a, TA98, TA100, TA102	0.01, 0.5 or 10 µmol/plate (3, 143 and 2853 µg/plate)[m]	Negative[a]	Karekar et al. (1996)
1600	Piperine	Reverse mutation (pre-incubation)	*S. typhimurium* TA97a, TA98, TA100, TA102	0.005, 0.05, 0.5 or 5 µmol/plate (1, 14, 143 and 1427 µg/plate)[m,n]	Negative[a]	Karekar et al. (1996)
1600	Piperine	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	1000 µg	Negative[a]	Andrews et al. (1980)
1607	Piperidine	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate (255 µg/plate)	Negative[a]	Florin et al. (1980)
1607	Piperidine	Reverse mutation	*S. typhimurium* TA1530, TA1531, TA1532, TA1964	1–5 mg/plate (1000–5000 µg/plate)[o]	Negative	Green & Savage (1978)
1607	Piperidine	Reverse mutation (microsomal assay)	*S. typhimurium* TA1530, TA1531, TA1532, TA1964	0.15 mol/l (12 772 µg/ml)[o,p]	Negative	Green & Savage (1978)
1607	Piperidine	Reverse mutation (host-mediated, mice)	*S. typhimurium* TA1950, TA1951, TA1952, TA1964	800 mg/kg bw[q]	Negative	Green & Savage (1978)
1607	Piperidine	Forward mutation	Mouse lymphoma L5178Y cells	3.03, 4.04, 5.05, 6.06 or 7.07 mmol/l (258, 344, 430, 516 and 602 µg/ml)[o]	Positive[e,f]	Wangenheim & Bolcsfoldi (1988)

Table 5 (contd)

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
1607	Piperidine	Forward mutation	Mouse lymphoma L5178Y cells	4.04, 5.05, 6.06, 7.07 or 8.08 mmol/l (344, 430, 516, 602 and 688 μg/ml)[o]	Negative[g]	Wangenheim & Bolcsfoldi (1988)
1607	Piperidine	Forward mutation	Mouse lymphoma L5178Y cells	2.0, 4.01 or 6.01 mmol/l (170, 341 and 512 μg/m)[o]	Negative[e]	Garberg et al. (1988)
1607	Piperidine	Forward mutation	Mouse lymphoma L5178Y cells	2.0, 4.01, 6.01 or 8.02 mmol/l (170, 341, 512 and 683 μg/ml)[o]	Equivocal[g,r]	Garberg et al. (1988)
1607	Piperidine	DNA repair	*E. coli* 343/591 uvrB$^-$/recA$^-$/lac$^+$ and uvrB$^+$/recA$^+$/lac$^+$	33.7 mmol/l (2870 μg/ml)[o,s]	Negative[e]	Hellmér & Bolcsfoldi (1992)
1607	Piperidine	DNA repair	*E. coli* 343/591 uvrB$^-$/recA$^-$/lac$^+$ and uvrB$^+$/recA$^+$/lac$^+$	101 mmol/l (8600 μg/ml)[o,s]	Negative[e]	Hellmér & Bolcsfoldi (1992)
1607	Piperidine	Single-strand DNA breaks	Rat hepatocytes	0.03, 0.3 or 3 mmol/l (2.6, 26 and 255 μg/ml)[o]	Negative	Sina et al. (1983)
1609	Pyrrolidine	Reverse mutation	*S. typhimurium* TA100	≤ 3 μmol/plate (213 μg/plate)[t]	Negative[a]	Florin et al. (1980)
1609	Pyrrolidine	Reverse mutation	*S. typhimurium* TA1530, TA1531, TA1532, TA1964	1–5 mg/plate (1000–5000 μg/plate)[c]	Negative	Green & Savage (1978)
1609	Pyrrolidine	Reverse mutation (microsomal assay)	*S. typhimurium* TA1530, TA1531, TA1532, TA1964	0.5 mol/l (35 561 μg/ml)[t]	Negative	Green & Savage (1978)
1609	Pyrrolidine	Reverse mutation (host-mediated, mice)	*S. typhimurium* TA1950, TA1951, TA1952, TA1964	800 mg/kg bw[q]	Negative	Green & Savage (1978)
1610	Trimethylamine	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	10–1000 μg/plate	Negative[a]	Mortelmans et al. (1986)
1611	Triethylamine	Reverse mutation TA1535, TA1537	*S. typhimurium* TA98, TA100,	10–10 000 μg/plate	Negative[a]	Zeiger et al. (1987)
1615	Piperazine	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33–3167 μg/plate	Negative[a]	Haworth et al. (1983)

Table 5 (contd)

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
In vivo						
1592	Acetamide	DNA damage (Comet assay)	Male ddy mice	2000 mg/kg bw[u]	Positive[v]	Sasaki et al. (2000)
1592	Acetamide	Micronuclei (bone marrow)	C57Bl/6 mice	2500 or 5000 mg/kg bw[w]	Negative	Mirkova (1996)
1592	Acetamide	Micronuclei (bone marrow)	Male CBA mice	5000 mg/kg bw[w]	Negative	Mirkova (1996)
1592	Acetamide	Micronuclei (bone marrow and peripheral blood)	Male CD1 mice	500–5000 mg/kg bw[x]	Negative	Morita et al. (1997)
1592	Acetamide	Micronuclei (bone marrow and peripheral blood)	Male BDF1 mice	1250–5000 mg/kg bw[x]	Negative	Morita et al. (1997)
1592	Acetamide	Micronuclei (bone marrow)	Female C57Bl/6 mice	3.39 mmol/kg bw (200 mg/kg bw)[y,z]	Positive	Chieli et al. (1987)
1594	1,6-Hexalactam	DNA damage (Comet assay)	Male ddy mice	2000 mg/kg bw[w]	Negative	Sasaki et al. (2000)
1594	1,6-Hexalactam	Replicative DNA synthesis	Male Fischer 344 rats	350 or 700 mg/kg bw[aa]	Negative	Uno et al. (1994)
1594 (1995)	1,6-Hexalactam	Replicative DNA synthesis	Male $B6C3F_1$ mice	250 or 500 mg/kg bw[w]	Negative	Miyagawa et al.
1594	1,6-Hexalactam	Mammalian spot	$(C57BlxT)F_1$ mouse embryos	400 or 500 mg/kg bw[w]	Positive[bb]	Fahrig (1989)
1594	1,6-Hexalactam	Mammalian spot	$(TxHT)F_1$ mouse embryos	500 mg/kg bw[cc]	Positive[dd]	Neuhäuser-Klaus & Lehmacher (1989)
1594	1,6-Hexalactam	Mammalian spot	$(TxHT)F_1$ mouse embryos	700 mg/kg bw[cc]	Negative	Neuhäuser-Klaus & Lehmacher (1989)
1594	1,6-Hexalactam	Sex-linked recessive lethal mutations	Male *D. melanogaster* larvae	5 mmol/l[ee] (566 µg/ml)[ff]	Negative	Vogel (1989)
1594	1,6-Hexalactam	Sex-linked recessive lethal mutations	Female *D. melanogaster* larvae	5 or 20 mmol/l[ee] (566 or 2263 µg/ml)[ff]	Positive	Vogel (1989)

Table 5 (contd)

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
1594	1,6-Hexalactam	Somatic mutation/ mitotic recombination	Female *D. melanogaster* larvae	2.5, 5, 10 or 20 mmol/l[ee] (283, 566, 1132 and 2263 μg/ml)[ff]	Positive	Vogel (1989)
1594	1,6-Hexalactam	Chromosomal aberrations (bone marrow)	Male and female $1C3F_1$ mice	1000 mg/kg bw[w]	Negative	Adler & Ingwersen (1989)
1600	Piperine	Micronuclei (bone marrow)	Male Swiss mice	10 or 20 mg/kg bw[w]	Negative	Karekar et al. (1996)
1600	Piperine	Micronuclei (bone marrow)	Male Swiss mice	1, 2 or 4 mg/kg bw[gg]	Negative	Muralidhara & Narasimhamurthy (1990)
1600	Piperine	Sperm morphology	Male Swiss mice	10 or 50 mg/kg bw per day[hh]	Negative	Karekar et al. (1996)
1600	Piperine	Sperm morphology	Male Swiss mice	35, 50 or 75 mg/kg bw per day[ii]	Negative	Daware et al. (2000)
1600	Piperine	Sperm morphology	Male Swiss mice	1, 2 or 4 mg/kg bw per day[jj]	Negative	Muralidhara & Narasimhamurthy (1990)
1600	Piperine	Dominant lethal mutations	Male and female Swiss mice	10 or 50 mg/kg bw[w]	Negative	Karekar et al. (1996)
1600	Piperine	Dominant lethal mutations	Male Swiss mice	4 mg/kg bw/day[jj]	Negative	Muralidhara & Narasimhamurthy (1990)
1607	Piperidine	Mitosis in adrenocortical cells	Male Wistar rats	100 mg/kg bw in dimethyl sulfoxide[w]	Negative	Danz & Urban (1979)
1607	Piperidine	Mitosis in adrenocortical cells	Male Wistar rats	100 mg/kg bw in 1% Tylose[w]	Negative	Danz & Urban (1979)
1607	Piperidine	Sperm morphology	Male hybrid mice	400 mg/kg bw per day[kk]	Negative	Bempong & Scully (1983)

