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Food and Chemical Toxicology 42 (2004) 157–185

Food and Chemical Toxicology

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Review

The FEMA GRAS assessment of cinnamyl derivatives used as flavor ingredients

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Received 2 July 2003; received in revised form 21 August 2003; accepted 31 August 2003

Abstract

This publication is the seventh in a series of safety evaluations performed by the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA). In 1993, the Panel initiated a comprehensive program to re-evaluate the safety of more than 1700 GRAS flavoring substances under conditions of intended use. Elements that are fundamental to the safety evaluation of flavor ingredients include exposure, structural analogy, metabolism, pharmacokinetics and toxicology. Flavor ingredients are evaluated individually and in the context of the available scientific information on the group of structurally related substances. Scientific data relevant to the safety evaluation of the use of cinnamyl derivatives as flavoring ingredients is evaluated.

Abbreviations: ABS, chromosomal aberration; ADH, alcohol dehydrogenase; ALD, aldehyde dehydrogenase; *B. subtilis*, *Bacillus subtilis*; CHO, Chinese hamster ovary; CoA, coenzyme A; DNA, deoxyribonucleic acid; ECETOC, European Centre for Ecotoxicology and Toxicology of Chemicals; *E. coli*, *Escherichia coli*; F, Female; FDA, United States Food and Drug Administration; FEMA, The Flavor and Extract Manufacturers Association; GRAS, Generally Recognized as Safe; GRASa, GRAS affirmed; GRASr, GRAS reaffirmed; IARC, International Agency for Research on Cancer; i.p., intraperitoneal; LD_{50}, median lethal dose; M, Male; MLA, mouse lymphoma cell assay; NAS, National Academy of Science; NCI, National Cancer Institute; NOEL, No observed effect level; NR, Not reported; NTP, National Toxicology Program; PPARα, peroxisome proliferator-activated receptor α; PE, polychromatic erythrocytes; ppm, parts per million; *S. typhimurium*, *Salmonella typhimurium*; SCE, sister chromatid exchanges; SLR, scientific literature review.

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0278-6915/$ - see front matter
doi:10.1016/j.fct.2003.08.021

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1. Chemical identity

This summary presents the key data relevant to the safety evaluation of cinnamyl alcohol, cinnamaldehyde, cinnamic acid (*trans*-3-phenylpropenoic acid), and 53 structurally related substances for their intended use as flavoring substances (Table 1). All members of this group are primary alcohols, aldehydes, or carboxylic acids, or their corresponding esters and acetals. The primary oxygenated functional group is located on a three-carbon saturated or unsaturated (i.e., at the 2,3-position) chain with a benzene ring at the 3 position (i.e., a 3-phenylpropyl or 3-phenyl-2-propenyl group). The aromatic ring also may be substituted with alkyl, alkoxy, or hydroxy substituents.

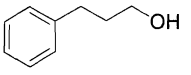
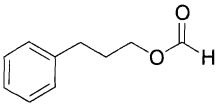
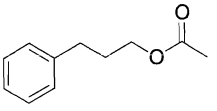
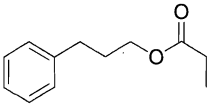
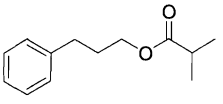
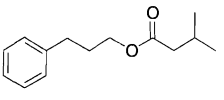
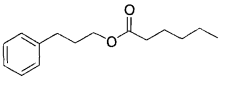
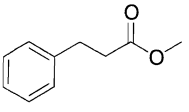
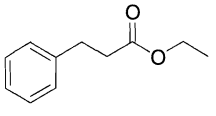
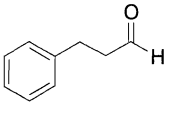
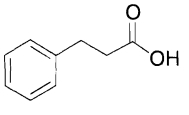
2. Exposure

2.1. Flavor use and natural occurrence

The total annual volume of the 56 cinnamyl derivatives used as flavoring ingredients is approximately 485,050 kg in the USA. (Lucas et al., 1999; NAS, 1970; 1982; 1987) (see Table 1). Approximately 93% of the total annual volume in the USA is accounted for solely by cinnamaldehyde (No. 22). Production volumes and intake values for each substance are reported in Table 1.

Cinnamyl compounds are a fundamental part of plant biochemistry. *trans*-Cinnamic acid is ubiquitous in the plant kingdom and is required for lignin formation in plants. It is derived from the action of L-phenylalanine ammonia lyase upon L-phenylalanine, forming ammonia and cinnamic acid (Goodwin and Mercer, 1972). Cinnamic acid is also converted to *p*-hydroxy cinnamic acid (*p*-coumaric acid) by plants. *p*-Coumaric acid is one of the more important precursors of lignins as it can be converted to polyphenolic alcohols which readily polymerize to form lignin (Goodwin and Mercer, 1972). Twenty-two of the 56 flavoring substances in this group have been detected as natural components of traditional foods (Maarse et al., 1999) (See Table 1). Quantitative natural occurrence data have been reported for 3-phenylpropyl acetate (No. 3), ethyl 3-phenylpropionate (No. 9), cinnamyl alcohol (No. 12), cinnamaldehyde (No. 22), cinnamic acid (No. 23), methyl cinnamate (No. 24), and

Table 1
Identity and exposure data for cinnamyl derivatives used as flavor ingredients

Flavoring ingredient	FEMA No.	CAS No. and structure	Most recent annual volume, kg[a]	Daily per capita intake ("eaters only")		Annual volume in naturally occurring foods, kg[b]	Consumption ratio[c]
				μg/d	μg/kg bw/d		
1. 3-Phenyl-1-propanol	2885	122-97-4 	236	31	0.5	+	NA
2. 3-Phenylpropyl formate	2895	104-64-3 	6[d]	1	0.02	–	NA
3. 3-Phenylpropyl acetate	2890	122-72-5 	68	9	0.1	140	2
4. 3-Phenylpropyl propionate	2897	122-74-7 	2	0.3	0.005	+	NA
5. 3-Phenylpropyl isobutyrate	2893	103-58-2 	122	16	0.3	–	NA
6. 3-Phenylpropyl isovalerate	2899	5452-07-3 	0.5[d]	0.1	0.001	–	NA
7. 3-Phenylpropyl hexanoate	2896	6281-40-9 	3[d]	0.5	0.008	–	NA
8. Methyl 3-phenylpropionate	2741	103-25-3 	23[d]	4	0.07	–	NA
9. Ethyl 3-phenylpropionate	2455	2021-28-5 	0.5	0.06	0.001	47	94
10. 3-phenylpropionaldehyde	2887	104-53-0 	145	19	0.3	+	NA
11. 3-Phenylpropionic acid	2889	501-52-0 	4	0.5	0.008	+	NA

(continued on next page)

Table 1 (*continued*)

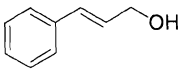
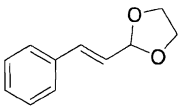
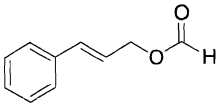
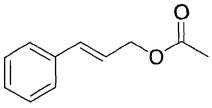
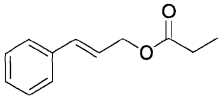
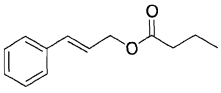
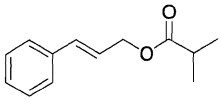
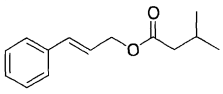
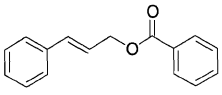
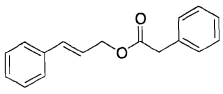
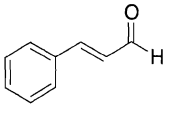
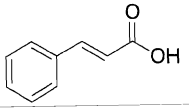
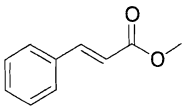
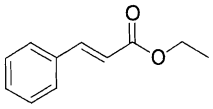
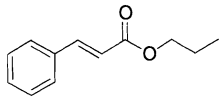
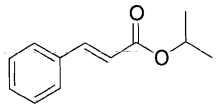
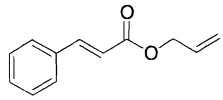
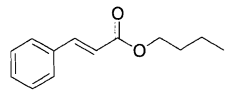
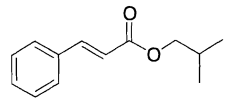
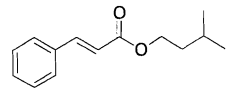
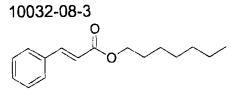
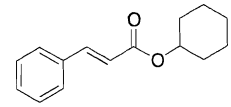
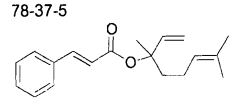
Flavoring ingredient	FEMA No.	CAS No. and structure	Most recent annual volume, kg[a]	Daily per capita intake ("eaters only")		Annual volume in naturally occurring foods, kg[b]	Consumption ratio[c]
				μg/d	μg/kg bw/d		
12. Cinnamyl alcohol	2294	104-54-1 	14651	1930	32	171	0.012
13. Cinnamaldehyde ethylene glycol acetal	2287	5660-60-6 	0.05	0.006	0.0001	–	NA
14. Cinnamyl formate	2299	104-65-4 	127	17	0.3	–	NA
15. Cinnamyl acetate	2293	103-54-8 	2250	296	5	+	NA
16. Cinnamyl propionate	2301	103-56-0 	191	25	0.4	–	NA
17. Cinnamyl butyrate	2296	103-61-7 	17	2	0.04	+	NA
18. Cinnamyl isobutyrate	2297	103-59-3 	163	22	0.4	–	NA
19. Cinnamyl isovalerate	2302	140-27-2 	64	8	0.1	–	NA
20. Cinnamyl benzoate		5320-75-2 	5[d]	1	0.01	–	NA
21. Cinnamyl phenylacetate	2300	7492-65-1 	10	1	0.02	–	NA
22. Cinnamaldehyde	2286	104-55-2 	450417	59328	989	38642	0.09
23. Cinnamic acid	2288	621-82-9 	331	44	0.7	183	1

Table 1 (*continued*)

Flavoring ingredient	FEMA No.	CAS No. and structure	Most recent annual volume, kg[a]	Daily per capita intake ("eaters only")		Annual volume in naturally occurring foods, kg[b]	Consumption ratio[c]
				µg/d	µg/kg bw/d		
24. Methyl cinnamate	2698	103-26-4 	6305	830	14	57	0.009
25. Ethyl cinnamate	2430	103-36-6 	481	63	1	292	1
26. Propyl cinnamate	2938	7778-83-8 	31	4	0.07	–	NA
27. Isopropyl cinnamate	2939	7780-06-5 	23	3	0.05	–	NA
28. Allyl cinnamate	2022	1866-31-5 	2	0.2	0.004	–	NA
29. Butyl cinnamate	2192	538-65-8 	1	0.2	0.003	–	NA
30. Isobutyl cinnamate	2193	122-67-8 	21	3	0.05	+	NA
31. Isoamyl cinnamate	2063	7779-65-9 	45	6	0.1	+	NA
32. Heptyl cinnamate	2551	10032-08-3 	390[d]	69	1	–	NA
33. Cyclohexyl cinnamate	2352	7779-17-1 	0.3	0.04	0.001	–	NA
34. Linalyl cinnamate	2641	78-37-5 	19	2	0.04	–	NA

(*continued on next page*)

Table 1 (*continued*)

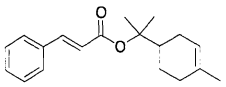
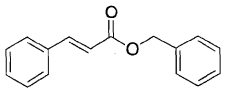
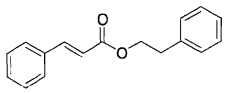
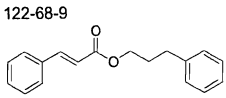
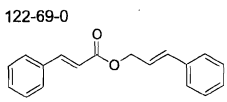
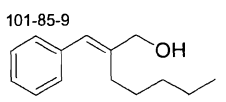
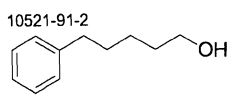
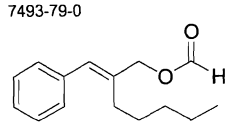
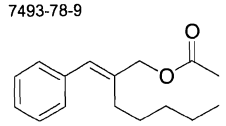
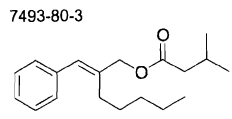
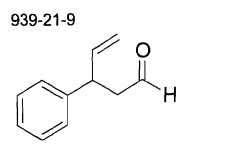
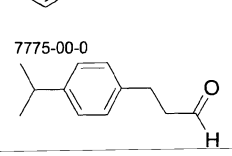
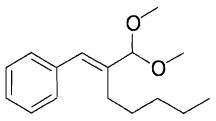
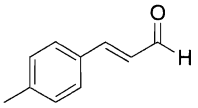
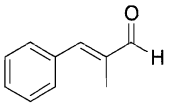
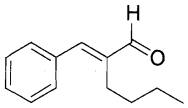
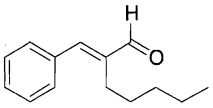
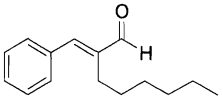
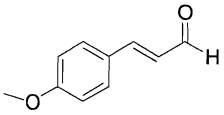
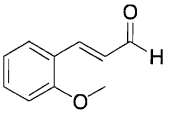
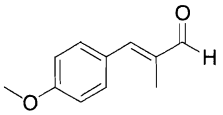
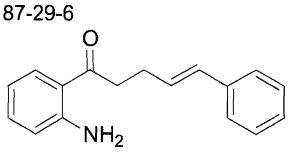
Flavoring ingredient	FEMA No.	CAS No. and structure	Most recent annual volume, kg[a]	Daily per capita intake ("eaters only")		Annual volume in naturally occurring foods, kg[b]	Consumption ratio[c]
				μg/d	μg/kg bw/d		
35. Terpinyl cinnamate	3051	10024-56-3 	4[d]	0.7	0.01	–	NA
36. Benzyl cinnamate	2142	103-41-3 	526	69	1	+	NA
37. Phenethyl cinnamate	2863	103-53-7 	381	50	0.8	–	NA
38. 3-Phenylpropyl cinnamate	2894	122-68-9 	281	37	0.6	–	NA
39. Cinnamyl cinnamate	2298	122-69-0 	277	36	0.6	+	NA
40. -Amylcinnamyl alcohol	2065	101-85-9 	9	1	0.02	–	NA
41. 5-Phenylpentanol	3618	10521-91-2 	1[d]	0.2	0.004	–	NA
42. -Amylcinnamyl formate	2066	7493-79-0 	4[d]	0.7	0.01	–	
43. -Amylcinnamyl acetate	2064	7493-78-9 	1991	263	4	–	NA
44. -Amylcinnamyl isovalerate	2067	7493-80-3 	4[d]	0.7	0.01	–	NA
45. 3-Phenyl-4-pentenal	3318	939-21-9 	16[d]	2	0.04	–	NA
46. 3-(*p*-Isopropylphenyl) propionaldehyde	2957	7775-00-0 	1	0.2	4	–	NA

