Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food and Chemical Toxicology 43 (2005) 1207–1240
www.elsevier.com/locate/foodchemtox

Review

The FEMA GRAS assessment of benzyl derivatives used as flavor ingredients

T.B. Adams [a,*], S.M. Cohen [b], J. Doull [c], V.J. Feron [d], J.I. Goodman [e], L.J. Marnett [f], I.C. Munro [g], P.S. Portoghese [h], R.L. Smith [i], W.J. Waddell [j], B.M. Wagner [k,l]

[a] *Flavor and Extract Manufacturers Association, 1620 I Street, N.W., Suite 925, Washington, DC 20006, United States*
[b] *Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198, United States*
[c] *Department of Pharmacology and Toxicology, University of Kansas Medical Center, Kansas City, KS 66103, United States*
[d] *TNO Nutrition and Food Research Toxicology, Zeist, The Netherlands*
[e] *Department of Pharmacology and Toxicology, Michigan State University, B440 Life Science Building East Lansing, MI 48824, United States*
[f] *Department of Biochemistry, Vanderbilt University, School of Medicine, Nashville, TN 37232, United States*
[g] *CanTox Inc., Mississauga, Ont., Canada*
[h] *Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN 55455, United States*
[i] *Division of Biomedical Sciences, Section of Molecular Toxicology, Imperial College, School of Medicine, South Kensington, London, SW7 2AZ, United Kingdom*
[j] *Department of Pharmacology and Toxicology, University of Louisville, School of Medicine, Louisville, KY 40292, United States*
[k] *School of Medicine, New York, New York University, NY 10016, United States*
[l] *Bernard M. Wagner Associates, Millburn, NJ 07078, United States*

Received 8 September 2004; accepted 26 November 2004

Abstract

This publication is the eighth 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 benzyl derivatives as flavoring ingredients is evaluated. The group of benzyl 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-observed-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 benzyl derivatives as natural components of traditional foods is greater than their intake as intentionally added flavoring substances.

Abbreviations: ABS, chromosomal aberration; AMS, Ames assay; *B. subtilis*, *Bacillus subtilis*; bw, body weight; CHO, Chinese hamster ovary; CoA, coenzyme A; DNA, deoxyribonucleic acid; *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; im, intramuscular; ip, intraperitoneal; iv, intravenous; LD_{50}, median lethal dose; M, Male; MLA, mouse lymphoma cell assay; NAS, National Academy of Science; NCI, National Cancer Institute; NOAEL, No-observed-adverse effect level; NR, not reported; NTP = National Toxicology Program; ppm = parts per million; SMVCE, sperm morphology and vaginal cytology examinations; *S. typhimurium*, *Salmonella typhimurium*; SCE, sister chromatid exchanges; SLR, scientific literature review; UDS, unscheduled DNA synthesis.

* Corresponding author. Tel.: +1 202 293 5800; fax: +1 202 463 8998.
E-mail address: tadams@therobertsgroup.net (T.B. Adams).

0278-6915/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2004.11.014

Keywords: Benzyl derivatives; Flavoring ingredients; FEMA GRAS

Contents

1. Chemical identity

This summary presents the key data relevant to the safety evaluation of benzyl alcohol, benzaldehyde, or benzoic acid and 34 structurally related substances for their intended use as flavoring ingredients (see Table 1). All members of this group are aromatic primary alcohols, aldehydes, carboxylic acids, or their corresponding esters or acetals. The structural features common to this group of substances include an oxygenated functional group bonded directly to a benzene ring that may be ring-substituted with alkyl substituents. The group contains the parent saturated alcohol, benzyl alcohol (No. 1), and related benzyl esters (No. 2–12); the corresponding aldehyde, benzaldehyde (No. 13), and related acetals (No. 14–17); the corresponding parent carboxylic acid, benzoic acid (No. 18), and related benzoate esters (No. 19–30). This group also includes 7 additional benzyl derivatives (No. 31–37) containing ring alkyl substituents.