Table 5 (contd)

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
1607	Piperidine	Sperm morphology	Male golden Syrian hamsters	400 mg/kg bw/day[kk]	Negative	Bempong & Scully (1983)
1609	Pyrrolidine	Mitosis in adreno-cortical cells	Male Wistar rats	100 mg/kg bw in 1% Tylose[w]	Negative	Danz & Urban (1979)
1609	Pyrrolidine	Mitosis in adreno-cortical cells	Male Wistar rats	100 mg/kg bw in dimethyl sulfoxide[w]	Positive	Danz & Urban (1979)

[a] With and without metabolic activation from rat liver microsomes (S9)
[b] Calculated from relative molecular mass for butyrlamine = 73.14 g/mol
[c] Calculated from relative molecular mass for tyramine = 137.18 g/mol
[d] Compound actually used was tyramine hydrochloride at concentrations of 0.101–7.59 mmol/l (18–1318 µg/ml) without metabolic activation, and 0.506–4.05 mmol/l (88–703 µg/ml) with metabolic activation
[e] Without metabolic activation
[f] Significant increases in mutation frequency only at cytotoxic doses
[g] With metabolic activation
[h] Tyrosine was nitrosated (incubated for 60 min at 37 °C with 25 mmol/l sodium nitrite) before test
[i] Calculated from relative molecular mass for acetamide = 59.07 g/mol
[j] Cytotoxic at 2000 µg/ml
[k] Toxic and precipitated at 1500 µg/plate
[l] Toxic and precipitated at 5000 µg/plate
[m] Calculated from relative molecular mass for piperine = 285.34 g/mol
[n] Toxic at 5 µmol/plate without metabolic activation
[o] Calculated from relative molecular mass for piperidine = 85.15 g/mol
[p] Highest non-cytotoxic concentration
[q] Intraperitoneal injection of *S. typhimurium* and intramuscular injection of test material
[r] Results did not meet the criteria for positive or negative classification
[s] Concentration at which a significant reduction in the number of colonies of each strain was observed; however, the highest concentration of piperidine tested was 1010 mmol/l.
[t] Calculated from relative molecular mass for pyrrolidine = 71.12 g/mol

Table 5 (contd)

[u] Administered as a single intraperitoneal injection
[v] Increased DNA damage observed in stomach, colon, lungs and bone marrow
[w] Administered as a single dose by gavage
[x] Single, double or quadruple intraperitoneal injections, separated by 24 h
[y] Administered by gavage 30 and 6 h before sacrifice
[z] Calculated from relative molecular mass for acetamide = 59.07 g/mol
[aa] Administered as a single subcutaneous injection
[bb] Frequency of spots of genetic relevance significantly increased relative to controls in one of three trials, only at the highest dose (500 mg/kg bw)
[cc] Administered at a single dose (route not specified)
[dd] Significant increase in spots of genetic relevance observed in one of four groups receiving 500 mg/kg bw
[ee] Administered in the diet
[ff] Calculated from relative molecular mass for 1,6-hexalactam = 113.16 g/mol
[gg] Two intraperitoneal injections at 0 and 24 h
[hh] Administered by gavage for 5 days
[ii] Administered orally for 5 consecutive days
[jj] Administered intraperitoneally for 5 days, followed by a 35-day maintenance period
[kk] Administered orally for 100 days; on day 40 and every subsequent 5 days, three mice were killed for examination of sperm morphology

In vitro

No mutagenicity was found in the standard Ames assay when various strains of *Salmonella typhimurium* (TA97a, TA98, TA100, TA102, TA1535, TA1537, TA1538, TA1530, TA1531, TA1532 and TA1964) were incubated with up to 10 000 μg/plate of ethylamine (No. 1579), isopropylamine (No. 1581), butylamine (No. 1582), isobutylamine (No. 1583), pentylamine (No. 1585), acetamide (No. 1592), 2-isopropyl-*N*-2,3-trimethybutyramide (No. 1595), *N*-isobutyl-(E,E)-2,4-decadienamide (No. 1598), piperine (No. 1600), piperidine (No. 1607), pyrrolidine (No. 1609), trimethylamine (No. 1610), triethylamine (No. 1611) or piperazine (No. 1615) with or without metabolic activation (Green & Savage, 1978; Haworth et al., 1978; Andrews et al., 1980; Florin et al., 1980; Haworth et al., 1983; Mortelmans et al., 1986; Zeiger et al., 1987; Karekar et al., 1996; King, 2003).

In a host-mediated assay in which *S. typhimurium* strain TA1950, TA1951, TA1952 or TA1964 was injected intraperitoneally into mice followed by an intramuscular injection of 800 mg/kg bw of piperidine or pyrrolidine, no mutagenicity was observed (Green & Savage, 1978).

There was no evidence of DNA damage when *Escherichia coli* 343/591 $uvrB^-/recA^-/lac^+$ or $uvrB^+/recA^+/lac^+$ was incubated with up to 1080 mmol/l (63 793 μg/ml) of acetamide (No. 1592) or up to 33.7 mmol/l (2870 μg/ml) of piperidine (No. 1607) (Hellmér & Bolcsfoldi, 1992). In the SOS Chromotest with *E. coli* PQ37, the *N*-nitroso derivative of tyramine (No. 1590) gave positive results (Ohshima et al., 1989).

Assays in mammalian cell lines have been performed with tyramine (No. 1590), acetamide (No. 1592), 2-isopropyl-*N*-2,3-trimethylbutyramide (No. 1595) and piperidine (No. 1607). Unscheduled DNA synthesis was not increased when WI-38 human cells were incubated with 125–2000 μg/ml of 2-isopropyl-*N*-2,3-trimethylbutyramide (Skinner, 1978). No single-strand DNA breaks were reported when 0.03–

1000 mmol/l (2–59 068 μg/ml) of acetamide or 0.0–3 mmol/l (2.6–255 μg/ml) of piperidine were incubated with rat hepatocytes (Sina et al., 1983). Mixed results have been reported with tyramine and piperidine in the mouse lymphoma forward mutation assay: positive results were reported for both compounds when tested at up to 823 and 688 μg/ml, respectively, in L5178Y mouse lymphoma cells with and without metabolic activation, but only at cytotoxic doses (Wangenheim & Bolcsfoldi, 1988). No mutagenic effects were reported when tyramine and 2-isopropyl-*N*,2,3-trimethylbutyramide were tested at concentrations up to 3500 and 1000 μg/ml, respectively, in L5178Y mouse lymphoma cells (Kirby et al., 1978; McGregor et al., 1988). No mutagenic effects were observed when piperidine was tested at concentrations up to 512 μg/ml without metabolic activation in L5178Y mouse lymphoma cells; however, equivocal results were obtained when metabolic activation was added (Garberg et al., 1988).

In vivo

In male and female C57Bl/6, male CBA, male CD_1 and male BDF_1 mice, a single dose of acetamide (No. 1592) of up to 5000 mg/kg bw did not induce micronuclei in bone marrow or peripheral blood when administered by gavage or intraperitoneal injection (Mirkova, 1996; Morita et al., 1997). Micronuclei were found in the bone marrow of female C57Bl/6 mice given 3.39 mmol/kg bw (approximately 200 mg/kg bw) of acetamide by gavage 30 and 6 h before termination; however, no dose–response relation was seen, as only a single dose was used (Chieli et al., 1987).

Piperine (No. 1600) did not induce micronuclei in the bone marrow of male Swiss mice given a single dose of 10 or 20 mg/kg bw by gavage (Karekar et al., 1996) or two intraperitoneal doses (at 0 and 24 h) for a total dose of up to 4 mg/kg bw (Muralidhara & Narasimhamurthy, 1990).

Male and female $1C3F_1$ mice were given a single dose of 1000 mg/kg bw of 1,6-hexalactam (No. 1594) by gavage, and bone marrow was sampled from groups of 10 animals after 24, 30 and 48 h. Colchicine was administered to the mice 1 h before sacrifice. No chromosomal aberrations were seen (Adler & Ingwersen, 1989). The Comet assay was used to quantify DNA damage in cells from organs of male ddy mice given either acetamide (No. 1592) or 1,6-hexalactam (No. 1594). No DNA damage was reported in mice given a single dose of 2000 mg/kg bw of 1,6-hexalactam by gavage; however, DNA damage was reported in the stomach, colon, lungs and bone marrow of mice given a single intraperitoneal injection of acetamide at 2000 mg/kg bw (Sasaki et al., 2000).

1,6-Hexalactam (No. 1594) did not induce replicative DNA synthesis in rat or mouse hepatocytes after treatment in vivo or in vitro at a dose of 350 or 700 mg/kg bw or 250 or 500 mg/kg bw, respectively (Uno et al., 1994; Miyagawa et al., 1995). In the mouse spot test, a single [route not stated but assumed to be intraperitoneal] injection of 1,6-hexalactam at a dose of up to 500 mg/kg bw significantly increased the frequency of spots over those in controls (Neuhäuser-Klaus & Lehmacher, 1989); however, statistically significant effects were observed in only one of three or four trials. It has been suggested that the colour spots observed were indicative of mitotic recombination and not mutation (Fahrig, 1989). Moreover, administration of 700 mg/kg bw in one trial did not significantly increase the frequency of spots over that in controls (Neuhäuser-Klaus & Lehmacher, 1989).