Table 1 (*continued*)

Flavoring ingredient	FEMA No.	CAS No. and structure	Most recent annual volume, kg[a]	Daily per capita intake ("eaters only")		Annual volume in naturally occurring foods, kg[b]	Consumption ratio[c]
				μg/d	μg/kg bw/d		
47. α-Amylcinnamaldehyde dimethyl acetal	2062	91-87-2 	0.05	0.006	0.0001	–	NA
48. *p*-Methylcinnamaldehyde	3640	1504-75-2 	7[d]	1	0.02	–	NA
49. α-Methylcinnamaldehyde	2697	101-39-3 	2926	385	6	+	NA
50. α-Butylcinnamaldehyde	2191	7492-44-6 	0.5[d]	0.08	0.001	+	NA
51. α-Amylcinnamaldehyde	2061	122-40-7 	172	23	0.4	+	NA
52. α-Hexylcinnamaldehyde	2569	101-86-0 	82	11	0.2	+	NA
53. *p*-Methoxycinnamaldehyde	3567	1963-36-6 	1510[d]	265	4	+	NA
54. *o*-Methoxycinnamaldehyde	3181	1504-74-1 	540	71	1	+	NA
55. *p*-Methoxy methylcinnam aldehyde	3182	65405-67-6 	0.4[d]	0.06	0.001	–	NA
56. Cinnamyl anthranilate	2295	87-29-6 	163[d]	29	0.5	–	NA

[a] Intake (μg/person/day) calculated as follows: [(annual volume, kg)×(1×10^9 μg/kg)]/[population×survey correction factor×365 days], where population (10%, "eaters only") = 26×10^6 for the U.S.A.; where correction factor = 0.6 for NAS surveys and 0.8 for the Lucas et al. U.S.A. survey representing the assumption that only 60% and 80% of the annual flavor volume, respectively, was reported in the poundage surveys (Lucas et al., 1999; NAS, 1970,1982, 1987). Intake (μg/kg bw/d) calculated as follows: [(μg/person per day)/body weight], where body weight = 60 kg. Slight variations may occur from rounding.

[b] Quantitative data for the United States reported by Stofberg and Grundschober, 1987

[c] The consumption ratio is calculated as follows: (annual consumption via food, kg)/(most recent reported volume as a flavoring substance, kg); NA = data not available.

[d] Annual volume reported in previous U.S.A. surveys (NAS, 1970, 1982, 1987).

ethyl cinnamate (No. 25), and indicate that intake of these substances are predominately from food (i.e., consumption ratio > 1) (Stofberg and Kirschman, 1985; Stofberg and Grunschober, 1987). Cinnamaldehyde has been detected in the oils derived from natural sources such as cinnamon, cinnamomum, and cassia leaf at levels up to 750,000 ppm (Maarse et al., 1999).

3. Hydrolysis, absorption, distribution, excretion and metabolism

3.1. Hydrolysis

Esters and acetals formed from the parent alcohol, aldehyde, or carboxylic acid are hydrolyzed prior to or during or after absorption. Once formed, cinnamyl alcohol, cinnamaldehyde and cinnamic acid have all been shown to be rapidly absorbed from the gut, metabolized and excreted primarily in the urine and, to a minor extent, in the feces. Results of numerous studies indicate that cinnamyl derivatives are absorbed, metabolized and excreted as polar metabolites within 24 h.

In general, esters containing an aromatic ring system are hydrolyzed in vivo by classes of enzymes recognized as carboxylesterases or esterases (Heymann, 1980), the most important of which are the A-esterases. In mammals, A-esterases occur in most tissues throughout the body (Anders, 1989; Heymann, 1980) but predominate in the hepatocytes (Heymann, 1980). Acetals are rapidly hydrolyzed in acidic medium (Morgareidge, 1962).

Esters of cinnamic acid and structurally related aromatic esters have been shown to hydrolyze rapidly to the component acid and alcohol. Oral administration of a single dose of 50 mg methyl cinnamate (No. 24)/kg bw resulted in the urinary excretion, after 24 h, of hippuric acid (66%) and benzoylglurcuronide (5%). This distribution of metabolites, nearly identical to that for cinnamic acid, indicates that rapid hydrolysis of the ester in vivo precedes metabolism of the acid (Fahelbum and James, 1977). Ethyl cinnamate (No. 25) administered subcutaneously to a cat also produced only cinnamic acid metabolites in the urine (Dakin, 1909). Incubation of benzyl cinnamate (No. 36) or benzyl acetate with simulated intestinal fluid (pH 7.5; pancreatin) at 37 °C for 2 h resulted in 80 and 50% hydrolysis, respectively (Grundschober, 1977). in vitro incubation of the structurally related aromatic acetal, 2-phenylpropanal dimethyl acetal (1 mM) with simulated gastric juice at 37 °C resulted in 97% hydrolysis in 1 h. Under the same experimental conditions, benzaldehyde propylene glycol acetal (1 mM) was 97% hydrolyzed in 5 h when compared with a blank incubation of the acetal and 0.1 N HCl under reflux (Morgareidge, 1962).

3.2. Absorption, distribution and excretion

In male Fischer 344 (F344) rats (4/group), 83%, 77%, or 79% of an oral dose of 2.5 mmol/kg bw of [3-^{14}C-d_5]-cinnamyl alcohol (335 mg/kg bw), [3-^{14}C-d_5]-cinnamaldehyde (330 mg/kg bw), or [3-^{14}C-d_5]-cinnamic acid (370 mg/kg bw), respectively, is excreted primarily in the urine within 24 h. Excretion in the feces accounted for only minor amounts of the administered alcohol (6.1%), aldehyde (15.8%), or acid (0.9%). Greater than 90% of the administered dose of any of the three substances is recovered in the urine and feces within 72 h. Administration of the same doses of the parent alcohol, aldehyde, or acid to groups of CD-1 mice by intraperitoneal injection results in a similar pattern of excretion in the urine and feces at 24 (75, 80 and 93%, respectively) and 72 h (>93%) (Nutley, 1990).

In a study (Sapienza et al., 1993) of tissue distribution and excretion of cinnamaldehyde, male F344 rats (8/group) were pretreated with single daily oral doses of 5, 50, or 500 mg/kg bw of cinnamaldehyde by gavage for 7 days. Twenty-four hours later, animals in each group received a single oral dose of [3-^{14}C]cinnamaldehyde equivalent to the pretreatment level. Groups of rats (8/group) receiving no pretreatment were also given single oral doses of 5, 50 or 500 mg/kg bw. Radioactivity is distributed primarily to the gastrointestinal tract, kidneys, and liver, after single- or multiple-dose oral administration. After 24 h, >80% of the radioactivity is recovered in the urine and <7% in the feces from all groups of rats, regardless of dose level. At all dose levels, a small amount of the dose is distributed to the fat. At 50 and 500 mg/kg bw, radioactivity could be measured in animals terminated 3 days after dosing. Except for the high dose pretreatment group, the major urinary metabolite is hippuric acid, accompanied by small amounts of cinnamic and benzoic acid. In the high dose pretreatment group, benzoic acid is the major metabolite, suggesting that saturation of the glycine conjugation pathway occurs at repeated high dose levels of cinnamaldehyde.

In a study of the effect of dose, species, and sex on the disposition of [3-^{14}C]cinnamaldehyde (Peters and Caldwell, 1994). A 2.0 or 250 mg/kg bw dose of cinnamaldehyde was administered to groups of male and female F344 rats (4/group) or CD1 mice (6/group) by intraperitoneal injection. Regardless of the dose level, species, or sex, greater than 85% of the radiolabel is recovered in the urine and feces within 24 h. Greater than 90% is recovered after 72 h. When 250 mg/kg bw of [3-^{14}C]cinnamaldehyde is administered orally to F344 rats, 98% is recovered from the urine (91%) and feces (7%) within 24 h (Peters and Caldwell, 1994). The effect of dose on the disposition of [3-^{14}C-d_5]-cinnamic acid in F344 rats and CD1 mice has also been studied. Five

dose levels of cinnamic acid in the range from 0.0005 mmol/kg bw to 2.5 mmol/kg bw were given orally to groups of F344 rats (4/group) or by intraperitoneal injection to groups of CD1 mice (4/group). After twenty-four hours, 73–88% of the radioactivity is recovered in the urine of rats and 78–93% in the urine of mice. After 72 h, 85–100% of the radioactivity is recovered from rats mainly in the urine (Caldwell and Nutley, 1986). In mice, the recovery is 89–100% within 72 h. Only trace amounts of radioactivity are present in the carcasses, indicating that cinnamic acid is readily and quantitatively excreted at all dose levels (Nutley et al., 1994). In summary, it appears that the parent alcohol, aldehyde, and acid undergo rapid absorption, metabolism, and excretion independent of dose (up to 250 mg/kg bw), species, sex, and mode of administration.

Cinnamic acid is rapidly absorbed and cleared from the blood in humans. Eleven adult human subjects each received a single intravenous dose of cinnamic acid, equivalent to 5 mg/kg bw. Analysis of the blood reveals cinnamic acid at 100% of the total dose within 2.5 min, declining to 0% after 20 min (Quarto di Palo and Bertolini, 1961).

A 1.5 mmol/kg bw oral dose (243 mg/kg bw) of methyl cinnamate is rapidly, and almost completely (95%), absorbed from the rat gut. Methyl cinnamate was partially hydrolyzed to cinnamic acid in the stomach (9%) and gut (40%). The rate of absorption from the gut was similar for cinnamic acid and methyl cinnamate. No ester was detected in the peripheral blood of rabbits or rats dosed with methyl cinnamate. Only traces were detected in portal and heart blood samples taken from dosed rats, indicating that almost complete hydrolysis of methyl cinnamate occurs during intestinal absorption (Fahelbum and James, 1977).

More sterically hindered esters are also readily hydrolyzed in vivo. Following administration of a single 250 mg/kg i.p. dose of [3-^{14}C]cinnamyl anthranilate to both rats and mice, greater than 91% of the radioactivity is eliminated within 24 h for both species (Keyhanfar and Caldwell, 1996).

3.3. Metabolism

3.3.1. Cinnamyl alcohol and cinnamaldehyde derivatives

The aromatic primary alcohols and aldehydes used as flavoring substances or formed by the hydrolysis of esters and acetals are readily oxidized to the corresponding cinnamic acid derivative (see Fig. 1). Human NAD^+ dependent alcohol dehydrogenase (ADH) catalyzes oxidation of primary alcohols to aldehydes (Pietruszko et al., 1973). Isoenzyme mixtures of NAD^+ dependent aldehyde dehydrogenase (ALD) (Weiner, 1980) catalyze oxidation of aldehydes to carboxylic acids. Aromatic alcohols and aldehydes have been reported to be excellent substrates for ADH (Sund and Theorell, 1963) and ALD (Feldman and Wiener, 1972), respectively. The urinary metabolites of cinnamyl alcohol and cinnamaldehyde are mainly those derived from metabolism of cinnamic acid (see Fig. 1).

Fifty-two percent of a 335 mg/kg bw oral dose of cinnamyl alcohol given to rats (4) is recovered in 0–24 h in the urine as the glycine conjugate of benzoic acid (hippuric acid). Ten minor metabolites cumulatively account for about 10% of the dose (Nutley, 1990). Administered to mice by intraperitoneal injection, cinnamyl alcohol undergoes functional group oxidation followed by β-oxidation and cleavage to yield benzoic acid that is subsequently excreted in the urine as the glycine conjugate, hippuric acid (Nutley, 1990).

In a study of the effect of species, route and dose on the metabolism of cinnamaldehyde, doses of 2 and 250 mg *trans*-[3-^{14}C]cinnamaldehyde/kg bw were given by i.p. injection to male and female F344 rats and CD1 mice (Peters and Caldwell, 1994). Doses of 250 mg/kg bw were administered via oral gavage to male rats and mice only. In both species and via both routes of administration, the major urinary metabolites form from oxidation of cinnamaldehyde to cinnamic acid,

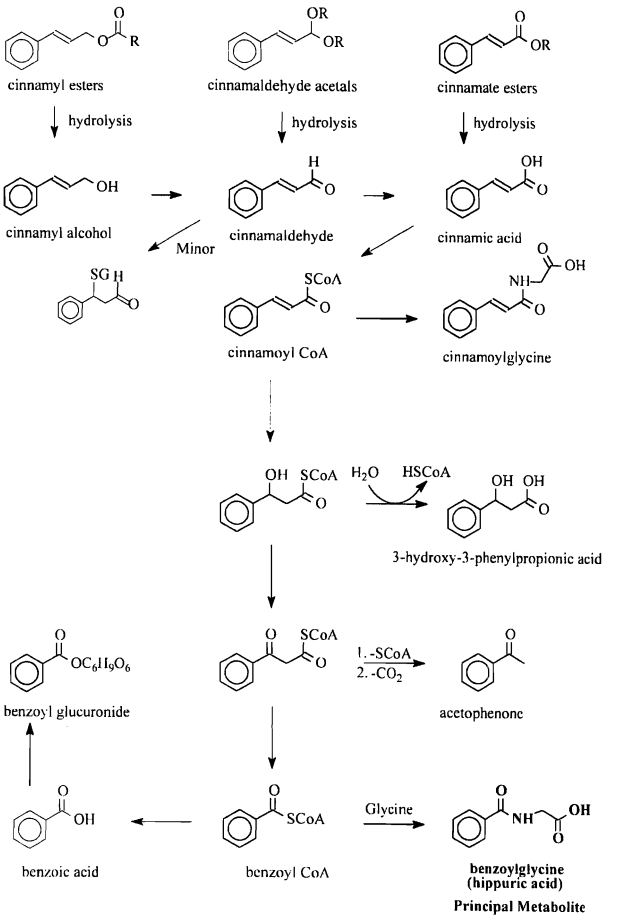


Fig. 1. Metabolism of cinnamyl derivatives.

which is subsequently oxidized in the β-oxidation pathway. The major urinary metabolite is hippuric acid (71–75% in mice and 73–87% in rats), accompanied by small amounts of 3-hydroxy-3-phenylpropionic acid (0.4–4%), benzoic acid (0.4–3%), and benzoyl glucuronide (0.8–7.0%). The glycine conjugate of cinnamic acid is formed to a considerable extent only in the mouse (4–13%). To a small extent, glutathione conjugation of cinnamaldehyde competes with the oxidation pathway. Approximately 6–9% of either dose is excreted in 24 h as glutathione conjugates of cinnamaldehyde. The authors concluded that the excretion pattern and metabolic profile of cinnamaldehyde in rats and mice are not systematically affected by sex, dose size, or route of administration (Peters and Caldwell, 1994).