The substances in this group were selected based on the criteria that: (1) all members contain a benzene ring substituted with a reactive primary oxygenated functional group or can be hydrolyzed to such a functional group, (2) the major pathway of metabolic detoxication involves hydrolysis and oxidation to yield the corresponding benzoic acid derivative which is excreted either as the free acid or the glycine conjugate, (3) they show a consistent pattern of toxicity in both short- and long-term studies, and (4) they exhibit no evidence of genotoxicity in standardized batteries of in vitro and in vivo assays. Metabolism studies in humans and animals and four 2-year bioassays performed on parent substances in this group provide the basis for a comprehensive metabolic and toxicological assessment of the group.

2. Exposure

2.1. Flavor use and natural occurrence

The total annual volume of the 37 benzyl derivatives is approximately 464,110 kg in USA (NAS, 1970, 1982,

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

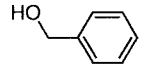
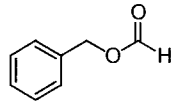
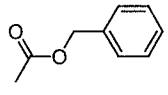
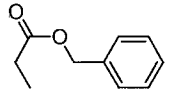
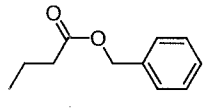
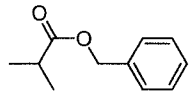
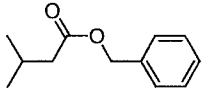
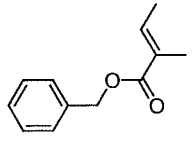
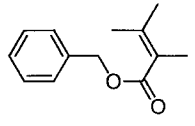
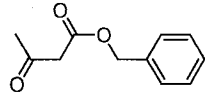
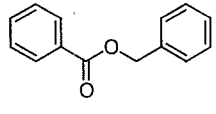
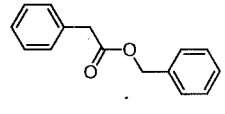
Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume (kg)[a]	Daily per capita intake ("eaters only")[b]		Annual volume in naturally occurring foods (kg)[c]	Consumption ratio[d]
				μg/d	μg/kg bw/d		
1. Benzyl alcohol	2137	100-51-6 	130,635	17,207	287	7099	0.05
2. Benzyl formate	2145	104-57-4 	386	51	1	12	0.03
3. Benzyl acetate	2135	140-11-4 	6486	854	14	3453	0.5
4. Benzyl propionate	2150	122-63-4 	748	99	2	+	NA
5. Benzyl butyrate	2140	103-37-7 	2209	291	5	+	NA
6. Benzyl isobutyrate	2141	103-28-6 	163	21	0.4	+	NA

Table 1 (*continued*)

Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume (kg)[a]	Daily per capita intake ("eaters only")[b]		Annual volume in naturally occurring foods (kg)[c]	Consumption ratio[d]
				μg/d	μg/kg bw/d		
7. Benzyl isovalerate	2152	103-38-8 	95	14	0.2	+	NA
8. Benzyl *trans*-2-methyl-2-butenoate	3330	37526-88-8 	0.2	0.03	0.0004	+	NA
9. Benzyl 2,3-dimethyl crotonate	2143	7492-69-5 	9[e]	2	0.03	–	NA
10. Benzyl acetoacetate	2136	5396-89-4 	0.5[f]	0.08	0.001	+	NA
11. Benzyl benzoate	2138	120-51-4 	31,797	4188	70	108	0.003
12. Benzyl phenylacetate	2149	102-16-9 	435	57	1	+	NA

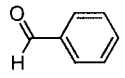
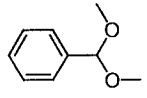
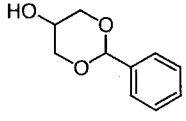
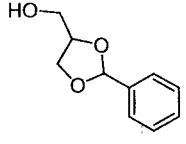
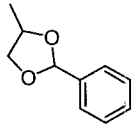
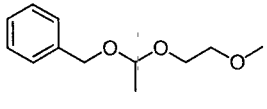
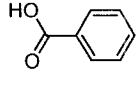
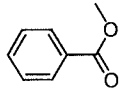
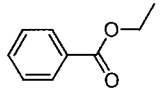
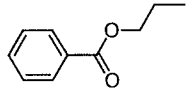
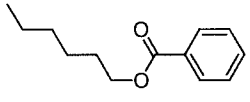
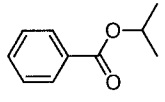
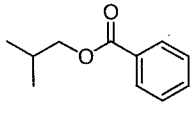
13. Benzaldehyde	2127	100-52-7 	273,516	36,027	600	57,956	0.2
14. Benzaldehyde dimethyl acetal	2128	1125-88-8 	2[g]	0.3	0.005	+	NA
15. Benzaldehyde glyceryl acetal	2129	1319-88-6  or 	2254	297	5	–	NA
16. Benzaldehyde propylene glycol acetal	2130	2568-25-4 	821	108	2	+	NA
17. Benzyl methoxyethyl acetal	2148	7492-39-9 	10[h]	2	0.03	–	NA
18. Benzoic acid	2131	65-85-0 	2604	343	6	+	NA