Female *Drosophila melanogaster* larvae fed up to 20 mmol/l (2263 μg/ml) of 1,6-hexalactam (No. 1594) showed sex-linked recessive lethal mutations and somatic mutation–mitotic recombination, whereas male larvae fed up to 5 mmol/l (566 μg/ml) did not have sex-linked recessive lethal mutations (Vogel, 1989).

Piperidine (No. 1607) and pyrrolidine (No. 1609) were tested for promoting activity in male Wistar rats given a single dose of 100 mg/kg bw of the test substance by gavage in dimethyl sulfoxide or 1% Tylose. The number of mitoses in the adrenal cortex was examined 36 h after dosing. Only administration of pyrrolidine in dimethyl sulfoxide caused a statistically significant increase (approximately twofold) in the number of mitoses over that in controls (Danz & Urban, 1979).

Piperine (No. 1600) and piperidine (No. 1607) did not cause mutations in male germ cells, as assessed by sperm shape abnormality and tests for dominant lethal mutations in mice and hamsters. Mice given piperine at doses up to 75 mg/kg bw per day by gavage or up to 4 mg/kg bw per day by intraperitoneal injection for 5 days showed no sperm shape abnormalities or dominant lethal mutations (Muralidhara & Narasimhamurthy, 1990; Karekar et al., 1996; Daware et al., 2000). In another study, an oral dose of 400 mg/kg bw per day of piperidine for 40–100 days did not induce sperm shape abnormalities in mice or hamsters (Bempong & Scully, 1983).

Conclusions

Negative results were reported in bacterial assays for reverse mutation with 15 aliphatic and aromatic amine and amide derivatives: ethylamine, isopropylamine, butylamine, isobutylamine, *sec*-butylamine, pentylamine, acetamide, 2-isopropyl-*N*,2,3-trimethylbutyramide, *N*-isobutyl (E,E)-2,4-decadienamide, piperine, piperidine, pyrrolidine, trimethylamine, triethylamine and piperazine.

Two substances, tyramine and piperidine, gave both positive and negative results in the mouse lymphoma assay, particularly at cytotoxic concentrations, while nitrosated tyramine gave positive results in the SOS Chromotest with *E. coli*.

Piperine and piperidine consistently gave negative results in a variety of studies in vivo, whereas acetamide, 1,6-hexalactam and pyrrolidine gave mainly negative results with some positive findings.

(f) Other relevant studies

Developmental and reproductive toxicity

Butylamine (No. 1582)

The hydrochloride salt of butylamine (mono-*n*-butylamine hydrochloride) was administered by gavage at a dose of 100, 400 or 1000 mg/kg bw per day to groups of 25 female Wistar rats from 6 to 15 days after mating with untreated male Wistar rats. As controls, another group of female Wistar rats received only the vehicle (double-distilled water) for the same period. The rats were observed for clinical signs of toxicity at least once daily, while their food consumption and body weights were measured on days 0, 1, 3, 6, 8, 10, 13, 15, 17 and 20 after mating. On day 20 after mating, all the rats were killed and necropsied. Major organs were examined macroscopically, and the uterus and placenta were weighed. For each rat, the numbers of corpora lutea, implantation sites, early or late resorptions and live or

dead fetuses were recorded. After removal from the uterus, the fetuses were sexed, weighed and examined for external abnormalities. Non-pregnant rats were excluded from the analyses of mean maternal food consumption, body and uterus weights and reproduction indices. All the rats survived to scheduled termination (day 20 after mating). Of the 25 mated female rats in each group, 22 were pregnant by day 20 after mating in the control group and at 400 mg/kg bw per day and 24 in each group at 100 and 1000 mg/kg bw per day. Food consumption was significantly reduced during most of the treatment period in rats at the highest dose when compared with controls. There were no significant effects on mean maternal body-weight gain, corpora lutea, implantation sites, percentage of pre-implantation loss, percentage of early or late resorptions or number of live fetuses per litter in treated rats when compared with controls. There were no abortions, premature births or dead fetuses reported in any group. In comparison with controls, rats at the highest dose had significantly lower mean weights of the gravid uterus, placenta and fetuses but significantly higher mean percentages of post-implantation loss, litters with malformations and fetuses per litter with malformations. The incidence of soft-tissue malformation was significantly higher in the fetuses of animals at the two higher doses (3% and 4%, respectively), while the incidence of external malformations (1%) was significantly higher in fetuses from dams at the highest dose than in controls. The number of litters or fetuses per litter with malformations increased with the dose of n-butylamine hydrochloride administered, reaching statistical significance only at the highest dose. This group also showed skeletal development retardation (incomplete skull and sternebral ossification) and malformations (filiform or kinked tail, enlarged cardiac ventricular chamber(s), malpositioned heart, aortic arch atresia and diaphragmatic hernia). In the group at the intermediate dose, soft-tissue skeletal malformations (aortic arch atresia, malpositioned heart and diaphragmatic hernia) were also observed. The NOEL was 400 mg/kg bw per day for maternal toxicity and 100 mg/kg bw per day for embryo or fetal toxicity (Gamer et al., 2002).

1,6-Hexalactam (No. 1594)

Groups of 20 pregnant Fischer 344 rats were given 0 (vehicle control), 100, 500 or 1000 mg/kg bw per day of 1,6-hexalactam in distilled water by gavage during days 6–15 of gestation. All the rats were observed daily for deaths or morbidity throughout the study. Clinical observations were made on days 0, 6, 11, 15 and 20 of gestation. Food consumption was measured at intervals ending on days 6, 11, 15 and 20 of gestation. On day 20 of gestation, all surviving dams were killed and the viscera were examined grossly; the uterus and ovaries of each dam were excised and weighed. For each litter, the number of corpora lutea per ovary, the number and placement of uterine implantations, resorption sites and live and dead fetuses were recorded. Fetuses were excised from their placenta, weighed and examined externally and internally. The survival rate of the group at 1000 mg/kg bw per day was significantly lower than that of controls, 9 of 20 dams at the highest dose dying during the study. While there was no significant difference in most clinical observations between control and treated rats, slightly higher incidences of urine stains, rough coat, vaginal discharge, bloody crust on or about the eyes, mouth or nose, hunched or thin appearance or depression were observed in treated groups. Mean maternal food consumption was significantly lower than that of controls during days 6–11 of gestation at the two higher doses and during days 11–15 at the intermediate dose.

Consequently, the mean maternal body weights were significantly lower in these groups during these periods when compared with controls. After treatment (days 15–20 of gestation), however, no significant differences from controls were reported. While the overall food consumption (days 0–20 of gestation) of animals at the intermediate dose was significantly lower than that of controls, there was no significant effect on mean maternal body weight. Pregnancy rate, mean ovarian and uterine weights and mean numbers of corpora lutea, implantations, resorptions and viable fetuses were not significantly different in treated and control groups. On a per litter and group basis, there were no significant effects on mean implantation efficiency, incidence of fetal viability, sex ratio or pup body weight and length; however, the mean incidence of resorption was significantly higher in dams at the highest dose than in controls. Although one dam at the highest dose had a distended uterus with clear fluid, gross pathological examination of treated rats revealed no remarkable changes. No skeletal anomalies or major malformations were observed in any of the pups examined, and only non-dose-related incidences of visceral anomalies were observed (Gad et al., 1987).

In a three-generation assay of reproductive toxicity, groups of 10 male and 20 female Fischer 344 rats were fed diets containing 0, 1000, 5000 or 10 000 ppm 1,6-hexalactam (equivalent to 0, 50, 250 and 500 mg/kg bw per day) for 10 weeks. All animals were observed once daily. Body weights, food consumption, physical appearance and behaviour were recorded at baseline and at weeks 4 and 10 of the 10-week treatment period. After 10 weeks of treatment, all animals were allowed to mate for 2 weeks. Each parental generation (P_1, P_2 and P_3) was allowed two breeding phases, the second 7–13 days after the first litter of offspring had been killed. The numbers of live and dead pups (by sex), individual body weights and any evidence of abnormality were recorded on days 1, 7 and 21 of lactation for each litter. After weaning of the F_{1b} pups, the P_1 rats were killed and subjected to complete gross necropsies, whereas P_2 and P_3 rats were killed and discarded without necropsy. All parental animals that died during the study were nevertheless subjected to complete gross necropsies. Additionally, major organs and tissues and any gross lesions from P_1 and F_{3b} rats were examined microscopically and macroscopically. No treatment-related effects on mortality, clinical signs, reproductive performance or gross appearance were reported in parental animals. Relative to controls, the mean food consumption was significantly reduced in P_2 males at the two higher doses, in P_3 males at the highest dose and in all parental generations of females at the highest dose. Consequently, P_2 and P_3 rats of each sex at 500 mg/kg bw per day had significantly lower body weights than controls throughout the 10-week treatment period. Histopathological examination of P_1 males at the highest dose revealed a slight increase in the severity of spontaneous nephropathy, accompanied by granular casts in 3/25 rats (12%) examined. The offspring showed no treatment-related effects on gross appearance, gross pathology, survival, number of pups, percentage of male pups or kidney weight. The only consistent effect reported in offspring was reduced body weight in males at the two higher doses and in females at the highest dose (all generations) on days 1, 7 and 21 of lactation. Male and female F_{3b} pups at the highest dose had significantly lower absolute kidney weights than controls, but no significant changes in relative kidney weights were observed. As there were no effects on reproductive performance or offspring survival, the NOEL was 10 000 ppm (500 mg/kg bw per day) (Serota et al., 1988).