The toxicokinetic profile of cinnamaldehyde has been investigated in male F344 rats (Yuan et al., 1992). Plasma levels of cinnamaldehyde (<0.1 μg/ml) and cinnamic acid (<1 μg/ml) are not measurable when rats (3–6/group) are administered a single oral dose of 50 mg/kg bw of cinnamaldehyde by gavage in corn oil. At dose levels of 250 and 500 mg/kg bw, plasma levels of cinnamaldehyde and cinnamic acid are approximately 1 and greater than 10 μg/ml, respectively. The bioavailability of cinnamaldehyde was calculated to be less than 20% at both dose levels. A dose-dependent increase in hippuric acid, the major urinary metabolite, occurs 6 h after gavage and continues over the next 18 h. Only small amounts of cinnamic acid are excreted in the urine either free or as the glucuronic acid conjugate. Urinary hippuric acid recovered over 50 h accounted for 72–81% over the dose range from 50 to 500 mg/kg bw.

Data from different studies suggest that conjugation of cinnamaldehyde with glutathione is dose-dependent. Approximately 15% of an oral dose of 250 mg cinnamaldehyde/kg bw administered to rats by gavage is excreted in the urine as two mercapturic acid derivatives, *N*-acetyl-*S*-(1-phenyl-3-hydroxypropyl)cysteine and *N*-acetyl-*S*-(1-phenyl-2-carboxyethyl)cysteine, in a ratio of four to one. At a dose of 2 mg/kg bw, rats excrete only 6% of cinnamaldehyde as glutathione conjugates. Approximately 9% of an oral dose of 125 mg cinnamyl alcohol/kg bw is excreted in the urine as *N*-acetyl-*S*-(1-phenyl-3-hydroxypropyl)cysteine (Delbressine et al., 1981).

3.3.2. Cinnamic acid

Intracellular cinnamic acid is converted to acylCoA esters (Nutley et al., 1994). CinnamoylCoA either conjugates with glycine, a reaction catalyzed by *N*-acyl transferase, or undergoes β-oxidation eventually leading to the formation of benzoylCoA. The reactions that form benzoic acid from cinnamic acid are reversible but the equilibrium favors formation of the benzoic acid CoA ester (Nutley et al., 1994). The equilibrium in the reaction of cinnamylCoA to yield benzoylCoA and acetylCoA represents a high capacity pathway for the metabolism of cinnamic acid. BenzoylCoA is in turn conjugated with glycine, yielding hippuric acid, or the CoA thioester is hydrolyzed to yield free benzoic acid which is then excreted (Nutley et al., 1994). CoA thioesters of carboxylic acids are obligatory intermediates in amino acid conjugation reactions (Hutt and Caldwell, 1990). The reactions in this sequence are of historical significance in biochemistry, since it was studies on cinnamic acid and fatty acids that revealed the β-oxidation pathway of fatty acid catabolism (Nutley et al., 1994). Regardless of dose or species, the β-oxidation pathway is the predominant pathway of metabolic detoxication of cinnamic acid in animals.

In an extensive study of the effect of dose on the conversion of cinnamic acid to benzoic acid, six dose levels in the range of 0.0005–2.5 mmol/kg (ca. 0.08–400 mg/kg bw) [^{14}C]- or [^{14}C/$^{2}H_5$]-cinnamic acid were administered orally to male F344 rats or by intraperitoneal injection to male CD-1 mice. In both species, 84–101% was recovered within 72 h with the majority (73–93%) recovered from the urine within 24 h. The metabolites identified at all dose levels included hippuric acid, benzoyl glucuronide, 3-hydroxy-3-phenyl-propionic acid, benzoic acid, and unchanged cinnamic acid. The major metabolite was hippuric acid at all dose levels (44–77%). At the highest dose given, (2.5 mmol/kg bw) the percentage of hippuric acid decreased while the percentages of benzoyl glucuronide and benzoic acid increased. Increased formation of benzoyl glucuronide (0.5–5%) and free benzoic acid (0.4–2%) at dose levels above 0.5 mmol/kg bw provide evidence that saturation of the glycine conjugation pathway occurs at these higher dose levels. The fact that 3-hydroxy-3-phenyl-propionic acid was only slightly changed over the dose range (0.2–0.9%) supports the conclusion that the β-oxidation pathway is not capacity-limited up to 2.5 mmol/kg bw cinnamic acid in the male rat (Nutley et al., 1994). The increasing role of glucuronic acid conjugation relative to glycine conjugation as dose size increases is a general trend observed in the metabolism of carboxylic acids (Caldwell et al., 1980).

In mice, glycine conjugation of cinnamic acid competes with the β-oxidation pathway, but only at low dose levels. However, as dose levels increase from 0.0005 to 2.5 mmol/kg bw, urinary hippuric acid increases from 44 to 67%, while cinnamoylglycine levels decrease from 29 to 2.4%. These results suggest that glycine *N*-acetyl transferase has high affinity but low capacity for cinnamic acid compared with benzoic acid. At the highest dose (2.5 mmol/kg bw), an increase in excreted free benzoic acid (0.8–8.6%) suggests that glycine conjugation of benzoylCoA is also capacity limited in mice. At all dose levels, the mouse excretes a small proportion of benzoyl glucuronide, which suggests that this conjugation reaction is of minimal importance in this species (Nutley et al., 1994).

Like cinnamic acid, the saturated analog, 3-phenyl-1-propanol, participates in the same metabolic pathway. When ring deuterated 3-phenylpropionic acid is administered orally to a human as a single dose (57 mg), deuterobenzoic acid corresponding to 110% of the dose is isolated from the alkaline hydrolyzed urine collected within 100 min of dosing (Pollitt, 1974).

Eleven adult volunteers received single intravenous doses of cinnamic acid, equivalent to 5 mg/kg bw. Analysis of the blood plasma revealed cinnamic acid at 100% of the total dose within 2.5 min declining to 0% after 20 min. Ninety minutes after dosing, urinalysis revealed hippuric acid, cinnamoylglucuronide, and benzoylglucuronide present in a ratio of 74:24.5:1.5 (Quarto di Palo and Bertolini, 1961). These data demonstrate that cinnamic acid is rapidly oxidized to benzoic acid metabolites, and excreted in the urine of humans.

3.3.3. Ring and chain substituted cinnamyl derivatives

The position and size of ring substituents play a role in the metabolism of cinnamyl derivatives. Cinnamyl derivatives containing α-methyl substituents (e.g. α-methylcinnamaldehyde, No. 49) participate in the β-oxidation and cleavage to yield mainly the corresponding hippuric acid derivative. A benzoic acid metabolite is isolated from the urine of dogs given either α-methylcinnamic acid or α-methylphenylpropionic acid (Kay and Raper, 1924). Substituents greater than C_1 located at the *alpha-* or *beta*-position, to some extent, inhibit β-oxidation (Kassahun et al., 1991; Deuel, 1957). In these cases, there may be direct conjugation of the carboxylic acid with glucuronic acid followed by excretion. While α-methylcinnamic acid undergoes oxidation to benzoic acid, α-ethyl- and α-propylcinnamic acids are excreted unchanged (Carter, 1941). α-Ethylcinnamic alcohol and α-ethylcinnamaldehyde administered orally to rabbits result in the urinary excretion of α-ethylcinnamic acid, in addition to small amounts of benzoic acid (Fischer and Bielig, 1940). These observations suggest that α-methylcinnamaldehyde undergoes oxidation to benzoic acid while higher homologues primarily are excreted unchanged or as the conjugated form of the cinnamic acid derivative.

Ortho (*o*) ring substituents (e.g. *o*-methoxycinnamaldehyde, No. 54) selectively inhibit oxidation of CoA esters of β-hydroxyacids within the β-oxidation pathway. In these cases, the hydroxyacid derivative is excreted unchanged as a glycine conjugate. The β-hydroxy derivative is a principal metabolite of *o*-methoxycinnamaldehyde (Samuelsen et al., 1986).

The glycine conjugates of *o*-methoxycinnamic and *o*-methoxyphenylpropionic acids are principal urinary metabolites of *o*-methoxycinnamaldehyde in rats. Relatively large amounts of the β-hydroxylated phenylpropionic acid derivatives are also detected, but only traces of benzoic and hippuric acid derivatives (i.e., products of further β-oxidation) are excreted. The detection of relatively large amounts of a β-hydroxylated derivative suggests that this metabolite is not readily oxidized, possibly due to steric hinderance of the ortho substituent (Solheim and Scheline, 1973).

In contrast, *para* (*p*-) ring substituents (e.g. 3-(*p*-isopropylphenyl)propionaldehyde, No. 46, and *p*-methylcinnamaldehyde, No. 48) may not significantly impact metabolism via β-oxidation. In male albino rats, *p*-methoxycinnamic acid has been shown to metabolize mainly to *p*-methoxybenzoic acid and its corresponding glycine conjugate (Solheim and Scheline, 1973). Similar results are reported with 3,4-dimethoxycinnamic acid, which is *meta* and *para* substituted (Solheim and Scheline, 1976). The structurally related substance *p*-tolualdehyde metabolizes to *p*-methylbenzoic acid without any apparent oxidation of the methyl group (Williams, 1959). Based on these observations, it may be concluded that the presence of side-chain alkyl substituents greater than C_1 and *ortho*-ring substituents inhibit the β-oxidation pathway. In these cases, the parent acid (cinnamic acid derivative) or an intermediary β-oxidation metabolite (e.g., β-hydroxy-3-phenylpropanoic acid derivative) is efficiently excreted as the glycine or glucuronic acid conjugate.

3.3.4. Cinnamyl anthranilate

Results of a 2-year bioassay with cinnamyl anthranilate stimulated numerous metabolic studies that are described below (NCI, 1980) (see Carcinogenicity Studies in Section 4.3.1). The results of these studies demonstrate the presence of the intact ester in the liver of mice given high dose levels of cinnamyl anthranilate.

At low dose levels in rodents, cinnamyl anthranilate is hydrolyzed to cinnamyl alcohol and anthranilic acid. However, at high dose levels (> 500 mg/kg bw/day) in mice, ester hydrolysis is incomplete, resulting in the in vivo presence of the intact ester (Keyhanfar and Caldwell, 1996). Saturation of the hydrolysis pathway has only been observed at high dose levels in mice (Keyhanfar and Caldwell, 1996; Caldwell and Viswalingam, 1989). A single dose of 250 mg cinnamyl anthranilate/kg administered by i.p. injection to both rats and mice. In the rat, 95 and 4% of the dose are recovered in the 24-h urine as hippuric acid and benzoic acid, respectively. No unchanged cinnamyl anthranilate is recovered. In mice, 77% of the dose is recovered as hippuric acid, 19% as benzoic acid and 2% as unchanged cinnamyl anthranilate (Keyhanfar and Caldwell, 1996). In a multiple dose study, male mice received intraperitoneal injections of 5, 10, 20, 50, 100 or 250 mg cinnamyl anthranilate/kg bw. Over all dose levels, the relative amounts of hippuric acid and benzoic acid present in the urine as metabolites is essentially unchanged. However, at dose levels greater than or equal to 10 mg/kg bw, unhydrolyzed cinnamyl anthranilate is detected in the urine. The relative amount of cinnamyl anthranilate increases with increasing dose levels of greater than 10 mg/kg bw (Keyhanfar and Caldwell, 1996).

In a dietary study, concentrations of 0, 100, 1000, 5000, 15,000 or 30,000 ppm, which corresponds to estimated daily intakes of 15, 150, 750, 2250 or 4500 mg/kg bw, respectively (FDA, 1993) of cinnamyl anthranilate were administered in feed to mice for 21 days. The two highest concentrations correspond to the same dose levels used in the NTP 2-year bioassay (NCI, 1980). In both the male and female mice, unchanged cinnamyl anthranilate is detected in the urine at dietary levels of greater than or equal to 5000 ppm (ca. 750 mg/kg bw/day) (Keyhanfar and Caldwell, 1996). There is no evidence of unhydrolyzed ester in the urine of humans administered a single i.p. injection of 250 mg cinnamyl anthranilate/kg bw (Keyhanfar and Caldwell, 1996).

Large doses of cinnamyl anthranilate administered to mice, resulting in saturation of the hydrolysis pathway, have also been associated with hepatic enzyme induction (Caldwell, 1992). The enzymic basis for the species differences in metabolism has been studied in hepatic microsomes of rats, mice, and humans. The results show that while cinnamyl anthranilate is hydrolyzed relatively slowly by hepatic microsomes of rat and human, the ester is essentially unreactive in mouse liver microsomes, with less than 10% hydrolysis occurring over a 24-h period (Caldwell, 1992). In mice, cinnamyl anthranilate was shown to cause a pattern of enzyme induction that is characteristic of peroxisome proliferation, including increases in cytochrome P450, lauric acid *omega*-hydroxylation and peroxisomal fatty-acid oxidation (Viswalingam et al., 1988). Peroxisome proliferation would not be expected in humans given the absence of the intact ester in human urine (Keyhanfar and Caldwell, 1996).

Although the lack of hydrolysis exhibited by cinnamyl anthranilate is not observed for other cinnamyl esters (Fahelbum and James, 1977; Grundschober, 1977; Dakin, 1909; Morgareidge, 1962), it resembles the hydrolytic behavior of other anthranilate esters. Hydrolysis studies performed in a number of in vitro systems including simulated intestinal fluid, simulated stomach juice, and freshly prepared rat liver homogenate (Gangolli and Shilling, 1968; Longland et al., 1977), in homogenates of pig liver and jejenum (Grundschober, 1977), and in vivo in the blood of guinea pigs (Pelling et al., 1980) indicated that methyl anthranilate and methyl *N*-methylanthranilate are resistant to ester hydrolysis. It is anticipated that the anthranilate moiety inhibits ester hydrolysis leading, in the case of cinnamyl anthranilate, to elevated in vivo concentrations of ester.