Rabbits

Groups of 25 pregnant New Zealand white rabbits were given 0 (vehicle control), 50, 150 or 250 mg/kg bw per day of 1,6-hexalactam in distilled water by gavage during days 6–28 of gestation. The rabbits were weighed on days 0, 6, 9, 12, 15, 18, 21, 24, 27 and 29 of gestation. Any rabbit that died during the study was examined macroscopically. On day 29 of gestation, all surviving does were killed and examined macroscopically. The uterus of each doe was excised, weighed and examined internally and externally for major malformations, minor anomalies and common skeletal variants. The same procedure was applied to any aborted fetuses. Four of the rabbits at the highest dose died during the study: three had convulsions immediately after dosing and died within 35 min, while the other rabbit, which died on day 28 of gestation, aborted on day 27 of gestation and was in a weakened condition. One control rabbit also died on day 29 of gestation, probably due to ulceration of the stomach. No adverse clinical findings were reported in animals at the two lower doses; however, rabbits at the highest dose had convulsions, rapid breathing and other incidental findings, including decreased food intake, decreased faecal volume and areas of alopecia. During the first few days of treatment (days 6–9 of gestation), rabbits at the two higher doses had a significant reduction in body weight compared with controls; however, the corrected body weights (body weight on day 29 of gestation minus weight of the gravid uterus) of treated and control groups were not significantly different. No abnormal findings were made at necropsy of treated does on day 29 of gestation. On a per litter and per group basis, the treated rabbits showed no statistically significant differences from controls in mean total corpora lutea and implantation sites, mean number of viable fetuses, sex ratio, litter weight, incidence of pre- or post-implantation losses and number of resorptions; however, mean fetal weight was significantly reduced at the two higher doses when compared with controls. With the exception of a significantly increased incidence of fetuses with unilateral or bilateral thirteenth ribs in the group at the highest dose, the incidence of major malformations and minor visceral or skeletal anomalies in the fetuses was deemed to be non-dose-related and considered not to be due to treatment. The authors concluded that, while maternal effects were observed at 250 mg/kg bw per day, no embryotoxic or teratogenic effects occurred (Gad et al., 1987).

*2-Isopropyl-*N*-2,3-trimethylbutyramide (No. 1595)*

Groups of 30 male and 30 female Sprague-Dawley rats were given 0 (vehicle control), 10, 50 or 100 mg/kg bw per day of 2-isopropyl-*N*-2,3-trimethylbutyramide in corn oil by gavage in a three-part study of growth, reproduction and teratogenesis. After the 63-day growth phase, male and female rats (F_0 generation) from the same dose groups were allowed to mate (reproduction phase). The treatment was administered to rats of each sex during the growth phase and until the end of the first mating. Female rats continued to be dosed with corn oil or 2-isopropyl-*N*-2,3-trimethylbutyramide throughout gestation until weaning. During gestation, body weights were measured twice a week for males and on days 0 and 19 of gestation for females. Food consumption was recorded twice a week for male rats and on days 6, 11, 15 and 19 of gestation for female rats. All the rats were observed daily for physical appearance, behaviour and pharmacological or toxicological symptoms. After the birth of the F_{1a} generation, maternal weights, number of viable fetuses per

litter, number of pups by sex per litter, weight of pups by sex per litter and number of deformed pups per litter were recorded. At weaning, the dams and F_{1a} pups were weighed; the pups were then killed and examined. The teratogenesis phase of the study began 21 days after the reproduction phase, whereby the same groups of F_0 male and female rats were mated once again; however, only the female rats continued to be dosed daily. As in the reproduction phase, female rats were weighed on days 0 and 19 of gestation, and their food intakes were recorded on days 6, 11, 15 and 19 of gestation. On day 19 of gestation, all the F_0 males and females were killed and examined grossly, and the fetuses were excised for examination for visceral or skeletal abnormalities. For each litter, the number of corpora lutea per ovary, the number and placement of uterine implantations, early and late resorbed fetuses, viable fetuses and any gross abnormalities were recorded.

During the 63-day growth phase, two control females, one male at 10 mg/kg bw per day, five females at the lowest dose, two males at 50 mg/kg bw per day, four females at the intermediate dose and one female at 100 mg/kg bw per day died due to gavage errors. These 15 rats were necropsied; no effects were observed that could be attributed to treatment. During the reproduction phase, another female at the intermediate dose died due to a gavage error. No treatment-related clinical signs or effects on body weight or food consumption were noted during the growth and reproduction phases of the study. Pregnancy rates were low in all groups, but no dose-related trends were observed. Gestation survival index, parturition index and lactation survival index were 100% in all groups. Mean live birth index and neonatal survival index were lower in the group at the highest dose; however, no dose-related trends were observed. The mean weanling survival index, mean sex ratio and mean pup body weights of treated groups were comparable to those of controls. No gross deformities were reported in any of the pups during the reproduction phase of the study. In the teratogenesis phase, no animals died and no treatment-related clinical signs were observed. There were no treatment-related effects on body weights or food consumption during the gestation period, and the mean body-weight changes were comparable in treated and control animals. There were no statistically significant differences in pregnancy rates between treated and control animals. The mean number of corpora lutea per group, the mean number of implantations per group and mean fetal body weights and lengths were comparable in treated and control groups. The mean implantation efficiency was slightly lower in the group at the highest dose than in controls. The mean incidence of resorptions was lower in the group at the lowest dose but higher in the groups at the two higher doses than in controls. Consequently, the mean incidence of fetal viability was higher in the group at the lowest dose and lower in the other two groups when compared with controls. The mean incidence of fetal mortality was lower in treated groups than in controls. No dose-related visceral or skeletal abnormalities or gross or microscopic findings were reported (Pence, 1980b).

Piperine (No. 1600)

Mice

In a series of studies conducted in Swiss albino mice, piperine was evaluated for effects on the estrous cycle, mating behaviour and growth of pups; effects on sperm morphology; anti-implantation activity and effect on fetal survival and sex ratio; and anti-fertility activity.

To study the possible effects of piperine on the estrous cycle, mating behaviour and growth of pups, adult female Swiss albino mice (number per group not stated) with a normal estrous cycle were given 0 (vehicle control), 10 or 20 mg/kg bw per day of piperine in 1% carboxymethyl cellulose by gavage for 14 days. The estrous cycle pattern was studied throughout treatment. Treatment with piperine appeared to lengthen the diestrous phase (2.08 ± 1.37 and 2.58 ± 2.09 days with 10 and 20 mg/kg bw per day, respectively, versus 1.33 ± 0.47 days for controls), resulting in an increase in duration (5.42 ± 1.54 and 5.50 ± 2.14 days at 10 and 20 mg/kg bw per day, respectively, versus 4.33 ± 0.37 days for controls), although the phases were not statistically significantly longer than that of the vehicle control. After the 14-day treatment, groups of six females were mated with healthy males to assess mating performance (determined from a vaginal plug or sperm-positive smear). Pregnant females were allowed to deliver, and the growth of their pups was monitored up to 21 days post partum. In the group at 20 mg/kg bw per day, mating performance was significantly reduced (50%) in comparison with controls and females at 10 mg/kg bw per day (both 83.3%). Fertility was significantly reduced, by 60% and 66.7% at the lowest and highest doses, respectively, compared with controls (100%); however, litter size and pup growth (litter body weight and length) were not affected by treatment.

A test for sperm shape abnormalities was carried out in young adult male Swiss albino mice given 0 (vehicle control), 35, 50 or 75 mg/kg bw per day of piperine in 1% carboxymethyl cellulose orally for 5 days. On day 35 of the study, the mice were killed and sperm was isolated for analysis. No significant abnormalities in sperm head morphology were reported.

The possible anti-implantation activity of piperine and effects on fetal survival and sex ratio were studied by mating groups of six adult female Swiss albino mice with males and treating the pregnant females with 0 (vehicle control), 10 or 20 mg/kg bw per day of piperine in 1% carboxymethyl cellulose by gavage on days 1–5 of gestation. On day 10 of gestation, the females were laparotomized to record the incidence of pregnancy and the size and number of implants. The pregnancies were allowed to continue to day 20 of gestation, at which time the fetuses were removed. Treatment with piperine decreased the overall reproductive capacity of female mice by inhibiting implantation. Only one of six females in each treated group had implants, whereas all six females in the vehicle control group had implants. No abnormalities were observed at the cellular level on histological examination of the ovary and uterus, and post-implantation survival and sex ratio were unaffected by treatment.

The possible anti-fertility activity of piperine was studied in groups of six female Swiss albino mice that had been laparotomized and injected 10 days before mating with: 50 µl of groundnut oil into both uterine horns; 50 µl of groundnut oil into the left horn and 50 µl of piperine into the right horn; or 50 µl of piperine into both horns. On day 14 of gestation, the females were examined for pregnancy and size and number of implants. There was no statistically significant reduction in the total number of implants in treated groups compared with controls (Daware et al., 2000).

Rats

In a study in adult albino rats (number and sex not specified), the possible anti-inflammatory activity of piperine on prostaglandin E1 was examined. The rats were given a single dose of 0 (vehicle control) or 20 mg/kg bw of piperine in

carboxymethyl cellulose by gavage. A positive control group of female rats was given 160 mg/kg bw of aspirin orally. To induce acute inflammation, prostaglandin E1 was injected into the right hind paw of each rat, and the percentage inflammation (paw volume) was measured 0, 30 and 60 min later. Both aspirin and piperine significantly reduced prostaglandin-induced inflammation of the paw. The authors suggested that the effects of piperine on specific reproductive parameters might be mediated, at least partly, through its effects on prostaglandin (Daware et al., 2000).

Piperidine (No. 1607)

Mice

In a study designed to evaluate the fertility of animals exposed to *N*-chloropiperidine (results not described here), piperidine was used as a negative control. Groups of male and female C57Bl/J6 × DBA2 hybrid mice treated orally for 100 days with 0 (vehicle control) or 400 mg/kg bw per day of piperidine in 0.001% ethanol were mated with similarly treated males. The positive control group consisted of animals given 250 mg/kg bw per day of ethylmethane sulfonate for 100 days, and the mating combination for this group was control female with ethylmethane sulfonate-treated male. Each group consisted of 15–25 females. On day 18 of gestation, 15–20 pregnant females were killed and the uteri were removed and examined for implantation sites and fetal death. Five females in each group were allowed to reach term, and the number of pups per dam was recorded. The fertility index (number of pregnant females:number of mated females) did not differ significantly between piperidine-treated animals and vehicle controls. While the frequency of offspring per pregnancy in piperidine-treated animals was comparable to that in vehicle controls, the number of dead fetuses in piperidine-treated animals was significantly higher than in vehicle controls. In comparison with positive controls, however, the incidence of fetal mortality per pregnancy was significantly lower in the piperidine-treated group. Additionally, on day 40 and every 5 days thereafter, three male rats per group were killed, and their vas deferentia were excised for investigation of sperm morphology. The frequency of sperm abnormalities was comparable in piperidine-treated and vehicle control groups of mice (Bempong & Scully, 1983).

Hamsters

In a study to examine the effect of piperidine on fertility in hamsters, groups of male and female inbred golden Syrian hamsters were given 0 (vehicle control) or 400 mg/kg bw per day of piperidine in 0.001% ethanol orally for 100 days and mated with corresponding males. The positive control group consisted of animals given 250 mg/kg bw per day of ethylmethane sulfonate for 100 days, and the mating combination for this group was control female with ethylmethane sulfonate-treated male. Each group consisted of 15–25 females. On day 18 of gestation, 15–20 pregnant females were killed and their uteri were removed and examined for implantation sites and fetal deaths. Five females in each group were allowed to reach term, and the number of pups per female was recorded. The fertility index (number of pregnant females:number of mated females) did not differ significantly between piperidine-treated animals and vehicle controls. The frequency of offspring per pregnancy in piperidine-treated animals was comparable to that in vehicle

controls. Additionally, on day 40 and every 5 days thereafter, three male hamsters per group were killed, and their vas deferentia were excised for investigation of sperm morphology. The frequency of sperm abnormalities was comparable in piperidine-treated and vehicle control groups (Bempong & Scully, 1983).

Trimethylamine (No. 1610)

Trimethylamine (0.75 mmol/l or 44.3 μg/ml) was incubated with 8-day-old embryos from CD-1 mice in culture for up to 42 h. Control embryos were cultured in the presence of saline at volumes equal to those used with trimethylamine. Within 2–4 h of incubation, time-dependent inhibition of embryonic growth was apparent (70% of control), accompanied by decreased incorporation of tritium-labelled thymidine, uridine and leucine into DNA, RNA and proteins, respectively. Trimethylamine also caused neural tube defects in 73% of the embryos. After 42 h of incubation, the DNA, RNA and protein contents of trimethylamine-treated embryos were approximately 50% those of controls. The effects were not related to changes in pH or osmolarity of the culture media. The authors suggested that trimethylamine has teratogenic effects and inhibits growth in cultured mouse embryos by reducing macromolecular synthesis (Guest & Varma, 1992).

Binding assay in vitro

N-Ethyl 2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601)

An assay was performed in vitro to assess the potential of *N*-ethyl 2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601) to bind to α2μ-globulin, as this compound has been reported to cause histological changes in male rat kidney that are similar to those seen in male rat-specific α2μ-globulin nephropathy. *N*-Ethyl 2-isopropyl-5-methylcyclohexanecarboxamide displaced ^{14}C-*d*-limonene epoxide (a known ligand for α2μ-globulin) with an affinity (IC_{50} approximately 20 μmol/l) similar to that of *d*-limonene (IC_{50} approximately 15 μmol/l) and lower than that of *d*-limonene-1,2-epoxide (IC_{50} approximately 3 μmol/l). The authors concluded that the histopathological changes seen in the kidneys of male rats given *N*-ethyl 2-isopropyl-5-methylcyclohexanecarboxamide were probably attributable to its affinity for α2μ-globulin, which causes male rat-specific α2μ-globulin nephropathy (Lehman-McKeeman & Caudill, 1994).

3. REFERENCES

Adler, I.D. & Ingwersen, I. (1989) Evaluation of chromosomal aberrations in bone marrow of $1C3F_1$ mice. *Mutat. Res.*, **224**, 343–345.

Akesson, B., Skerfving, S. & Mattiasson, L. (1988) Experimental study on the metabolism of triethylamine in man. *Br. J. Ind. Med.*, **45**, 262–268.

Akesson, B., Vinge, E. & Skerfving, S. (1989) Pharmacokinetics of triethylamine and triethylamine-*N*-oxide in man. *Toxicol. Appl. Pharmacol.*, **100**, 529–538.

Amoore, J.E., Gumbmann, M.R., Booth, A.N. & Gould, D.H. (1978) Synthetic flavors: efficiency and safety factors for sweaty and fishy odorants. *Chem. Senses Flavour*, **3**, 307.

Andrews, A.W., Fornwald, J.A. & Luinsky, W. (1980) Nitrosation and mutagenicity of some amine drugs. *Toxicol. Appl. Pharmacol.*, **52**, 237–244.

Audunsson, G. (1988) Determination of low parts per billion levels of amines in urine by liquid membrane sample cleanup directly coupled to a gas-liquid chromatograph. *Anal. Chem.*, **60**, 1340–1347.

Ayesh, R., Mitchell, S.C., Zhang, A. & Smith, R.L. (1993) The fish odour syndrome: biochemical, familial, and clinical aspects. *Br. Med. J.*, **307**, 655–657

Beckett, A.H. & Sheikh, A.H. (1973) Proceedings: In vitro metabolic *N*-oxidation of the minor tobacco alkaloids, (–)methylanabasine and (–)anabasine to yield a hydroxylamine and nitrone in lung and liver homogenates. *J. Pharmaceut. Pharmacol.*, **25** (Suppl.), 171P.

Bellander, T. Osterdahl, B.G. & Hagmar L. (1985) Formation of *N*-mononitrosopiperazine in the stomach and its excretion in the urine after oral intake of piperazine. *Toxicol. Appl. Pharmacol.*, 80, 193–198.

Bempong, M.A. & Scully, F.E., Jr (1983) Seminal cytology and reproductive toxicology of *N*-chloropiperidine. *J. Am. Coll. Toxicol.*, **2**, 209–219.

Bernheim, F. & Bernheim, M.J.C. (1938) The oxidation of mescaline and certain other amines. *J. Biol. Chem.*, **123**, 317–326.

Bhat, B.G. & Chandrasekhara, N. (1986a) Studies on the metabolism of piperine: absorption, tissue distribution and excretion of urinary conjugates in rats. *Toxicology*, **40**, 83–92.

Bhat, B.G. & Chandrasekhara, N. (1986b) Lack of adverse influence of black pepper, its oleoresin and piperine in the weanling rat. *J. Food Saf.*, **7**, 215–223.

Bhat, B.G. & Chandrasekhara, N. (1987) Metabolic disposition of piperine in the rat. *Toxicology*, **44**, 99–106.

Block, W. (1953) [On the physiology of ^{14}C-radioactive mescaline in animals. Part IV. Comparative studies with ^{14}C-mescaline and ^{14}C-β-phenylethylamine.] *Z. Naturforsch. B: Chem. Biochem. Biophys. Biol.*, **8B**, 440–444 (in German with English translation).

Bond, G.G. & Nixon, G.A. (1980) Acute oral toxicity (LD_{50}) study. TGSE 1776, T0332.04, WS-23. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Bray, H.G., James, S.P., Thorpe, W.V., Wasdell, M.R. & Wood, P.B. (1949) The fate of certain organic acids and amides in the rabbit. 9. Lower aliphatic amides. *Biochem. J.*, **45**, 467–471.

ten Brink, B., Damink, C. Joosten, H.M. & Huis in't Veld, J.H. (1990) Occurrence and formation of biologically active amines in foods. *Int. J. Food Microbiol.*, **11**, 73–84

Cheever, K.L., Richards, D.E. & Pltnick, H.B. (1982) The acute oral toxicity of isomeric monobutylamines in the adult male and female rat. *Toxicol. Appl. Pharmacol.*, **63**, 150–152.