4. Toxicological studies

4.1. Acute toxicity

Oral LD_{50} values have been reported for 39 of the 55 substances in this group. In rats, LD_{50} values are in the range of 1520 to greater than 5000 mg/kg bw, demonstrating that the oral acute toxicity of these cinnamyl derivatives is extremely low (Denine and Palanker, 1973; Jenner et al., 1964; Keating, 1972; Levenstein, 1972, 1974, 1975, 1976; Moreno, 1971, 1972, 1973, 1974, 1975, 1976, 1977, 1981, 1982; Opdyke, 1974; Russell, 1973; Schafer et al., 1983; Weir and Wong, 1971; Wohl, 1974; Zaitsev and Rakhmanina, 1974). LD_{50} values are in the range of 913 to greater than 5000 mg/kg bw in mice (Colaianni, 1967; Draize et al., 1948; Harada and Ozaki, 1972; Levenstein, 1975; Schafer and Bowles, 1985; Zaitsev and Rakhmanina, 1974), and 3130 to greater than 5000 mg/kg bw in guinea pigs (Draize et al., 1948; Zaitsev and Rakhmanina, 1974) (see Table 2).

4.2. Short-term toxicity

Studies performed for cinnamyl alcohol, the corresponding aldehyde, two cinnamate esters, two α-alkyl-substituted cinnamaldehyde derivatives, two alkoxy-substituted cinnamaldehyde derivatives, and a mixture of five cinnamyl derivatives show no evidence of any toxicity at dose levels exceeding the estimated daily per capita intake of the respective cinnamyl derivative by at least three orders of magnitude (see discussion below and Table 2). Data on the structurally related ester cinnamyl anthranilate is also included, even though it is no longer used as a flavoring substance (voluntarily discontinued in 1986).

Daily doses of 53.5 mg/kg bw of cinnamyl alcohol (No. 12), 68 mg/kg bw of cinnamaldehyde (No. 22), or 80 mg/kg bw of ethyl cinnamate (No. 25), each equivalent to 2% of the LD_{50} for the respective substance, were each administered in a sunflower oil solution (0.2 ml/100 g bw) to white rats (12 males/group, strain not identified) by oral intubation once daily for 4 months. Liver function tests were performed on animals at days 40 and 140. Increased (26%) blood serum fructose diphosphate aldolase activity was observed in the cinnamyl alcohol and ethyl cinnamate group at day 140. Activity of serum cholinesterase and alanine aminotransferase, as well as levels of blood serum SH groups, exhibited no change compared to controls. The authors concluded that none of the three cinnamyl derivatives caused any significant pathological change in the liver of rats (Zaitsev and Rakhmanina, 1974).

Groups (10/sex/group) of male and female Osborne-Mendel rats were maintained on a diet containing either 0 (control), 1000, 2500 or 10,000 ppm cinnamaldehyde (No. 22) for a total of 16 weeks. These dietary concentrations correspond to average daily intakes of 50, 125, or 500 mg/kg bw/day, respectively (FDA, 1993). Measurement of body weight and food intake recorded weekly showed no significant difference between test and control animals at any dose level. At termination, hematological examinations revealed normal values. At necropsy, no differences were reported between major

Table 2
Acute and short-term toxicity studies for cinnamyl derivatives used as flavor ingredients

	Flavoring ingredient	Oral acute studies		Short-term studies			
		Oral LD_{50} mg/kg bw (species)	Reference	Species, sex[a]	Time (days)/route	NOEL (mg/kg bw)	Reference
1	3-Phenyl-1-propanol	2300 (Rat)	Moreno (1976)				
1	3-Phenyl-1-propanol	2250 (Rat)	Weir and Wong (1971)				
2	3-Phenylpropyl formate	4000 (Rat)	Levenstein (1975)				
3	3-Phenylpropyl acetate	4700 (Rat)	Moreno (1973)				
4	3-Phenylpropyl propionate	> 5000 (Rat)	Moreno (1973)				
5	3-Phenylpropyl isobutyrate	> 5000 (Rat)	Levenstein (1975)				
8	Methyl 3-phenylpropionate	4200 (rat)	Moreno (1981)				
10	3-Phenylpropionaldehyde	5000 (Rat)	Russell (1973)				
11	3-Phenylpropionaldehyde	913 (mouse)	Schafer and Bowles (1985)				
12	Cinnamyl alcohol	2675 (Rat)	Zaitsev and Rakhmanina (1974)	Rat, M	120/oral	53.5[b]	Zaitsev and Rakhmanina (1974)
12	Cinnamyl alcohol	2000 (Rat)	Moreno (1973)				
12	Cinnamyl alcohol	2000 (Rat)	Opdyke (1974)				
14	Cinnamyl formate	2900 (Rat)	Denine and Palanker (1973)				
15	Cinnamyl acetate	3300 (Rat)	Moreno (1972)				
16	Cinnamyl propionate	3400 (Rat)	Moreno (1973)				
17	Cinnamyl butyrate	5000 (Rat)	Levenstein (1976)				
18	Cinnamyl isobutyrate	5000 (Rat)	Moreno (1977)				
19	Cinnamyl isovalerate	> 5000 (Rat)	Moreno (1973)				
20	Cinnamyl benzoate	4000 (Rat)	Moreno (1975)				
22	Cinnamaldehyde	3400 (Rat)	Schafer et al. (1983)	Rat, M	120/oral	68	Zaitsev and Rakhmanina (1974)
22	Cinnamaldehyde	3350 (Rat)	Zaitsev and Rakhmanina (1974)	Rat; M/F	84/oral	227	Trubeck Laboratories (1958a)
22	Cinnamaldehyde	2225 (Mouse)	Harada and Ozaki (1972)	Rat; M/F	84/oral	103[c]	Trubek Laboratories (1958b)
22	Cinnamaldehyde			Rat; M/F	91/oral	625	NTP (1995)
22	Cinnamaldehyde			Rat; M/F	112/oral	125	Hagen et al. (1967)
22	*trans*-Cinnamaldehyde			Mouse; M/F	730	540	NTP (2002)
22	*trans*-Cinnamaldehyde			Rat; M/F	730	200	NTP (2002)
23	Cinnamic acid	4454[d] (Rat)	Levenstein (1976)				
23	Cinnamic acid	> 5000 (Rat, Mouse, Guinea pig)	Zaitsev and Rakhmanina (1974)				
23	Cinnamic acid	3400 (Rat)	Zaitsev and Rakhmanina (1974)				
24	Methyl cinnamate	2610 (Rat)	Weir and Wong (1971)	Rat; M/F	84/oral	3[c]	Trubek Laboratories (1958b)
25	Ethyl cinnamate	4000 (Rat)	Zaitsev and Rakhmanina (1974)	Rat; M	120/oral	80	Zaitsev and Rakhmanina (1974)
25	Ethyl cinnamate			Rat; M/F	84/oral	3[c]	Trubeck Laboratories (1958b)
26	Propyl cinnamate	7305[e] (Mouse)	Draize et al. (1948)				
26	Propyl cinnamate	3130[f] (Guinea pig)	Draize et al. (1948)				
27	Isopropyl cinnamate	> 5000 (Rat)	Moreno (1982)				
28	Allyl cinnamate	1520 (Rat)	Jenner et al. (1964)				
29	Butyl cinnamate	> 5000 (Rat)	Moreno (1977)				
30	Isobutyl cinnamate	> 5000 (Rat)	Levenstein (1975)				
31	Isoamyl cinnamate	> 5000 (Rat)	Moreno (1974)				
34	Linalyl cinnamate	> 39,040 (Mouse)	Colaianni (1967)	Rat, M/F	119/oral	500	Hagen et al. (1967)
36	Benzyl cinnamate	3280 (Rat)	Levenstein (1972)	Rat; M/F	133/oral	500	Hagen et al. (1967)
37	Phenethyl cinnamate	5000 (Rat)	Moreno (1975)				
37	Phenethyl cinnamate	> 5000 (Mouse)	Levenstein (1975)				
38	3-Phenylpropyl cinnamate	> 5000 (Rat)	Keating (1972)				
39	Cinnamyl cinnamate	4200 (Rat)	Wohl (1974)	Rat; M/F	84/oral	3[c]	Trubeck Laboratories (1958b)
40	αAmylcinnamyl alcohol	4000 (Rat)	Denine and Palanker (1973)				
43	α-Amylcinnamyl acetate	> 5000 (Rat)	Moreno (1974)				
47	α-Amylcinnamaldehyde dimethyl acetal	> 5000 (Rat)	Moreno (1974)				
49	α-Methylcinnamaldehyde	2000 (Rat)	Russell (1973)	Rat; M	90/oral	221	Trubeck Laboratories (1958c)
49	α-Methylcinnamaldehyde			Rat; M/F	84/oral	3[c]	Trubeck Laboratories (1958b)
50	α-Butylcinnamaldehyde	4400 (Rat)	Moreno (1977)				
51	α-Amylcinnamaldehyde	3730 (Rat)	Jenner et al. (1964)	Rat; M, F	98/oral	287.3 (M) 320.3 (F)	Carpanini et al. (1973)
51	αAmylcinnamaldehyde			Rat; M,F	90/oral	6.1 (M) 6.6 (F)	Oser et al. (1965)
52	α-Hexylcinnamaldehyde	3100 (Rat)	Moreno (1971)				
54	*o*-Methoxycinnamaldehyde	5000 (Rat)	Levenstein (1974)	Rat; M,F	90/oral	47.1 (M) 52.5 (F)	Posternak et al. (1969)
55	*p*-Methoxy-α-methylcinnamaldehyde			Rat; M,F	90/oral	2.43 (M) 2.74 (F)	Posternak et al. (1969)

[a] M = Male; F = Female. If not listed, sex was not specified in the report.
[b] This study was performed at either a single dose or multiple dose levels that produced no adverse effects. Therefore, this dose level is not a true NOEL, but is the highest dose tested that produced no adverse effects. The actual NOEL would be higher.
[c] The test substance was administered as a component of a mixture.
[d] Calculated, based on a reported LD50 of 3.57 ml/kg (Levenstein, 1976) and a density of 1.2475 (CRC, 1989).
[e] Calculated, based on a reported LD50 of 7 ml/kg (Draize et al., 1948) and a density of 1.0435 (CRC, 1989).
[f] Calculated, based on a reported LD50 of 3 ml/kg (Draize et al., 1948) and a density of 1.0435 (CRC, 1989).

organ weights of test and control animals. Gross examination of the tissue of all animals was unremarkable. Histopathological examination of 6-8 animals, equally represented by gender, in the high-dose group revealed a slight hepatocyte swelling and a slight hyperkeratosis of the stomach (Hagan et al., 1967).

Groups of male and female rats (20/sex/group) were maintained on a diet containing cinnamaldehyde at levels calculated to result in the approximate daily intake of either 0 (control), 58, 114, or 227 mg/kg bw for 12 weeks. Observations of general condition and behavior, as well as measurements of bodyweight, food intake, and efficiency of food utilization were recorded regularly. No statistically significant differences between test and control animals were noted. At week 12 of experimentation, hematological examination revealed normal blood hemoglobin levels, and urine analysis revealed the absence of glucose in either sex and only trace levels of albumin in male urines (attributed to the possible presence of semen). At necropsy, measurement of liver and kidney weights revealed no significant difference between test and control groups. Gross examination revealed occasional occurrence of respiratory infections in animals from all groups. Histopathological examination revealed no evidence of adverse effects that could be related to administration of the test substance (Trubeck Laboratories, 1958a).

In a 13-week study, groups of 10 male and 10 female F344/N rats were administered 0, 1.25, 2.5, 5.0, or 10.0% microencapsulated cinnamaldehyde in the diet. These dietary levels correspond to estimated daily intakes of 0, 625, 1250, 2500 or 5000 mg/kg bw, respectively (FDA, 1993). Necropsies were performed on all survivors and histopathological examinations were performed on the two highest dose groups and the control group. There were no early deaths and no cinnamaldehyde-related clinical observations of toxicology. Group mean terminal body weight values were similar to untreated controls for the male and the female vehicle control group. However, the group mean body weight values decreased for males and females in the 2.5, 5.0, and 10.0% dose groups. Food consumption for treated male and female rats was depressed during the first study week and was attributed to taste aversion. Hematological evaluations did not show any overt cinnamaldehyde-related toxicity. Clinical chemistry parameters that were increased by treatment included bile salts and alanine transaminase levels (male and female 10.0% dose group), suggesting mild cholestasis. There were no morphological alterations to the liver based on microscopic examination. Gross necropsy findings were limited to the stomach of the 2.5, 5.0, and 10.0% dose groups (NTP, 1995).

Charles River CD rats (10–16/group) were maintained for 90 days on diets containing either *o*-methoxycinnamaldehyde (No. 54) at levels calculated to result in the approximate daily intake of 0 (control), or 47.1 mg/kg bw for males and 52.5 mg/kg bw for females or *p*-methoxy-α-methylcinnamaldehyde (No. 55) at levels calculated to result in the approximate daily intake of 2.43 mg/kg bw for males and 2.74 mg/kg bw for females. Control groups received basal diets only. Control and test groups, each consisting of 10–16 male and female Charles River CD rats, were housed in pairs of the same sex and given ad libitum access to water and food. The concentration of the test material in the diet was adjusted during the study to maintain constant levels of dietary intake. Clinical observations recorded daily and food consumption and body weights determined weekly failed to show any differences between test and control animals. Hematological examinations and blood urea determinations performed on 50% of the animals at week 7 and again on all animals at week 13 reveal normal values. At necropsy, measurement of liver and kidney weights showed no difference in absolute or relative organ weights between test and control groups. Histopathological examination on a wide range of tissues and organs failed to reveal any lesions that could be associated with administration of the test substances (Posternak et al., 1969).

Rats (5/sex/dose) were maintained on a diet containing α-methylcinnamaldehyde (No. 49) at levels calculated to result in an average daily intake of 0, 58, 115 or 221 mg/kg bw for 90 days. Observations of growth and food intake volume were recorded weekly with results of regular examinations of physical appearance, behavior, and efficiency of food utilization. At week 12 of experimentation, urine samples were collected from both male and females and analyzed for presence of sugar and albumin, and blood samples were taken for determination of hemoglobin level. Neither measurements of bodyweight, general observations, hematology, clinical chemistry, urinalysis, nor histopathology revealed any statistically significant differences between test and control animals at any dietary level (Trubeck Laboratories, 1958c).