Cheng, M.C. (1982) A subchronic oral toxicity study of T0332.04 in rats. Sugy No. 80063, Vol. 1. Hazleton Laboratories, Madison, Wisconsin, USA. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Chieli, E., Aliboni, F., Saviozzi, M. & Malvaldi, G. (1987) Induction of micronucleated erythrocytes by primary thioamides and their metabolites in the mouse. *Mutat. Res.*, **192**, 141–143.

Ciugudeanu, M., Gabor, S., Gocan, M., Ivanescu, G. & Buzas, A. (1985) Experimental research on the acute toxicity of butanol, mono-, di- and tributylamine. *Rev. Chim.*, **32**, 667–670.

Cork, M.J., Cooke, N.J. & Mellor, E. (1990) *Pruritis ani. Br. Med. J.*, **301**, 1398.

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

Damani, L.A. & Crooks, P.A. (1982) Oxidative metabolism of heterocyclic ring systems. In: Jakoby, W.B., ed., *The Enzymatic Basis of Detoxication*, New York, Academic Press, 2nd Ed., pp. 291–323.

Danz, M. & Urban, H. (1979) Carcinogens, promotors and suspicious drugs: their identical short-term effect in a promoting activity test (PAT). *Exp. Pathol.*, **17**, 181–184

Davies, D.F., Wolfe, K.M. & Perry, H.M. (1954) Studies on primary amines II. Their natural occurrence in urine of normotensive and hypertensive subjects. *J. Lab. Clin. Med.*, **43**, 620–632.

Daware, M.B., Mujumdar, A.M. & Ghaskadbi, S. (2000) Reproductive toxicity of piperine in Swiss albino mice. *Plant Med.*, **66**, 231–236.

Deal, D.L., Chandrasurin, P., Shockor, J., Minick, D.J., Findlay, J.W.A. & McNulty, M.J. (1992) Disposition and metabolism of triprolidine in mice. *Drug Metab. Disposition*, **20**, 920–927.

von Euler, U.S. (1945) The occurrence and determination of piperidine in human and animal urine. *Acta Pharmacol.*, **1**, 29–59.

Fahrig, R. (1989) Possible recombinogenic effect of caprolactam in the mammalian spot test. *Mutat. Res.*, **224**, 373–375.

Farber, T.M. (2003) Reexamination of the hematology findings from the 28-day rat feeding study on gold root extract performed by Product Safety Laboratories. ToxaChemica, International, Kensington, Maryland, USA. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Fassett, D. (1978) Conversion of inhalation dose in parts per million to a dose in mg/kg. Unpublished personal communication to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Fischer, C., Schönberger, F., Meese, C.O. & Eichelbaum, M. (1990) Determination of the enantiomers of flecainide and two major metabolites in man by gas chromatography/mass spectrometry using negative ion chemical ionization and stable isotope labelled internal standards. *Biomed. Environ. Mass Spectrom.*, **19**, 256–266.

Flaks, B., Trevan, M.T. & Flaks, A. (1983) An electron microscope study of hepatocellular changes in the rat during chronic treatment with acetamide. Parenchyma, foci and neoplasms. *Carcinogenesis*, **4**, 1117–1125.

Fleischman, R.W., Baker, J.R., Hagopian, M., Wade, G.G., Hayden, D.W., Smith, E.R., Weisburger, J.H. & Weisburger, E.K. (1980) Carcinogenesis bioassay of acetamide, hexanamide, adipamide, urea and p-tolylurea in mice and rats. *J. Environ. Pathol. Toxicol.*, **3**, 149–170.

Florin, I., Rutberg, L., Curvall, M. & Enzell, C.R. (1980) Screening of tobacco smoke constituents for mutagenicity testing using Ames test. *Toxicology*, **18**, 219–232.

Forgue, S.T., Kari, P. & Barbhaiya, R. (1987) *N*-Oxidation of *N*-methylpyrrolidine released in vivo from cefepime. *Drug Metab. Disposition*, **15**, 808–815.

Gad, S.C., Robinson, K., Serota, D.G. & Colpean, B.R. (1987) Developmental toxicity studies of caprolactam in the rat and rabbit. *J. Appl. Toxicol.*, **7**, 317–327.

Gage, J.C. (1970) The subacute inhalation toxicity of 109 industrial chemicals. *Br. J. Ind. Med.*, **27**, 1–18.

Gamer, A.O., Heliwig, J. & van Ravenzwaay, B. (2002) Developmental toxicity of oral *n*-butylamine hydrochloride and inhaled *n*-butlyamine in rats. *Food Chem. Toxicol.*, **40**, 1833–1842.

Garberg, P., Åkerblom, E.-L. & Bolcsfoldi, G. (1988) Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution. *Mutat. Res.*, **203**, 155–176.

Green, N.R. & Savage, J.R. (1978) Screening of safrole, eugenol, their ninhydrin positive metabolites and selected secondary amines for potential mutagenicity. *Mutat. Res.*, **57**, 115–121

Greenblatt, M., Mirvish, S. & So, B.T. (1971) Nitrosamine studies: induction of lung adenomas by concurrent administration of sodium nitrite and secondary amines in Swiss mice. *J. Natl Cancer Inst.*, **46**, 1029–1034.

Guest, I. & Varma, D.R. (1992) Teratogenic and macromolecular synthesis inhibitory effects of trimethylamine on mouse embryos in culture. *J. Toxicol. Environ. Health*, **36**, 27–41.

Haworth, S., Koo, S. & Lawlor, T. (1978) *Salmonella*/mammalian–microsome plate incorporation mutagenesis assay of compound #T0332.01 (MRI #78). EG_G Mason Research Institute, Rockville, Maryland, USA. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W. & Zeiger, E. (1983) *Salmonella* mutagenicity test results for 250 chemicals. *Environ. Mutag.*, **Suppl. 1**, 3–142.

Hellmér, L. & Bolcsfoldi, G. (1992) An evaluation of the *E. coli* K-12 uvrB/recA DNA repair host-mediated assay. I. In vitro sensitivity of the bacteria to 61 compounds. *Mutat. Res.*, **272**, 145–160.

Honegger, C.G. & Honegger, R. (1960) Volatile amines in brain. *Nature*, **185**, 530–532.

Hunter, B., Benson, H.G., Street, A.E., Heywood, R. & Prentice, D.E. (1974) WS-3 (*N*-ethyl 2-isopropyl-5-methylcyclohexanecarboxamide) toxicity following oral administration for 22 weeks. Huntingdon Research Centre, Huntingdon, Cambridgeshire, England. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

International Organization of the Flavor Industry (1995) European inquiry on volume use. Private communication to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Ishitoya, Y., Baba, S. & Hashimoto, I. (1973) Gas-chromatographic separation and identification of aliphatic amines in blood. *Clin. Chim. Acta*, **46**, 55–61.

Iwamoto, T. (1957) Biochemical studies of putrefaction amines. IX. Distribution and change of trimethylamine in cat organs after its parenteral administration. *Yakugaku Zasshi*, **77**, 1189–1191.

James, R. (1974) Appendix 4, animal studies—summary of results. *N*-Ethyl-2-isopropyl-5-methylcyclohexane. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Kaiser, C., Swagzdis, J.E., Flanagan, T.L., Lester, B.M., Burghard, G.L., Green, H. & Zirkle C.L. (1972) Metabolism of diphenidol. Urinary products in humans and dogs. *J. Med. Chem.*, **15**, 1146–1150.

Karekar, V.R., Mujumdar, A.M., Joshi, S.S., Dhuley, J., Shinde, S.L. & Ghaskadbi, S. (1996) Assessment of genotoxic effect of piperine using *Salmonella typhimurium* and somatic and germ cells of Swiss albino mice. *Arzneimittel-Forsch.*, **46**, 972–975.

Kase, Y., Miyata, T., Kamikawa, Y. & Kataoka, M. (1969a) Pharmacological studies on alicyclic amines (II). Central actions of piperidine, pyrrolidine and piperazine. *Jpn. J. Pharmacol.*, **19**, 300–314.

Kase, Y., Kataoka, M. & Miyata, T. (1969b) An improved method for the determination of micro amounts of piperidine in living materials. *Jpn. J. Pharmacol.*, **19**, 354–362.

Kearney, E.B., Salach, J.I., Walker, W.H., Seng, R. & Singer, T.P. (1971) Structure of the covalently bound flavin of monoamine oxidase. *Biochem. Biophys. Res. Communications*, **42**, 490–496.

King, M.-T. (2003) Mutagenicity study of HR 03/G05015 in the *Salmonella typhimurium*/mammalian microsome reverse mutation assay (Ames-test). Project No. AM00103N. King & Harnasch GmbH, Kirchzarten. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Kirby, P.E., Branch, M.L. & Pizzarello, R.F. (1978) L5178Y mouse lymphoma mutagenesis assay of compound #T0332.01 (MRI #78). EG_G Mason Research Institute, Rockville, Maryland, USA. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Kirkel, A.Z., Tischenko, L.N. & Aksenova, L.N. (1991) Comparative characteristics of membrane bound and cytoplasmic monoamine oxidases in human placenta. *Vop. Med. Khim.*, **37**, 25–28.

Kumar, R., Siddiqi, M., Fazili, Z., Wacker, C.-D., Spiegelahalder, B. & Preussmann, R. (1992) Effects of dietary nitrate on endogenous nitrosation of piperazine in humans. *Cancer Lett.*, **65**, 139–143.

Lehman-McKeeman, L.D. & Caudill, D. (1994) Determination of the potential for WS-23 to bind to α2μ-globulin. Experimental summary. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Lin, J. & Cashman, J.R. (1997) Detoxication of tyramine by the flavin-containing monooxygenase: stereoselective formation of the trans oxime. *Chem. Res. Toxicol.*, **10**, 842–852.

Loit, A.O. & Filov, V.A. (1964) Toxicity of aliphatic amines and its modification in homologous series. *Gig. Tr. Prof. Zabol.*, **8**, 23–28.

Lovenberg, W. (1973) Some vaso- and psychoactive substances in food: amines, stimulants, depressants and hallucinogens. In: *Toxicants Occurring Naturally in Foods*, Washington DC, National Academy of Sciences Press, 2nd Ed., pp. 170–188.

Lucas, C.D., Putnam, J.M. & Hallagan, J.B. (1999) *1995 Poundage and Technical Effects Update Survey*, Washington DC, Flavor and Extract Manufacturers Association of the United States.

Lynch, D.W. Moorman, T.R., Lewis, T.R., Stober, P., Hamlin, R.D. & Schueler, R.L. (1990) Subchronic inhalation of triethylamine vapor in Fischer F344 rats: organ system toxicity. *Toxicol. Ind. Health*, **6**, 403–414.

March, J. (1992) *Advanced Organic Chemistry, Reactions, Mechanisms, and Structure*, New York, Wiley, 4th Ed., pp. 884–885.

Masumoto, H., Ohta, S. & Hirobe, M. (1991) Application of chemical cytochrome P-450 model system to studies on drug metabolism. IV. Mechanism of piperdine metabolism pathways via an iminium intermediate. *Drug Metab. Disposition*, **194**, 768–780.

McEwen, C.M., Jr (1965) Human plasma monoamine oxidase. I. Purification and identification. *J. Biol. Chem.*, **240**, 2003–2010.

McGregor, D.B., Riach, C.G., Brown, A., Edwards, I., Reynolds, D., West, K. & Willington, S. (1988) Reactivity of catecholamines and related substances in the mouse lymphoma L5178Y cell assay for mutagens. *Environ. Mol. Mutag.*, **11**, 523–544.

McNulty, M.J., Deal, D.L., Page, T.L., Chandrasurin, P. & Findlay, J.W. (1992) Disposition of triprolidine in the male beagle dog. *Drug Metab. Disposition*, **20**, 928–935.

Medvedev, A.E., Kirkel, A. & Kamyshanskaya, N.S. (1995) Influence of ethanol administration on the activity and compartmentation of rat liver monoamine oxidases. *Alcohol Alcoholism*, **30**, 729–735.

Meffin, P., Robertson, A.V., Thomas, J. & Winkler, J. (1973) Neutral metabolites of mepivacaine in humans. *Xenobiotica*, **3**, 191–196.

Midgley, I., Hood, A.J., Chasseaud, L.F., Brindley, C.J., Baughman, S. & Allan, G. (1992) Percutaneous absorption of co-administered *N*-methyl-2-[^{14}C]pyrrolidinone and 2-[^{14}C]-pyrrolidinone in the rat. *Food Chem. Toxicol.*, **30**, 57–64.

Mirkova, E.T. (1996) Activities of the rodent carcinogens thioacetamide and acetamide in the mouse bone marrow micronucleus assay. *Mutat. Res.*, **352**, 23–30.

Miyagawa, M., Takasawa, H., Sugiyama, A., Inoue, Y., Murata, T., Uno, Y. & Yoshikawa, K. (1995) The in vivo–in vitro replicative DNA synthesis (RDS) test with hepatocytes prepared from male B6C3F$_1$ mice as an early prediction assay for putative nongenotoxic (Ames-negative) mouse hepatocarcinogens. *Mutat. Res.*, **343**, 157–183.

Miyata, K. (1995) Summary of 28-day repeated-dose oral toxicity study of WS-3. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Moore, G.E. (2002) 28-day dietary toxicity study in rodents. Study No. 11326. Product Safety Labs, East Brunswick, New Jersey, USA. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Morita, T., Asano, N., Awogi, T., Sasaki, Y.F., Sato, S., Shimada, H., Sutou, S., Suzuki, A., Wakata, A., Sofuni, T. & Hayashi, M. (1997) Evaluation of the rodent micronucleus assay to screen IARC carcinogens (group 1, 2A, and 2B). The summary report of the 6th collaborative study by CSGMT/JEMS. *Mutat. Res.*, **389**, 3–122.

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

Moskvitina, T.A. & Medvedev, A.E. (2001) Different sensitivity of mitochondrial and cytosolic monoamine oxidases to in vivo but not in vitro inhibition by specific irreversible inhibitors. *Med. Sci. Monit.*, **7**, 17–19.

Mosnaim, A.D., Inwang, E.E., Sugerman, J.H. & Sabelli, H.C. (1973) Identification of 2-phenylethylamine in human urine by infrared and mass spectroscopy and its quantification in normal subjects and cardiovascular patients. *Clin. Chim. Acta*, **46**, 407–413.

Müller, H. & Immendorfer, I. (1942) The characteristics of trimethylamine and trimethylamine oxide in the metabolism. *Hoppe-Seyler Z. Physiol. Chem.*, **275**, 267–276.

Muralidhara & Narasimhamurthy, K. (1990) Lack of genotoxic effects of piperine (the active principle of black pepper) in albino mice. *J. Food Saf.*, **11**, 39–48.

Myers, R.C. & Ballantyne, B. (1997) Comparative acute toxicity and primary irritancy of various classes of amines. *Toxic Subst. Mech.*, **16**, 151–193.

National Toxicology Program (1982) *Carcinogenesis Bioassay of Caprolactam in F344 Rats and B6C3F$_1$ Mice*, Technical Report Series No. 214, NIH Publication No. 81-1770, esearch Triangle Park, North Carolina, US Department of Health and Human Services, National Institute of Environmental Health Sciences.

Neuhäuser-Klaus, A. & Lehmacher, W. (1989) The mutagenic effect of caprolactam in the spot test with (TxHT)F$_1$ mouse embryos. *Mutat. Res.*, **224**, 369–37?.

Neurath, G.B., Dünger, M., Orth, D. & Pein, F.G. (1987) *trans*-3′-Hydroxycotinine as a main metabolite in urine of smokers. *Int. Arch. Occup. Environ. Health*, **59**, 199–201.

Nijssen, B., van Ingen-Visscher, K. & Donders, J. (2003) *Volatile Compounds in Food 8.1*, Zeist, Netherlands, Centraal Instituut Voor Voedingsonderzioek TNO. http://www.voeding.tno.nl/vcf/VcfNavigate.cfm.

Nixon, G. & Alden, C. (1978) 14-Day pilot intubation study (YE-193; TGSE-1540; P78-045). Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Nnane, I.P. & Damani, L.A. (2001) Pharmacokinetics of trimethylamine in rats, including the effects of a synthetic diet. *Xenobiotica*, **31**, 749–755.

Ohshima, H., Friesen, M., Malaveille, C., Brouet, I., Hautefeuille, A. & Bartsch, H. (1989) Formation of direct-acting genotoxic substances in nitrosated smoked fish and meat products: identification of simple phenolic precursors and phenyldiazonium ions as reactive products. *Food Chem. Toxicol.*, **27**, 193–203.

Okano, Y., Miyata, T., Hung, S.-H., Motoya, T., Kataoka, M., Takahama, K. & Kase, Y. (1978) Metabolites of piperidine in rat urine. *Jpn. J. Pharmacol.*, **28**, 41–47.

Panoutsopoulos, G.I., Kouretas, D., Gounaris, E.G. & Beedham, C. (2004) Metabolism of 2-phenylethylamine and phenylacetaldehyde by precision-cut guinea pig fresh liver slices. *Eur. J. Drug Metab. Pharmacokinet.*, **29**, 111–118.

Pearson, A.M., Sleight, S.D., Cornforth, D.P. & Akoso, B.T. (1982) Effects of N-nitrosopyrrolidine, nitrite and pyrrolidine on tumour development in mice as related to ingestion of cured meat. *Meat Sci.*, **7**, 259–268.

Pence, D.H. (1980a) Subchronic oral toxicity study in rats. T-0332.03. Hazleton Laboratories, Falls Church, Virginia, USA. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Pence, D.H. (1980b) Reproduction and teratology study in rats. T-0332.03. Final report. Hazleton Laboratories, Falls Church, Virginia, USA. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Perry, T.L., Hansen, S., Foulks, J.G. & Ling, G.M. (1965) Aliphatic and aromatic amines of cat brain. *J. Neurochem.*, **12**, 397–405.