Groups of male and female rats (CFE strain; 15/sex/group) were maintained on a diet containing 0 (control), 80, 400 or 4000 ppm α-amylcinnamaldehyde (No. 51) for 14 weeks. Additional groups of five male and five female rats were maintained on diets containing 400 and 4000 ppm α-amylcinnamaldehyde for 2 and 6 weeks. The respective mean dietary intakes over the 14-week period were reported to be 0, 6.1, 29.9, and 287.3 mg/kg bw/day for males and 0, 6.7, 34.9, and 320.3 mg/kg bw/day for females (Carpanini et al., 1973). Measurement of bodyweight, food and water consumption revealed no significant differences between treated and control groups. Hematological examinations (hemoglobin content, hematocrit, erythrocyte and leucocyte counts, and individual leucocyte counts) and blood chemistry determinations conducted at 2, 6, and 14 weeks revealed normal values. Reticulocyte counts performed only on control and the high dose groups showed no significant differences. Urine analysis performed during the final

week of treatment revealed no difference in cell content and renal concentration tests for test and control groups. Measurement of organ weights at autopsy revealed a statistically significant increase in relative liver weight in males ($P<0.01$) and females ($P<0.05$) at the 4000 ppm dietary level after 14 weeks, increased stomach weights in males at the 400 ppm level after 6 weeks, and increased relative kidney weight in males ($P<0.01$) at 4000 ppm after 14 weeks. The relative organ weight increases were not associated with any evidence of histopathology. Microscopic examination of prepared tissues from all major organs revealed no evidence of histopathological changes that could be associated with administration of the test material in the diet (Carpanini et al., 1973).

In a study on the same substance, groups of male and female rats (15/sex) were maintained on a diet containing α-amylcinnamaldehyde (No. 51) at levels calculated to result in the approximate daily intake of 6.1 mg/kg bw for males and 6.6 mg/kg bw for females for a total of 90 days. Bodyweight measurements, food consumption, and observations of general condition were recorded regularly. Hematological and clinical chemistry examinations were conducted on 8 rats of each sex at week 6 and again on all animals at week 12 of experimentation. Neither measurements of growth, hematology, clinical chemistry, nor histopathology at necropsy revealed any evidence of toxic effects (Oser et al., 1965).

A mixture of flavorings containing 897 ppm cinnamaldehyde (No. 22) and 25 ppm each of methyl cinnamate (No. 24), ethyl cinnamate (No. 25), cinnamyl cinnamate (No. 39), and α-methylcinnamaldehyde (No. 49) was added to the diet of rats (12/sex/group) for 12 weeks, resulting in the approximate daily intake of 110 mg/kg bw (male) and 119 mg/kg bw (female) [approximately equivalent to 103 mg/kg bw of cinnamaldehyde and 3 mg/kg bw of each of the other components (FDA, 1993)]. Weekly measurement of body weight and food intake revealed a decreased weight gain in treated males compared to controls animals. The decrease was not statistically significant. There was a statistically significant decrease in efficiency of food utilization for male ($P<0.01$) and female ($P<0.05$) test groups compared to their respective control group. At week 12, measurement of blood hemoglobin, urinary sugar, and urinary albumin levels in three animals of each sex revealed normal values. At necropsy, liver, kidney, and brain weights were within normal limits for both sexes. Gross examination revealed no observable differences between test and control groups (Trubeck Laboratories, 1958b).

Groups (10/sex/group) of male and female Osborne-Mendel rats were provided a diet containing either 0 (control), 1000, 2500 or 10,000 ppm linalyl cinnamate (No. 34) for 17 weeks or 0 (control), 1000 or 10,000 ppm benzyl cinnamate (No. 36) for 19 weeks. These dietary levels correspond to estimated daily intakes of 0, 50, 125 or 500 mg/kg bw per day of linalyl cinnamate or 0, 50 or 500 mg/kg bw per day of benzyl cinnamate, respectively (FDA, 1993). Diets were prepared weekly. Analysis of old diet preparations revealed a 4% weekly loss of linalyl cinnamate. Dietary loss of benzyl cinnamate was not determined. Measurement of body weight and food intake recorded weekly showed no significant differences between test and control animals at any intake level. At termination, hematological examinations revealed no significant differences between test and control animals. At necropsy, no differences were reported between major organ weights of test and control animals. Gross examination of tissue of all animals was unremarkable and histopathological examination of 6–8 animals, equally represented by gender, from the high-dose group and the control group revealed no treatment-related lesions (Hagan et al., 1967).

4.3. Carcinogenicity studies on cinnamyl anthranilate, cinnamaldehyde, and anthranilic acid

4.3.1. Cinnamyl anthranilate

Groups of 50 F344 rats or 50 B6C3F1 mice of each sex were fed cinnamyl anthranilate in diets containing 0, 15,000 or 30,000 ppm for 103 weeks and then observed for an additional 2–3 weeks (NCI, 1980). The dietary levels of 15,000 and 30,000 ppm are calculated to provide an average daily intake of 2250 and 4500 mg/kg bw per day, respectively (FDA, 1993). Control groups consisted of 50 untreated rats and 50 untreated mice of each sex. All surviving animals were terminated and necropsied at 105–107 weeks. Dose-related reductions in mean body weight gain occurred in all groups of dosed male and female rats and mice. Mean body weight gains for high dose groups of both sexes of mice were as much as 30% lower than those for respective control groups (NCI, 1980).

Pathological findings. Renal non-neoplastic and neoplastic lesions. An increased incidence of chronic renal inflammation was observed in control (35/48), low- (47/50) and high-dose (44/49) groups of male rats. An increased incidence of renal mineralization in the low- (17/50) and high-dose group (30/49) was observed in male rats when compared to controls (0/48). The lower incidence of renal mineralization (controls, 2/48; low dose 0/50; high dose, 3/50) and chronic inflammation (controls, 9/48; low dose 9/50; high dose, 16/50) in all groups of female rats suggest that renal toxicity is less pronounced in the female rat than in the male rat. No increased incidences of renal toxicity or renal neoplasms were reported for dosed groups of male or female mice.

Tubular adenomas (2/50) and adenocarcinomas (2/50) of the renal cortex were reported in the high-dose group of male rats but were not statistically significant as compared with controls (0/48). No renal tumors were

observed in control or low-dose groups of male rats or in any group of female rats or mice. Based on the historical incidence among male controls at the laboratory (0/634) and the incidence in all laboratories in the NTP Testing Program (8/1538, 0.37%), the NTP report concluded the following: "Under the conditions of these 2-year dietary studies, there was evidence of carcinogenicity of cinnamyl anthranilate in male F344 rats based on the increased incidence of renal tubule adenomas and adenocarcinomas." (NCI, 1980).

Chronic renal nephropathy (i.e., inflammation and mineralization) and renal tubule neoplasms were reported when cinnamyl anthranilate was administered to male rats in the diet for 2 years. Although treated female rats also exhibited a slight increase in the incidence of renal inflammation, they did not show any renal tubular neoplasms. The data indicate that renal toxicity and subsequent neoplasms are sex and species-specific effects that occur only at chronic high levels of intake (>2000 mg/kg bw/d). The sensitivity of the male rat to this type of kidney toxicity is apparently due to spontaneous nephropathy during aging, which may be exacerbated by administration of high dose levels of the test material. Similar findings have been observed at high intake levels of other substances (NTP, 1992, 1993a, 1993b). When species and sex sensitivity are combined with the facts that dosed groups of male rats showed significantly lower growth rates (30% lower), and that the increase in the incidence of neoplasms was not statistically significant, there is no clear evidence that the incidence of these neoplasms is related to administration of cinnamyl anthranilate in the diet. The renal effects of cinnamyl anthranilate in the male rat are a species- and sex-specific phenomena and reflect the sensitivity of the male rat kidney to chronic progressive nephropathy, focal hyperplasia, and specific tumorigenic responses (Adams et al., 1996, 1998). The relationship of age to the induction of kidney tumors by various chemical agents in laboratory rodents in now a well recognized phenomenon (Hard, 1998).

Pancreatic acinar-cell neoplasms in male rats. The incidence of pancreatic acinar-cell adenomas (2/45) and carcinomas (1/45) was increased in the high-dose males (3/45; 7%) compared with controls (0/42). The difference was not statistically significant. However, according to the NTP, the incidence of this type of neoplasm in aging F344 control rats is extremely low [historical incidence for controls in participating NTP laboratories (6/1538; 0.28%)]. Therefore, the NTP considered occurrence of these neoplasms to be related to administration of the test material.

Since completion of the 2-year bioassay with cinnamyl anthranilate, other carcinogenicity studies have established a relationship between peroxisome proliferation and the appearance of pancreatic acinar-cell neoplasms in the male F344 rat. The sex-specific phenomenon also has been observed when F344 male rats were exposed to high dose levels of other peroxisome proliferators (e.g. butyl benzyl phthalate and hypolipidemic drugs, clofibrate and nafenopin) (Malley et al., 1995; NTP, 1997a; Reddy and Qureshi, 1979; Svoboda and Azanoff, 1979). It appears that the effect on the rat pancreas is secondary to the effect of these substances on the liver.

The sequence of pancreatic acinar cell hypertrophy, hyperplasia, and adenomas in male rats is affected by several factors including steroids, growth factors such as cholecystokinin (CCK), growth factor receptor, and diet. Studies show that testosterone stimulates, and estrogen inhibits, the growth of pancreatic acinar-cell neoplasms in rats (Lhotse et al., 1987a,b; Sumi et al., 1989; Longnecker, 1987; Longnecker and Sumi, 1990). Cholecystokinin has been shown to stimulate adaptive and neoplastic changes of pancreatic acinar cells (Longnecker, 1987). The impact of diet on stimulation of CCK and the subsequent appearance of acinar cell neoplasms in male rats has also been reported (Longnecker, 1987; NTP, 1997b). In rat bioassays, the corn oil vehicle has been shown to increase the incidence of pancreatic acinar call neoplasms (Longnecker, 1987). Also, the incidence of pancreatic acinar-cell neoplasms induced by benzyl phthalate was 10/50 for male rats fed ad libitum, but 0/10 for rats placed on a restricted feed protocol for 2 years. The latter study clearly demonstrated the effect of excess caloric intake on the incidence of pancreatic acinar cell neoplasms. In summary, the appearance of these neoplasms is sex, species, dose, and even diet specific.

Apparently, prolonged peroxisome proliferation inhibits bile flow leading to cholestasis (Lu et al., 2000; Marrapodi and Chiang, 2000). The cholestasis, in turn, leads to a decrease in trypsin activity and an increase in monitor protein in the gut lumen which stimulates cholecystokinin (CCK) (Obourn, 1997a,b). CCK then acts on CCK receptors on pancreatic acinar cells leading to hyperplasia and eventually adenomas. This is a high dose phenomenon in rats and is unlikely to occur in humans. Several human studies of hypolipidemic drugs that are recognized peroxisome proliferators in rodents have failed to show any significant difference in cancer deaths between treated patients and placebo-treated group (IARC, 1996). Also, acinar cell neoplasms are extremely rare in humans. These results are expected, since humans and rodents show quantitative difference in their response to peroxisome proliferators. Apparently, increased CCK levels in humans do not stimulate acinar cell proliferation, because humans possess a relatively small number of CCK receptors compared with the rat.

Given this more recent data and the lack of any correspondence between bioassay results and human studies with peroxisome proliferators, it is concluded that

the increased incidence of acinar-cell neoplasms in the F344 male rat are associated with peroxisomal proliferation induced by high dose levels of cinnamyl anthranilate. This effect is specific to the male F344 rat and, therefore, is not relevant to the human health assessment of cinnamyl anthranilate.

Hepatocellular neoplasms in mice. Neoplastic and non-neoplastic lesions associated with administration of cinnamyl anthranilate to mice developed principally in the liver (Table 3). Treated groups of male and female mice showed evidence of lipoidosis, hemosiderosis, and hyperplasia of hepatocytes. There was a statistically significant increase in the incidence of combined hepatocellular adenomas and carcinomas [control, 14/48; 15,000 ppm or 2250 mg/kg bw, 30/50 ($P=0.003$); 30,000 ppm or 4500 mg/kg bw, 37/47 ($P<0.001$)] in male mice compared with that of the control group (Table 3). However, the increase in the incidence of hepatocellular carcinomas (control, 6/48; 15,000 ppm or 2250 mg/kg, 7/50; 30,000 ppm or 4500 mg/kg, 12/47) was not statistically significant. There was a statistically significant increase in the incidence of hepatocellular carcinomas [control, 1/50; 15,000 ppm or 2250 mg/kg bw, 8/49 ($P=0.014$); 30,000 ppm or 4500 mg/kg bw, 14/49 ($P<0.001$)] and combined adenomas and carcinomas [control, 3/50; 15,000 ppm or 2250 mg/kg bw, 20/49 ($P<0.001$); 30,000 ppm or 4500 mg/kg bw, 33/49 ($P<0.001$)] in dosed groups of female mice. Four high-dose and two low-dose females were diagnosed as having both adenomas and carcinomas.

The NTP report concluded the following: "Based on increased incidences of hepatocellular adenomas, and hepatocellular adenomas and carcinomas, cinnamyl anthranilate was considered carcinogenic for male and female B6C3F1 mice receiving 15,000 or 30,000 ppm cinnamyl anthranilate in the diet" (NCI, 1980).

Since performance of the original bioassay (NCI, 1980), additional studies on over 70 substances have established a direct correlation between the increased incidence of hepatocarcinogenicity and the induction of peroxisome proliferation in rodent livers (Ashby et al., 1994). Studies performed by the European Centre for Ecotoxicity and Toxicology of Chemicals (ECETOC) (1992) show that peroxisome proliferators form a discrete category of rodent liver carcinogens, the carcinogenicity of which does not involve direct genotoxic mechanisms.

Histological evidence of peroxisome proliferation in rodents is reflected by an increased peroxisome/mitochondrial ratio which is correlated with increases in target organ weights, total cytochrome P-450 content, and activities in microsomal lauric acid hydroxylation, carnitine acetyl transferase, and cyanide (CN^-) insensitive palmitoyl-CoA (Reddy et al., 1980, 1986; Reddy and Lalwai, 1983; Barber et al., 1987). Peroxisome proliferation is a transcription-mediated process involving the peroxisome proliferator-activated receptor (PPARα) in the hepatocyte nucleus. The role of PPARα in the induction of hepatocarcinogenicity in the mouse has been clearly established (Peters et al., 1997). Carcinogenicity studies with mice genetically modified to remove PPARα show no evidence of either peroxisome proliferation or carcinogenicity. Given that levels of expression of PPARα in humans is 1–10% of levels found in the rat or mouse (Palmer et al., 1994, 1998), it is not unexpected that humans are refractory to peroxisome proliferation following chronic exposure to potent rodent peroxisome proliferators. No significant evidence of peroxisome proliferation has been observed in human studies with several potent hypolipidemic drugs that are peroxisome proliferators (reviewed in Doull et al., 1999; Ashby et al., 1994). Based on these observations, it is concluded that the hepatocarcinogenic response in rodents is not relevant to the human health assessment of cinnamyl anthranilate.

Summary. When the above information is combined with data on metabolism and enzyme induction, it may be concluded that hepatic peroxisome proliferation is both a rodent-specific and dose-dependent phenomenon induced by the intact ester cinnamyl anthranilate (Viswalingam et al., 1988; Keyhanfar and Caldwell, 1996; Caldwell, 1992). Specifically, repeated-dose metabolism studies have shown that above a threshold dose greater than 500 mg/kg bw/day, intact cinnamyl anthranilate given i.p. or in the diet to mice shows a dose-dependent increase in liver weight, total cytochrome P-450, microsomal lauric acid hydroxylation and cyanide (CN^-) insensitive palmitoyl-CoA activity, and peroxisome/mitochondria ratio in hepatic cells (Caldwell, 1992; Viswalingam et al., 1988). These markers for peroxisome proliferation correspond to dose levels at which saturation of the hydrolysis pathway leads to the presence of the intact ester in vivo. Therefore, peroxisome proliferation caused by cinnamyl anthranilate is a dose-dependent effect. In addition, the results of chronic

Table 3
Incidences of hepatocellular neoplasms associated with administration of cinnamyl anthranilate to mice in the diet for 2 years

	Control	15,000 ppm	30,000 ppm
1. Male Mice			
Hepatocellular adenoma	8/48	23/50	25/47
Hepatocellular carcinoma	6/48	7/50	12/47
Combined Rates[a]	14/48 (29%)	30/50 (60%)	37/47 (79%)
2. Female Mice			
Hepatocellular adenoma	2/50	12/49	19/49
Hepatocellular carcinoma	1/50	8/49	14/49
Combined rates[b]	3/50 (6%)	20/49 (41%)	33/49 (67%)

[a] Historical incidence for 2-year dietary studies with control groups (mean ± std. dev.): 112/257(47%).
[b] Historical incidence: 37/273 (14%).

studies on the hydrolysis product, anthranilic acid, and on the intermediary metabolite cinnamyl alcohol, provide additional evidence for this mechanism of action.

4.3.2. trans-*Cinnamaldehyde*

In a 2-year bioassay on *trans*-cinnamaldehyde (NTP, 2002), groups of 50 F344/N rats and B6C3F1 mice of both sexes were administered diets containing 0, 1000, 2100, or 4100 ppm of *trans*-cinnamaldehyde in modified corn starch and sucrose microcapsules. The microcapsules were coated with modified corn starch. The dietary load of microencapsulated *trans*-cinnamaldehyde was maintained at 1.25%. A vehicle control group (50/sex) received placebo microcapsules (1.25%) in the diet and an untreated control (50/sex) was maintained on the stardard NTP-2000 feed. Analysis of the diet every 9–12 weeks demonstrated that the diet was homogeneous throughout the study. The dietary levels were estimated to provide an average daily intake of 0, 50, 100 or 200 mg/kg bw of *trans*-cinnamaldehyde in rats and 125, 270 or 540 mg/kg bw of *trans*-cinnamaldehyde in mice.

Food and water was made available ad libitum to animals housed either individually (male mice), 2–3 per cage (male rats) of 5 per cage (female rats and mice). All animals were observed twice daily and body weights were recorded initially, on days 8 and 36, and then every 4 weeks to completion of the study. Complete necropsies and histopathological examinations were performed on all animals at the conclusion of the study. The urine of randomly selected male and female rats (10/sex/group) from each treated group was collected and analyzed for hippuric acid, the principal metabolite of *trans*-cinnamaldehyde.

Survival in male rats at the highest feeding level (4100 ppm) was greater than that for the vehicle control group. Mean body weight in males in the 4100 ppm group and in the 2100 ppm group after week 94 were less than that of the vehicle control group. Throughout the study, the rate of hippuric acid excretion reported as the hippuric acid/creatinine ratio was proportional to dose, supporting the conclusion that the primary metabolic pathway was not saturated over the 2 years of exposure in rats. There was no increase in the incidence of either non-neoplastic or neoplastic lesions in any group of treated male or female rats.

In mice, there was no dose-related decrease in survival for either sex of B6C3F1 mice. Mean body weight of the 2100 and 4100 ppm groups was generally less than that for the vehicle control group. Although squamous cell papillomas [1(M) and 3(F)] and carcinoma [1(M) and 1(F)] were reported in the 2100 ppm group (4% in males and 8% in females), the incidence of these lesions was within the historical control range (0–6%) for animals maintained on an NTP 2000 diet. Also there was no significant increase in this type of lesion in the higher dose group (4100 ppm). Although there was no evidence of a statistically significant increase in the incidence of neoplasms in any group treated with *trans*-cinnamaldehyde, there was a statistically significant decrease in the incidence of hepatocellular adenomas and carcinomas in male mice in the 2100 and 4100 ppm groups and a negative trend in female mice compared with the vehicle control group. NTP researchers had previously correlated (Haseman et al., 1997) the decreased incidence of liver neoplasms with decreased body weights in previous NTP studies using the NTP 2000 diet. The NTP Board of Scientific Counselors Technical Report Review Subcommittee met for a peer review of the recently issued draft NTP Technical Report on *trans*-cinnamaldehyde (NTP, 2002). The Subcommittee concluded: "Under the conditions of these 2-year feed studies there was no evidence of carcinogenic activity of trans-cinnamaldehyde in male or female F344/N rats exposed to 1000, 2100, or 4100 ppm. There was no evidence of carcinogenic activity of trans-cinnamaldehyde in male or female B6C3F1 mice exposed to 1000, 2100, or 4100 ppm."

4.3.3. Conclusion

The lack of any evidence of carcinogenicity in either rats or mice at levels exceeding 4000 ppm of the diet is consistent with the results of other bioassays in which aldehydes (e.g. citral) (NTP, 2002) or reactive substances (e.g. benzyl acetate) (NTP, 1993b) were provided in microencapsulated form administered in the diet. A comparison of the 2-year bioassay results for dietary administration of microencapsulated cinnamaldehyde to the gavage administration of a structurally related aromatic aldehyde, benzaldehyde (NTP, 1993a), provides a basis for evaluating the effect of route of administration on selected carcinogenic endpoints, specifically the increased incidence of forestomach papillomas and squamous cell carcinomas in rodent species. The increased incidence of forestomach hyperplasia, papillomas and eventually the appearance of squamous cell carcinomas in gavage studies using high concentrations of an irritating aldehyde confirm the impact of the mode of administration on the toxicological sequelae in the rodent forestomach. Future design of 2-year bioassays studies with low molecular weight, irritant substances should avoid the use of gavage as a mode of administration.

The lack of any evidence of carcinogenicity in the 2-year bioassay for *trans*-cinnamaldehyde provides further clarification for the mechanism by which hepatic neoplasms are induced in B6C3F1 mice exposed to high dose levels of a related cinnamyl ester, cinnamyl anthranilate (NCI, 1980). The toxicology data are also consistent with previously reported dose-dependent metabolic data on cinnamyl anthranilate.

At low dose levels, cinnamyl anthranilate is adequately hydrolyzed to cinnamyl alcohol and anthranilic acid (Keyhanfar and Caldwell, 1996). Cinnamyl alcohol

is then readily oxidized in the liver to yield cinnamaldehyde, then cinnamic acid, and eventually hippuric acid (Keyhanfar and Caldwell, 1996; Nutley, 1990; Teuchy et al., 1971). However, at elevated dietary levels, those exceeding 15,000 ppm in mice, the hydrolysis of cinnamyl anthranilate approaches saturation leading to accumulation of unhydrolyzed ester in the liver compartment. This phenomenon is accompanied by a pattern of hepatic enzyme induction that is characteristic of peroxisome proliferation (Caldwell, 1992; Caldwell and Viswalingam, 1989; Keyhanfar and Caldwell, 1996; Viswalingam et al., 1998).

In an earlier GRAS article (Newberne et al., 2000), it was concluded that the hepatic neoplasms in the B6C3F1 mouse in the NTP bioassay are secondary responses to peroxisome proliferation, a rodent-specific and dose-dependent phenomenon induced by the intact ester cinnamyl anthranilate (Caldwell, 1992; Caldwell and Viswalingam, 1989; Keyhanfar and Caldwell, 1996; Viswalingam et al., 1988). If the intact ester is responsible for induction of peroxisome proliferation and subsequent appearance of liver neoplasms, then the hydrolysis products (anthranilic acid and cinnamyl alcohol) or their liver metabolites (cinnamaldehyde or cinnamic acid) should show no evidence of hepatocarcinogenicity in bioassay studies in the same species and strain at similar or higher levels of exposure. The results of the bioassay studies for trans-cinnamaldehyde and anthranilic acid support this hypothesis.

An intake of 15,000 ppm (i.e., the LOAEL for peroxisome proliferation in the cinnamyl anthranilate study) corresponds to a potential production of 7945 ppm of cinnamyl alcohol and 8240 ppm of anthranilic acid.[5] There was no evidence of carcinogenicity reported when B6C3F$_1$ mice were maintained on diets of 1) 25,000 or 50,000 ppm anthranilic acid 5 days per week for 78 weeks and then observed for an additional 26–27 weeks (NCI, 1980) or 2) 1000, 2100 or 4100 ppm microencapsulated *trans*-cinnamaldehyde for 2 years (NTP, 2002). The lack of any evidence of hepatocarcinogenicity for the hydrolysis products supports a mechanism of action in which high concentrations of the intact ester are responsible for the onset of peroxisome proliferation and the eventual appearance of liver tumors.

The FEMA Expert Panel considers that the lack of any carcinogenic effect in either species of rodent in 2-year chronic studies supports the current recognition of GRAS for *trans*-cinnamaldehyde for its intended use as a flavoring substance. The Panel concludes that these data also support the conclusion that cinnamyl anthranilate is GRAS for its intended use as a flavoring substance given its historically low level of use by the flavor industry (NAS, 1970). This material was voluntarily withdrawn from use as a flavoring substance more than a decade ago.

[5] Molecular weight alcohol or acid/Molecular weight ester X dietary level (ppm).

4.4. Genotoxicity studies

4.4.1. In vitro

The results of in vitro studies are summarized in Table 4. Incubation of cinnamaldehyde (*trans* and unspecified regiochemistry), cinnamyl alcohol (*trans* and unspecified regiochemistry), cinnamic acid, α-methylcinnamaldehyde, cinnamyl acetate, benzyl cinnamate, cyclohexyl cinnamate, α-amylcinnamaldehyde, α-hexylcinnamaldehyde, *p*-methoxy-α-methylcinnamaldehyde, 3-phenylpropionaldehyde, or cinnamyl anthranilate in *Salmonella typhimurium*, including strains TA92, TA94, TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA2637 produced no evidence of mutagenicity with a few exceptions. Assays were performed at concentrations ranging up to 10,000 μg/plate and in some instances the level of cytotoxicity, both in the absence and presence of metabolic activation (S9 fraction) obtained from the livers of Aroclor 1254 or methylcholanthrene-induced Sprague-Dawley rats or Syrian hamsters (Azizan and Blevins, 1995; Dillon et al., 1992; Dunkel and Simon, 1980; Eder et al., 1980; 1982a, b; 1991; Florin et al., 1980; Fujita and Sasaki, 1987; Huang et al., 1985; Ishidate et al., 1984; Kasamaki et al., 1982; Kato et al., 1989; Lijinsky and Andrews, 1980; Lutz et al., 1980; 1982; Marnett et al., 1985; Mortelmans et al., 1986; Neudecker et al., 1983; NTP, 2002; Prival et al., 1982; Sekizawa and Shibamoto, 1982; Tennant et al., 1987; Wild et al., 1983).

A few weakly positive to positive results were reported for cinnamaldehyde in *Salmonella typhimurium* strain TA100 using the pre-incubation method (Dillon et al., 1992; Ishidate et al., 1984; NTP, 2002). However, the majority of similar studies in strain TA100, including a recent study using a prolonged pre-incubation time (120 min), and others using the standard plate incorporation method, did not find any evidence of mutagenicity in the TA 100 strain (Azizan and Blevins, 1995; Eder et al., 1982a, b; 1991; Kasamaki et al., 1982; Kato et al., 1989; Lijinsky and Andrews, 1980; Lutz et al., 1982; Neudecker et al., 1983; Prival et al., 1982; Sasaki and Endo, 1978; Sekizawa and Shibamoto, 1982).