Piyachaturawat, P., Glinsukon, T. & Toskulkao, C. (1983) Acute and subacute toxicity of piperine in mice, rats and hamsters. *Toxicol. Lett.*, **16**, 351–359.

Posternak, J.M., Linder, A. & Vodoz, C.A. (1969) Summaries of toxicological data. Toxicological tests on flavouring matters. *Food Cosmet. Toxicol.*, **7**, 405–407.

Power, F.W. & Sherwin, C.P. (1927) The detoxication of putrefactive products by the human body. *Arch. Intern. Med.*, **39**, 60–66.

Product Safety Laboratories (2004) 28-day dietary toxicity study in rodents. Draft clinical pathology summary. Lymphocyte effects in male and female rats. Study No. 11326. Product Safety Laboratories, Dayton, New Jersey, USA. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Pugh, C.E.M. & Quastel, J.H. (1937) Oxidation of aliphatic amines by brain and other tissues. *Biochem. J.*, **31**, 286–291.

Rechenberger J. (1940) Über die flüchtigan Alkylamine im menschlichen Stoffwechsel II. Mitteilung: Ausscheidung im Harn nach oraler Zufuhr. *Z. Physiol. Chem.*, **265**, 275–284 (in German).

Richter, D. (1937) CCXLIX. Adrenaline and amine oxidase. *Biol. Chem. Biochem. J.*, **31**, 2022–2028

Ross, S.B. & Renyi, A.L. (1971) Uptake and metabolism of beta-phenethylamine and tyramine in mouse brain and heart slices. *J. Pharm. Pharmacol.*, **23**, 276–279.

Rozman, E. Galceran, M.T., Anglada, L. & Albet, C. (1994) Metabolism of ebrotidine, a new H2-receptor antagonist, in human urine. *J. Pharm. Sci.*, **83**, 252.

Sasaki, Y.F., Sekihashi, K., Izumiyama, F., Nishidate, E., Saga, A., Ishida, K. & Tsuda, S. (2000) The Comet assay with multiple mouse organs: comparison of Comet assay results and carcinogenicity with 208 chemicals selected from the IARC Monographs and US NTP carcinogenicity database. *Crit. Rev. Toxicol.*, **30**, 629–799.

Schweizer, A.E., Fowlkes, R.L., McMakin, J.H. & Whyte, T.E., Jr (1978) Amines (lower aliphatic). In: Mar, H.F., Othmer, D.F., Overberger, C.G. & Seaborg, G.T., eds, *Kirk-Othmer Encyclopedia of Chemical Technology*, New York, Wiley, 3rd Ed., Vol. 2, pp. 272–283.

Schwen, R.J. (1982) Excretion and tissue distribution of (^{14})C after an oral dose of a capsaicin analog, *N*-(vanillyl)-[1-(^{14})C]-nonanamide. *Fed. Proc.*, **41**, 1558

Seakins, J.W.T. (1971) The determination of urinary phenylacetylglutamine as phenylacetic acid. Studies on its origin in normal subjects and children with cystic fibrosis. *Clin. Chim. Acta*, **35**, 121–131.

Serota, D.G., Hoberman, A.M., Friedman, M.A. & Gad, S.C. (1988) Three-generation reproduction study with caprolactam in rats. *J. Appl. Toxicol.*, **8**, 285–293.

Simenhoff, M.L. (1975) Metabolism and toxicity of aliphatic amines. *Kidney Int.*, **7**, S314–S317.

Sina, F., Bean, C.L., Dysart, G.R., Taylor, V.I. & Bradley, M.O. (1983) Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. *Mutat. Res.*, **113**, 357–391.

Singh, N., Kulshrestha, V.K., Srivastava, R.K. & Kohli, R.P. (1973) Studies on the analeptic activity of some Piper longum alkaloids. *Indian J. Med. Res.*, **8**, 1.

Skinner, W.A. (1978) Unscheduled DNA synthesis assay of Procter & Gamble compound #T0332.01. SRI International, Menlo Park, California, USA. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Smith, J.L., Wishnok, J.S. & Deen, W.M. (1994) Metabolism and excretion of methylamines in rats. *Toxicol. Appl. Pharmacol.*, **125**, 296–308.

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

Smyth, H.F., Jr, Carpenter, C.P., Weil, C.S., Pozzani, U.C. & Strieger, J.A. (1962) Range-finding toxicity data: List VI. *Ind. Hyg. Assoc. J.*, **23**, 95.

Smyth, H.F., Jr, Carpenter, C.P., Weil, C.S., Pozzani, U.C., Striegel, J.A. & Nycum, J.S. (1969) Range-finding toxicity data: List VII. *Am. Ind. Hyg. Assoc. J.*, **30**, 470–476

Snyder, F.H., Goetze, H. & Oberst, F.W. (1946) Metabolic studies on derivatives of beta-phenylethylamine. *J. Pharmacol. Exp. Ther.*, **86**, 145–150.

Stofberg, J. & Kirschman, J.C. (1985) The consumption ratio of flavoring materials: a mechanism for setting priorities for safety evaluation. *Food Chem. Toxicol.*, **23**, 857–860.

Stofberg, J. & Grundschober, F. (1987) Consumption ratio and food predominance of flavoring materials. *Perfum. Flavorist*, **12**, 27.

Suter, C.M. & Weston, A.W. (1941) Some fluorinated amines of the pressor type. *J. Chem. Soc. Perkin Trans.*, **63**, 60.

Til, H.P., Falke, H.E., Prinsen, M.K. & Willems, M.I. (1997) Acute and subacute toxicity of tyramine, spermidine, putrescine and cadaverine in rats. *Food Chem. Toxicol.*, **35**, 337–348.

Unger, P.D. & Friedman, M.A. (1980) High-pressure liquid chromatography of caprolactam and its metabolites in urine and plasma. *J. Chromatogr.*, **187**, 429–435.

Uno, Y., Takasawa, H., Miyagawa, M., Inoue, Y., Murata, T. & Yoshikawa, K. (1994) An in vivo–in vitro replicative DNA synthesis (RDS) test using rat hepatocytes as an early prediction assay for nongenotoxic hepatocarcinogens screening of 22 known positives and 25 non carcinogens. *Mutat. Res.*, **320**, 189–205.

Valoti, M., Morón, J.A., Benocci, A., Sgaragli, G. & Unzeta, M. (1998) Evidence of a coupled mechanism between monoamine oxidase and peroxidase in the metabolism of tyramine by rat intestinal mitochondria. *Biochem. Pharmacol.*, **55**, 37–43.

Van den Berg, C.M., Kemper, E.M. & Azzaro, A.J. (2003) Tyramine pharmacokinetics and reduced bioavailability with food. *J. Clin. Pharmacol.*, **43**, 604–609.

Van den Heuvel, M.J., Clark, D.G., Fielder, R.J., Koundakjian, P.P., Oliver, G.J.A., Pelling, D., Tomlinson, N.J. & Walker, A.P. (1990) The international validation of a fixed-dose procedure as an alternative to the classical LD_{50} test. *Food Chem. Toxicol.*, **28**, 469–482.

Vogel, E.W. (1989) Caprolactam induces genetic alterations in early germ cell stages and in somatic tissue of *D. melanogaster*. *Mutat. Res.*, **224**, 339–342.

Wangenheim, J. & Bolcsfoldi, G. (1988) Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. *Mutagenesis*, **3**, 193–205.

Williams, R.T. (1959) *Detoxication Mechanisms—The Metabolism of Aliphatic Amines and Amides and Various Compounds Derived from Them*, London, Chapman & Hall Ltd.

Wnorowski, G. (1995) 14-day dietary toxicity study: rats. Study No. 3652. Product Safety Labs, East Brunswick, New Jersey, USA. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Wranne, L. (1956) Urinary excretion of trimethylamine and trimethylamine oxide following trimethylamine-administration to normals and to patients with liver disease. *Acta Med. Scand.*, **113**, 433–441.

Wu, W.N., Pritchard, J.F., Ng, K.T., Hills, J.F., Uetz, J.A., Yorgey, K.A., McKown, L.A. & O'Neill, P.J. (1992) Disposition of bepridil in laboratory animals and man. *Xenobiotica*, **22**, 153–169.

Yam, J., Reer, P.J. & Bruce, R.D. (1991) Comparison of the up-and-down method and the fixed-dose procedure for acute oral toxicity testing. *Food Chem. Toxicol.*, **29**, 259–263.

Zaeva, G.N., Ordynskaya, N.N., Dubinina, L.I., Osipenko, N.I. & Ivanov, V.N. (1974) Substantiation of maximum permissible concentration of pyrrolidine in the work place atmosphere. *Gig. Tr. Prof. Zabol.*, **18**, 29–32

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

Zhang, A.Q., Mitchell, S. & Smith, R. (1993) Fish odour syndrome: verification of carrier detection test. *J. Inherited Metab. Dis.*, **18**, 669–674.

Zhang, A.Q., Mitchell, S.C. & Smith, R.L. (1998) Dimethylamine formulation in the rat from various related amine precursors. *Food Chem. Toxicol.*, **36**, 923–927.