Ames/*Salmonella typhimurium* assays using a pre-incubation method with *o*-methoxycinnamaldehyde produced negative to weak positive results (Eder et al., 1991; Mortelmans et al., 1986). Of these two studies, the weak evidence of mutagenicity was reported in strain TA100 with metabolic activation (Mortelmans et al., 1986) using two different activation systems, whereas negative results were obtained in strains TA1535, TA1537, and TA98 both with and without metabolic activation. In a second study using tester strain TA100, negative results were reported without metabolic acti-

Table 4
In vitro genotoxicity studies for cinnamyl derivatives used as flavoring ingredients

Agent	Test system	Test object	Concentration of agent	Results	Reference
10. 3-Phenylpropionaldehyde	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 μmol/plate (402 μg/plate)	Negative[a]	Florin et al. (1980)
10. 3-phenylpropionaldehyde	Sister chromatid exchange	Chinese hamster ovary cells	33.3 μM (4468 μg)	Negative[b]	Sasaki et al. (1989)
12. Cinnamyl alcohol	Ames test[c]	*S. typhimurium* TA1537, TA1538, TA98, TA100, TA1535	3000 μg/plate	Negative[a]	Sekizawa and Shibimoto (1982)
12. Cinnamyl alcohol	Rec-assay	*B. subtilis* M45 (rec$^-$) & H17 (rec$^+$)	21 μg/disk	Negative[b]	Oda et al. (1979)
12. Cinnamyl alcohol	Rec-assay	*B. subtilis*, H17 or M45	1.0 mg/disk (1000 μg/disk)	Positive[b]	Sekizawa and Shibimoto (1982)
12. Cinnamyl alcohol	Rec-assay	*B. subtilis* M45 (rec$^-$) & H17 (rec$^+$)	10 μl/disk (10,400 μg/disk)	Positive[b]	Yoo (1986)
12. Cinnamyl alcohol	Mutation	*E. coli* WP2 *uvrA*	3000 μg/plate	Negative[b]	Sekizawa and Shibimoto (1982)
12. Cinnamyl alcohol	Mutation	*E. coli* WP2 *uvrA*	4.0 mg/plate (4000 μg/plate)	Negative[b]	Yoo (1986)
12. Cinnamyl alcohol	Sister chromatid exchange	Chinese hamster ovary cells	33.3 μM (4468 μg)	Negative[b]	Sasaki et al. (1989)
15. Cinnamyl acetate	Sister chromatid exchange	Chinese hamster ovary cells	33.3 μM (5868 μg)	Negative[b]	Sasaki et al. (1989)
22. Cinnamaldehyde	Ames test[c]	*S. typhimurium* TA1537, TA1538, TA98, TA100, TA1535	600 μg/plate	Negative[a]	Sekizawa and Shibamoto (1982)
22. *trans*-Cinnamaldehyde	Ames test	*S. typhimurium* TA1537, TA98, TA100, TA1535	10 mg/plate (10,000 μg/plate)	Negative[a]	Prival et al. (1982)
22. Cinnamaldehyde	Ames test (preincubation method)	*S. typhimurium* TA104	0.8 μmoles (105 μg)	Negative[a]	Marnett et al. (1985)
22. Cinnamaldehyde	Ames test (preincubation method)	*S. typhimurium* TA1537, TA92, TA94, TA98, TA100, TA1535	0.5 mg/plate (500 μg/plate)	Positive[a,d]	Ishidate et al. (1984)
22. *trans*-Cinnamaldehyde	Ames test (plate incorporation and preincubation methods)	*S. typhimurium* TA1537, TA1538, TA98, TA100, TA1535	500 μg/plate	Negative[a]	Lijinsky and Andrews (1980)
22. *trans*-Cinnamaldehyde	Ames test	*S. typhimurium* TA98, TA100	500 μg/plate	Negative[a]	Kasamaki et al. (1982)
22. Cinnamaldehyde	Ames test (preincubation method)	*S. typhimurium* TA97, TA98, TA100	1 mg/ml (1000 μg/ml)	Negative[a]	Azizan and Blevins (1995)
22. *trans*-Cinnamaldehyde	Ames test (preincubation method)	*S. typhimurium* TA98, TA100, TA104	Not reported	Negative[a]	Kato et al. (1989)
22. *trans*-Cinnamaldehyde	Ames test (preincubation method)	*S. typhimurium* TA1537, TA98, TA100, TA1535	100 μg/plate	Negative[a]	Mortelmans et al. (1986)
22. *trans*-Cinnamaldehyde	Ames test (preincubation method)	*S. typhimurium* TA100	5 μmoles/plate (661 μg/plate)	Negative[a]	Neudecker et al. (1983)
22. *trans*-Cinnamaldehyde	Ames test (preincubation method)	*S. typhimurium* TA100, TA1535, TA1537, TA98	333 μg/plate	Negative[a]	NTP (2002)
22. *trans*-Cinnamaldehyde	Ames test (preincubation method)	*S. typhimurium* TA100, TA102, TA104	300 μg/plate	Negative[a] Weakly Positive[e]	NTP (2002)
22. Cinnamaldehyde	Mutation	*E. coli* WP2 *uvrA*	600 μg/plate	Negative[b]	Sekizawa and Shibimoto (1982)
22. Cinnamaldehyde	Mutation	*E. coli* WP2 *uvrA*	0.8 mg/plate (800 μg/plate)	Negative[b]	Yoo (1986)
22. Cinnamaldehyde	Rec-assay	*B. subtilis*, H17 or M45	0.2 mg/disk (200 μg/disk)	Positive[b]	Sekizawa and Shibimoto (1982)
22. Cinnamaldehyde	Rec-assay	*B. subtilis* M45 (rec$^-$) & H17 (rec$^+$)	10 μl/disk (10,500 μg/disk)	Positive[b]	Yoo (1986)
22. Cinnamaldehyde	Rec-assay	*B. subtilis* M45 (rec$^-$) & H17 (rec$^+$)	10 μl/disk (10,500 μg/disk)	Positive[a]	Kuroda et al. (1984)
22. Cinnamaldehyde	Rec-assay	*B. subtilis* M45 (rec$^-$) & H17 (rec$^+$)	21 μg/disk	Negative[b]	Oda et al. (1979)
22. Cinnamaldehyde	Sister chromatid exchange	Chinese hamster ovary cells	33.3 μM (4401 μg)	Negative[b]	Sasaki et al. (1987)
22. Cinnamaldehyde	Chromosome aberration assay	Chinese hamster fibroblasts	0.015 mg/ml (15 μg/ml)	Positive[b]	Ishidate et al. (1984)
22. Cinnamaldehyde	Chromosome aberration assay	Chinese hamster B241 cells	20 nM (2.6 μg)	Positive[b]	Kasamaki and Urasawa (1985)
22. Cinnamaldehyde	Chromosome aberration assay	Chinese hamster B241 cells	10 nM (1.3 μg)	Positive	Kasamaki et al. (1982)
22. *trans*-Cinnamaldehyde	Chromosome aberration assay	Chinese hamster ovary cells	18.3 μg/ml 100 μg/ml	Negative[b] Negative[f]	Galloway et al. (1987)
22. *trans*-Cinnamaldehyde	Sister chromatid exchange	Chinese hamster ovary cells	6.8 μg/ml	Weak Positive[b]	Galloway et al. (1987)
22. Cinnamaldehyde	DNA strand breaks	Mouse L1210 lymphoma cells	500 μmol (66,080 μg)	Positive[b]	Eder et al. (1993)
22. Cinnamaldehyde	Cytotoxicity	Mouse L1210 lymphoma cells	10 μg/ml	Positive[b]	Moon and Pack (1983)
22. Cinnamaldehyde	Mutation	Chinese hamster V79 cells	100 μM (13,216 μg)	Negative[b]	Fiorio and Bronzetti (1994)
22. Cinnamaldehyde	Micronucleus assay	Hep-G2 cells	500 μg/ml	Weak Positive[b]	Sanyal et al. (1997)
23. Cinnamic acid	Ames test (plate incorporation and preincubation methods)	*S. typhimurium* TA1537, TA1538, TA98, TA100, TA1535	1000 μg	Negative	Lijinsky and Andrews (1980)
23. Cinnamic acid	Rec-assay	*B. subtilis* M45 (rec$^-$) & H17 (rec$^+$)	25 μg/disk	Negative[b]	Oda et al. (1979)
23. Cinnamic acid	Rec-assay	*B. subtilis* M45 (rec$^-$) & H17 (rec$^+$)	2.0 mg/disk (2000 μg/disk)	Negative[b]	Yoo (1986)
23. Cinnamic acid	Sister chromatid exchange	Chinese hamster ovary cells	33.3 μM (4934 μg)	Positive[b]	Sasaki et al. (1989)
24. Methyl cinnamate	Rec assay	*B. subtilis* M45 (rec$^-$) & H17 (rec$^+$)	20 μg/disk	Negative[b]	Oda et al. (1979)
24. Methyl cinnamate	Sister chromatid exchange	Chinese hamster ovary cells	33.3 μM (5401 μg)	Positive[b]	Sasaki et al. (1989)
25. Ethyl cinnamate	Ames test (preincubation method)	*S. typhimurium* TA1537, TA92, TA94, TA98, TA100, TA1535	5.0 mg/plate (5000 μg/plate)	Negative	Ishidate et al. (1984)
25. Ethyl cinnamate	Chromosome aberration	Chinese hamster fibroblasts	0.063 mg/l (63 μg/ml)	Equivocal[b]	Ishidate et al. (1984)
25. Ethyl cinnamate	Rec-assay	*B. subtilis* M45 (rec$^-$) & H17 (rec$^+$)	20 μg/disk	Negative[b]	Oda et al. (1979)

(*continued on next page*)

Table 4 (*continued*)

Agent	Test system	Test object	Concentration of agent	Results	Reference
25. Ethyl cinnamate	Sister chromatid exchange	Chinese hamster ovary cells	33.3 μM (5868 μg)	Positive[b]	Sasaki et al. (1989)
28. Allyl cinnamate	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 μg/plate)	Negative	Wild et al. (1983)
33. Cyclohexyl cinnamate	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 μg/plate)	Negative	Wild et al. (1983)
36. Benzyl cinnamate	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 μmol/plate (715 μg/plate)	Negative	Florin et al. (1980)
36. Benzyl cinnamate	Rec-assay	*B. subtilis* M45 (rec^-) & H17 (rec^+)	1.0 mg/disk (1000 μg/disk)	Negative[b]	Yoo (1986)
40. α-Amylcinnamyl alcohol	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 μg/plate)	Negative	Wild et al. (1983)
49. α-Methylcinnamaldehyde	Ames test (preincubation method)	*S. typhimurium* TA100	4 μmoles/plate (585 μg/plate)	Negative	Neudecker et al. (1983)
49. α-Methylcinnamaldehyde	Ames test (preincubation method)	*S. typhimurium* TA98, TA100, TA1535, TA1537	500 μg/plate	Negative	Mortelmans et al. (1986)
49. α-Methylcinnamaldehyde	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 μg/plate)	Negative	Wild et al. (1983)
51. α-Amylcinnamaldehyde	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 μg/plate)	Negative	Wild et al. (1983)
51. α-Amylcinnamaldehyde	Ames test (preincubation method)	*S. typhimurium* TA97, TA102	1.0 mg/plate (1000 μg/plate)	Negative	Fujita and Sasaki (1987)
52. α-Hexylcinnamaldehyde	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 μg/plate)	Negative	Wild et al. (1983)
54. *o*-Methoxycinnamaldehyde	Ames test (preincubation method)	*S. typhimurium* TA98, TA100, TA1535, TA1537	666 μg/plate	Positive	Mortelmans et al. (1986)
55. *p*-Methoxy-alpha-methyl-cinnamaldehyde	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 μg/plate)	Negative	Wild et al. (1983)

[a] With and without metabolic activation,
[b] Without metabolic activation.
[c] Method included both plate incorporation (without metabolic activation) and preincubation method (with metabolic activation).
[d] Positive results in strain TA 100 only.
[e] Only in strain TA100 with mouse S9.
[f] With metabolic activation.

vation (Eder et al., 1991). No standard plate incorporation Ames test data were available for *o*-methoxycinnamaldehyde, which may be expected to behave similarly to the other cinnamyl compounds based on structural and metabolic similarities.

There was no evidence of mutagenicity in assays (several using the pre-incubation method) in which *Escherichia coli* strains WP2 *uvr*A, PQ37, and Sd-4-73 were incubated with cinnamaldehyde, cinnamyl alcohol, cinnamic acid, α-methylcinnamaldehdye, and α?amylcinnamaldehyde (Eder et al., 1991; 1993; Kato et al., 1989; Ohta et al., 1986; Sekizawa and Shibamoto, 1982; Szybalski, 1958; Yoo, 1986).

In the Rec assay in *Bacillus subtilis*, overall positive results were reported for cinnamaldehyde and cinnamyl alcohol, whereas cinnamic acid, ethyl cinnamate, methyl cinnamate, and benzyl cinnamate gave negative results in all tests using this assay (Kuroda et al., 1984; Oda et al., 1979; Sekizawa and Shibamoto, 1982; Yoo, 1986). Assays in isolated mammalian cells produced mixed but positive results for cinnamyl esters overall. Cinnamaldehyde produced equivocal to positive results in the forward mutation assay in L5178Y mouse lymphoma cells both with and without metabolic activation, but the reports describing these tests did not provide sufficient details on the methodology, test concentrations, or cytotoxic effects to adequately evaluate the results (Palmer, 1984; Rudd et al., 1983). In L1210 mouse lymphoma cells, DNA strand breaks were observed, but only at cytotoxic concentrations of cinnamaldehyde (Eder et al., 1993).

Tests for the induction of sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells exposed to cinnamaldehyde produced negative results at low concentrations and weakly positive results at concentrations approaching cytotoxic levels, suggesting only weak SCE activity (Galloway et al., 1987; Sasaki et al., 1987). A dose-dependent increase in SCE was reported only when cultures were pre-treated with mitomycin C (Sasaki et al., 1987); however, in the absence of SCE activity by cinnamaldehyde alone, the activity in conjunction with mitomycin contributes little to the evaluation of the potential SCE activity. Cinnamaldehyde was reported to induce chromosome aberrations at low concentrations (i.e., <15 μg/ml) in Chinese hamster fibroblasts and B241 cells tested with and without metabolic activation (Ishidate et al., 1984; Kasamaki et al., 1982; Kasamaki and Urasawa, 1985). However, higher concentrations were negative in CHO cells, both with and without metabolic activation in a well-conducted, repeated assay (Galloway et al., 1987). Transformation assays showed mixed activity for cinnamaldehyde, with positive results obtained at near-cytotoxic concentrations or after multiple generations of growth, and with negative results obtained in human HAIN-55 cells (Kasamaki et al., 1987; Matthews et al.,

1993). Subcutaneous injection of these transformed cells into nude mice led to the formation of nodules at the site of injection and neoplastic growth in the spleen (Kasamaki et al., 1987). Negative results were obtained with cinnamaldehyde in the mutation assay in Chinese hamster V79 cells (Fiorio and Bronzetti, 1994), while a weakly positive increase in the incidence of micronucleated Hep-G2 cells was reported (Sanyal et al., 1997).

Cinnamyl anthranilate did not increase chromosomal aberrations (ABS) or the frequency of chromatid breaks and exchanges (SCE) in Chinese hamster ovary cells with or without metabolic activation at concentrations of 40 or 30 μg/ml, respectively (Tennant et al., 1987).

The results with the other cinnamyl compounds in isolated mammalian cells were, in general, comparable to those obtained with cinnamaldehyde. SCE was not observed in CHO cells exposed to cinnamyl alcohol, cinnamic acid, ethyl cinnamate, methyl cinnamate, cinnamyl acetate, or 3-phenylpropionaldehyde. Pretreatment with mitomycin C resulted in increased SCE in assays with cinnamic acid, methyl cinnamate, and ethyl cinnamate but not cinnamyl alcohol, cinnamyl acetate, or 3-phenylpropionaldehyde (Sasaki et al., 1989). Cinnamyl alcohol, cinnamic acid, cinnamyl cinnamate, and *o*-methoxycinnamaldehyde have been reported to produce a dose related increase in the incidence of reversions in L5178Y mouse lymphoma cells with and without metabolic activation (Palmer, 1984).

Results of the L5178Ytk±mouse lymphoma cells (MLA) assay have yielded equivocal results. Cinnamyl anthranilate induced an increase in trifluorothymidine resistance when incubated at a concentration of 10 μg/ml with metabolic activation, but showed no mutagenic activity without metabolic activation (Tennant et al., 1987). No mutagenic activity was detected in a MLA assay performed at 40 μg/ml without S-9 activation. With S-9 activation, mutational frequency increased but only at concentrations approaching those causing cytolethality (18–31 μg/ml) (Myhr and Caspary, 1991). Other reports (Palmer, 1984; Rudd et al., 1983) of positive responses in the MLA assay failed to report concentration and cytolethality data.

The positive results obtained in MLA assays were at near-lethal concentrations in studies reporting cell lethality. The results of the MLA for simple aliphatic and aromatic substances have been shown to be inconsistent with the results of other standardized genotoxicity assays (Heck et al., 1989; Tennant et al., 1987). Culture conditions of low pH and high osmolality, which may occur upon incubation with substances (aldehydes, carboxylic acids, lactones, hydrolyzed esters) having a potentially acidifying influence on the culture medium, have been shown to produce false-positive results in this and other assays (Heck et al., 1989).

4.4.2. In vivo

The results of in vivo studies are summarized in Table 5. The majority of information relating to in vivo administration of cinnamyl compounds pertains to cinnamaldehyde. An increase in the frequency of sex-linked recessive lethal mutations was reported when *Drosophila melanogaster* was injected with 20,000 ppm cinnamaldehyde. However, no increase in the frequency of mutations occurred when *Drosophila melanogaster* were fed 800 ppm cinnamaldehyde for 3 days. Reciprocal translocations were not observed in either assay (Woodruff et al., 1985). In mammalian test systems, there was no evidence of an increase in unscheduled DNA synthesis in hepatocytes when rats or mice were administered 1000 mg cinnamaldehyde/kg bw by oral gavage (Mirsalis et al., 1989). In the rodent micronucleus assay, the frequency of micronuclei was not increased when rats or mice were given 1700 mg/kg bw or 1100 mg/kg bw, respectively, of cinnamaldehyde by oral gavage (Mereto et al., 1994) or when mice were administered 500 mg/kg bw by intraperitoneal injection (Hayashi et al. 1984, 1988). The frequency of micronucleated bone marrow cells in mice that had been exposed to X-rays decreased after 500 mg cinnamaldehyde was administered by injection (Sasaki et al., 1990).

In one study (Mereto et al., 1994), an increase in micronucleated cells was reported in rat and mouse hepatocytes, and in rat (but not in mouse) forestomach cells after oral gavage dosing with up to 1100 (rats) or 1700 (mice) mg cinnamaldehyde/kg bw. No increase in liver or forestomach micronuclei were observed at dose levels less than or equal to 850 mg/kg bw. No DNA fragmentation was observed in the rat hepatocytes or gastric mucosa cells. An increase in the incidence and size of GGT-positive foci in hepatocytes of rats pretreated with *N*-nitrosodiethylamine and then administered 500 mg cinnamaldehyde/kg bw/day by oral gavage for 14 days was observed (Mereto et al., 1994).

The positive in vivo findings with cinnamaldehyde in the rat forestomach and in the liver of both rats and mice are inconsistent with negative results observed in the standard bone marrow assays or peripheral blood assays and are observed at dose levels that far exceed those resulting from intake of cinnamaldehyde in foods. It has been reported that cinnamaldehyde given at oral doses of greater than or equal to 500 mg/kg bw results in the depletion of hepatocellular glutathione levels (Swales and Caldwell, 1991, 1992, 1993). Therefore, increases in micronuclei were reported at dose levels (1100 and 1700 mg/kg bw) that appear to affect cellular defense mechanisms (i.e., glutathione depletion). Based on the fact the micronuclei formation is dose-dependent, it appears that induction of micronuclei is a threshold phenomenon, which occurs at intake levels orders of magnitude greater than intake of cinnamaldehyde as a flavoring ingredient. Also, the bolus doses resulting from gavage administration likely produce

Table 5
In vivo genotoxicity studies for cinnamyl derivatives used as flavoring substances

Agent	Test system	Test object	Concentration of agent	Results	Reference
22. *trans*-Cinnamaldehyde	Sex-linked recessive lethal mutations	*Drosophila melanogaster*	800 ppm (800 μg/g)	Negative	Woodruff et al. (1985)
22. *trans*-Cinnamaldehyde	Sex-linked recessive lethal mutations	*Drosophila melanogaster*	20,000 ppm (20,000 μg/g)	Positive	Woodruff et al. (1985)
22. *trans*-Cinnamaldehyde	Reciprocal translocation mutations	*Drosophila melanogaster*	20,000 ppm (20,000 μg/g)	Negative	Woodruff et al. (1985)
28. Allyl cinnamate	Sex-linked recessive lethal mutations	*Drosophila melanogaster*	1 mM (188,000 μg)	Negative	Wild et al. (1983)
40 α-Amylcinnamyl alcohol	Sex-linked recessive lethal mutations	*Drosophila melanogaster*	45 mM (9,194,000 μg)	Negative	Wild et al. (1983)
49. α-Methylcinnamaldehyde	Sex-linked recessive lethal mutations	*Drosophilia melanogaster*	5 mM (731,000 μg)	Negative	Wild et al. (1983)
51. α-Amylcinnamaldehyde	Sex-linked recessive lethal mutations	*Drosophila melanogaster*	10 mM (2,023,000 μg)	Negative	Wild et al. (1983)
52. α-Hexylcinnamaldehyde	Sex-linked recessive lethal mutations	*Drosophila melanogaster*	10 mM (2,163,000 μg)	Negative	Wild et al. (1983)
22. Cinnamaldehyde	Unscheduled DNA synthesis	Rat and mouse hepatocytes	1,000,000 μg/kg	Negative	Mirsalis et al. (1989)
22. Cinnamaldehyde	Micronucleus assay	Mouse bone marrow cells	500,000 μg/kg	Negative	Hayashi et al. (1984,1988)
22. *trans*-Cinnamaldehyde	Micronucleus assay	Mouse peripheral blood cells	4,950,000 μg/kg	Negative	NTP (2002)
22. *trans*-Cinnamaldehyde	Micronucleus assay	Rat and mouse hepatocytes	1,700,000 μg/kg (mice) 1,100,000 μg/kg (rats)	Positive	Mereto et al. (1994)
22. *trans*-Cinnamaldehyde	Micronucleus assay	Rat and mouse bone marrow	1,700,000 μg/kg (mice) 1,100,000 μg/kg (rats)	Negative	Mereto et al. (1994)
22. Cinnamaldehyde	Nuclear anomalies[a]	Rat and mouse forestomach mucosa cells	1,700,000 μg/kg (mice) 1,100,000 μg/kg (rats)	Negative (mice) Positive (rat)	Mereto et al. (1994)
22. *trans*- cinnamaldehyde	DNA fragmentation	Rat hepatocytes and gastric mucosa cells	1,100,000 μg/kg	Negative	Mereto et al. (1994)
22. Cinnamaldehyde	Induction of hyperplastic foci	Rat hepatocytes	500,000 μg/kg/day[b]	Positive	Mereto et al. (1994)
28. Allyl cinnamate	Micronucleus assay	Mouse bone marrow cells	282,000 μg/kg	Negative	Wild et al. (1983)
40. α-Amylcinnamyl alcohol	Micronucleus assay	Mouse bone marrow cells	510,000 μg/kg	Negative	Wild et al. (1983)
49. α-Methylcinnamaldehyde	Micronucleus assay	Mouse bone marrow cells	438,000 μg/kg	Negative	Wild et al. (1983)
51. α-Amylcinnamaldehyde	Micronucleus assay	Mouse bone marrow cells	1,213,000 μg/kg	Negative	Wild et al. (1983)
52. α-Hexylcinnamaldehyde	Micronucleus assay	Mouse bone marrow cells	756,000 μg/kg	Negative	Wild et al. (1983)

[a] Includes%micronuclei, pyknosis, and karyorrhexis.
[b] Rats were initiated with *N*-nitrosodiethylamine then administered cinnamaldehyde by oral gavage for 14 consecutive days.

much greater exposures to both the forestomach and liver, as compared with dietary admixture administration. The authors (Mereto et al., 1994) acknowledged these facts and concluded that the data did not justify the conclusion that cinnamaldehyde was clastogenic. As a result of the apparent threshold for micronuclei induction and the lack of activity in the remainder of the in vivo studies, the results obtained with bolus, high-dose exposures occurring in the liver and forestomach are not considered relevant to the safety of cinnamaldehyde from use as a flavoring ingredient. In a more recent study (NTP, 2002) no increase in micronucleated peripheral blood cells was observed when B6C3F1 mice (5/dose/sex) were maintained on diets supplemented with 0 (control), 4100, 8200, 16,500 or 33,000 ppm daily for 3 months. These dietary levels correspond to average daily intakes of 0, 615, 1230, 2475 or 4950 mg/kg bw per day (FDA, 1993).

In other submammalian and mammalian in vivo tests, Wild et al. (1983) reported negative results in the sex-linked recessive lethal mutation assay in *Drosophila melanogaster* and in the micronucleus assay in mouse bone marrow cells, each after the administration of α-methylcinnamaldehyde, allyl cinnamate, α-amylcinnamyl alcohol, α-amylcinnamaldehyde, or α-hexylcinnamaldehyde.

Cinnamyl anthranilate did not induce sex-linked recessive lethal mutations or reciprocal translocations in male *Drosophila melanogaster* when incorporated into the diet at 5 mM for three days (Wild et al., 1983). No sex-linked recessive lethal mutations were observed when male *Drosophila melanogaster* were maintained on 5000 ppm cinnamyl anthranilate for three days or were given 2000 ppm cinnamyl anthranilate by intraperitoneal injection daily for three days (Foureman et al., 1994).

Cinnamyl anthranilate was administered to male F344/N rats at a dose level of 1000 mg/kg bw. Pancreatic cells failed to exhibit any evidence of unscheduled DNA synthesis (Steinmetz and Mirsalis, 1984). No increase in micronucleated polychromatic erythrocytes (PE) was observed 30 hours after groups male and female NMRI mice (5/dose/sex) were given single intraperitoneal injections of 2533, 1901, or 761 mg cinnamyl anthranilate/kg bw (Wild et al., 1983). No increase in micronucleated PE was reported when male B6C3F1 mice were given 500, 1000 or 2000 mg cinnamyl anthranilate/ kg bw daily by intraperitoneal injection for three consecutive days (Shelby et al., 1993).

4.4.3. Conclusion

Cinnamyl alcohol and related compounds lack direct mutagenic or genotoxic activity, as indicated by the negative results obtained in bacterial test systems. The mixed results in the Rec assay and in the various antimutagenicity studies are associated with cytotoxicity, as noted by Sekizawa and Shibamoto (1982). Evidence of genotoxic activity was observed in isolated mammalian cells, with the cinnamyl compounds producing chromosome aberrations and/or mutations in the respective test systems regardless of the presence or absence of metabolic activation; however, the reported in vitro activity did not translate into mutagenic, clastogenic, or genotoxic activity in vivo.

4.5. Other relevant studies

Female rats were orally administered a 53.5 mg/kg bw dose of cinnamyl alcohol (No. 12) on either day 4 (implantation) or on days 10–12 (organogenesis) of gestation. On day 20 of gestation, all animals were terminated and fetuses removed for examination. Neither measurements of fetal bodyweight, length, nor survival number revealed any significant differences between test and control animals. Histopathological examinations revealed a slight reduction in skeletal ossification of the extremities. Examination of the sagital sections revealed no anomalies in relation to palatal structure, eyes, brain, or other internal organs (Maganova and Saitsev, 1973).

In a second study, female rats were orally administered a 53.5 mg/kg bw dose of cinnamyl alcohol once per day for the entire course of pregnancy. On day 20 of gestation, 50% of animals from both test and control groups were terminated and the fetuses removed for examination. Neither measurements of fetal bodyweight, liver nucleic acids, number of survivors, nor examination of bone development revealed any significant differences between test and control animals. The remaining females from both groups delivered normally. Neither measurements of offspring bodyweight, survival number, nor size and general development at birth or at one month revealed significant differences between test and controls (Zaitsev and Maganova, 1975).

Rats were administered 5, 25 or 250 mg/kg bw/day cinnamaldehyde (No. 22) by gavage in olive oil on days 7–17 of gestation. A control group was included; however, it was not stated whether or not the controls received the olive oil vehicle. The number of dams treated per group was 15, 14, 16, and 15 for the control, low-, mid-, and high-dose groups, respectively. Fetal abnormalities observed included: poor cranial ossification in all dose groups; increased incidences of dilated pelvis/reduced papilla in the kidney as well as dilated ureters in the low- and mid-dose groups; and an increase in the number of fetuses with two or more abnormal sternebrae in the mid-dose group. However, these effects were not dose related and may be attributed to a decrease in maternal weight gain that was noted in the mid- and high-dose groups (Mantovani et al., 1989).

Female rats were orally administered 0, 5 or 50 mg cinnamic acid (No. 23)/kg bw once daily for the entire course of pregnancy. On day 20 of gestation, 50% of the females from all groups were terminated and the fetuses removed for examination. Fetal body weight measurements, number of survivors, bone development, and hepatic nucleic acids were determined and no significant differences between test and control animals were noted. The remaining females from both treated and control groups delivered normally on days 22–23 of gestation. Neither measurements of offspring bodyweight, size, survival number, nor general development at birth or one month following revealed any significant differences between test and control animals (Zaitsev and Maganova, 1975).

5. Recognition of GRASr status

The group of cinnamyl derivatives discussed here was determined to be generally recognized as safe (GRAS) under conditions of intended use as flavor ingredients by the FEMA Expert Panel in 1965. In 1978, the Panel evaluated the available data and affirmed the GRAS status of these flavor ingredients (GRASa). In 1993, the Panel initiated a comprehensive program to reevaluate the status of all FEMA GRAS flavor ingredients concurrent with a systematic revision of the FEMA Scientific Literature Reviews (SLRs). The group of cinnamyl derivatives was reaffirmed as GRAS (GRASr) based, in part, on their self-limiting properties as flavoring substances in food; their rapid absorption, metabolic detoxication, and excretion in humans and other animals; their low level of flavor use; the wide margins of safety between the conservative estimates of intake and the no adverse effect levels determined from subchronic and chronic studies and the lack of significant genotoxic and mutagenic potential. This evidence of safety is supported by the fact that the intake of cinnamyl derivatives as natural components of traditional foods is much greater than their intake as intentionally added flavoring substances.

6. Correction

In the Safety Assessment of allylalkoxybenzene derivatives used as flavor ingredients–methyl eugenol and estragole published by FCT in 2002, the publication was erroneously referred to as the seventh in the series. That publication was actually the sixth publication in the series of safety evaluations performed by FEMA's Expert Panel.

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