Table 1 (*continued*)

Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume (kg)[a]	Daily per capita intake ("eaters only")[b]		Annual volume in naturally occurring foods (kg)[c]	Consumption ratio[d]
				μg/d	μg/kg bw/d		
19. Methyl benzoate	2683	93-58-3 	1719	226	4	26	0.02
20. Ethyl benzoate	2422	93-89-0 	794	105	2	1070	1
21. Propyl benzoate	2931	2315-68-6 	1[e]	0.2	0.004	+	NA
22. Hexyl benzoate	3691	6789-88-4 	1	0.2	0.004	1711	1711
23. Isopropyl benzoate	2932	939-48-0 	1	0.2	0.004	+	NA
24. Isobutyl benzoate	2185	120-50-3 	6	1	0.01	+	NA

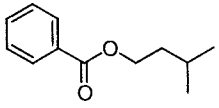
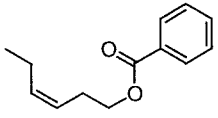
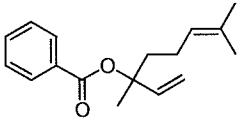
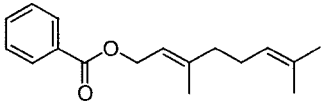
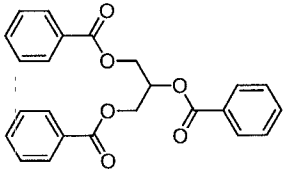
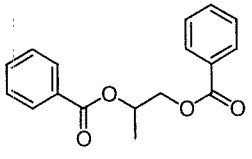
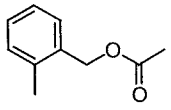
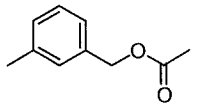
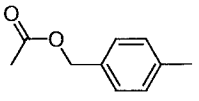
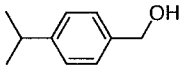
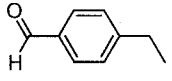
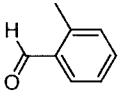
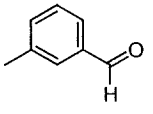
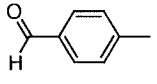
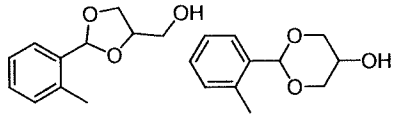
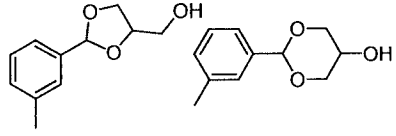
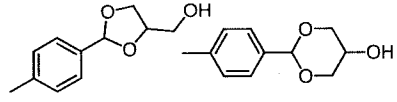
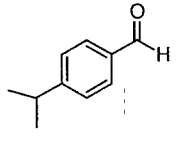
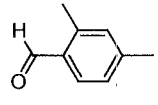
25. Isoamyl benzoate	2058	94-46-2 	250	33	1	216	1
26. *cis*-3-hexenyl benzoate	3688	25152-85-6 	1[e]	0.1	0.002	138	138
27. Linalyl benzoate	2638	126-64-7 	14[e]	2	0.03	+	NA
28. Geranyl benzoate	2511	94-48-4 	0.2	0.03	0.0004	–	NA
29. Glyceryl tribenzoate	3398	614-33-5 	370[f]	65	1	–	NA
30. Propylene glycol dibenzoate	3419	19224-26-1 	104[f]	18	0.3	–	NA

Table 1 (*continued*)

Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume (kg)[a]	Daily per capita intake ("eaters only")[b]		Annual volume in naturally occurring foods (kg)[c]	Consumption ratio[d]
				μg/d	μg/kg bw/d		
31. Methylbenzyl acetate (mixed *o*, *m*, *p*)	3702	17373-93-2  17369-57-2  2216-45-7 	0.5[g]	0.08	0.001	+	NA
32. *p*-Isopropylbenzyl alcohol	2933	536-60-7 	2	0.3	0.004	+	NA
33. 4-Ethylbenzaldehyde	3756	4748-78-1 	45[h]	8	0.1	+	NA
34. Tolualdehydes (mixed *o*, *m*, *p*)	3068	1334-78-7 (mixture) 529-20-4  620-23-5  104-87-0 	8618	1135	19	+	NA

35. Tolualdehyde glyceryl acetal (mixed *o*, *m*, *p*)	3067	1333-09-1  1333-11-5  4757-23-7 	5	1	0.01	–	NA
36. Cuminaldehyde	2341	122-03-2 	7	1	0.02	+	NA
37. 2,4-Dimethylbenzaldehyde	3427	15764-16-6 	1	0.1	0.002	+	NA

[a] From Lucas et al. (1999) or NAS (1970, 1982, 1987).

[b] 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 USA; where correction factor = 0.6 for NAS surveys and 0.8 for the Lucas et al. USA 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.

[c] Quantitative data for United States reported by Stofberg and Grundschober (1987).

[d] 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.

[e] NAS (1970).

[f] NAS (1982).

[g] NAS (1987).

[h] The volume cited is the anticipated annual volume, which was the maximum amount of flavor ingredient estimated to be used annually by the manufacturer at the time the material was proposed for flavor use.

1987; Lucas et al., 1999) (see Table 1). Approximately 94% of the total annual volume in USA is accounted for by three substances in the group [benzyl alcohol (No. 1), benzaldehyde (No. 13), and benzyl benzoate (No. 11)]. Approximately 59% of the total annual volume in USA is accounted for by benzaldehyde. The daily *per capita* intake [1] of each agent is reported in Table 1.

In addition to exposure through food and flavor sources, benzoic acid has been found endogenously in the human body. Endogeneus benzoic acid is formed through the phenylalanine–tyrosine pathway (JECFA, 1980, 1997).

Thirty (30) of the 37 substances have been reported to occur naturally in foods. They have been detected in a wide variety of fruits (apple, avocado, blackberry, blueberry, cherry, cranberry, melon, papaya, plum, raspberry, strawberry), vegetables (artichokes, asparagus, beans, cabbage, corn, leek, mushroom, potato, tomato), meats (cooked beef, cooked and roasted chicken, cured pork, shell fish), cheeses (gruyere, cheddar, mozzarella, parmesan), teas, and wines (Maarse et al., 1999).

Quantitative natural occurrence data is reported for 10 substances in the group. Five of these substances show that human oral exposure occurs predominantly in food (i.e., consumption ratio greater than or equal to 1), while the other five substances have consumption ratio less than 1 (See Table 1) (Stofberg and Kirschman, 1985; Stofberg and Grundschober, 1987).

3. Hydrolysis, absorption, distribution, excretion and metabolism

3.1. Hydrolysis

In general, aromatic esters are hydrolyzed in vivo through the catalytic activity of carboxylesterases or esterases (Heymann, 1980), the most important of which are the A-esterases. These enzymes are found throughout mammalian tissues (Anders, 1989; Heymann, 1980), but predominate in hepatocytes (Heymann, 1980).

Hydrolysis of benzyl and benzoate esters to yield the corresponding alcohols and carboxylic acids and hydrolysis of acetals to yield benzaldehyde and simple aliphatic alcohols have been reported in several in vitro experiments (see Fig. 1).

Benzyl acetate (No. 3) when incubated with rat plasma in vitro was rapidly hydrolyzed to benzyl alcohol (No. 1) with peak alcohol concentration after 4 minutes. No plasma benzyl alcohol was observed in vivo following gavage or feed administration of benzyl acetate to rats; however high plasma levels of hippuric acid and benzoic acid were detected. The absence of benzyl acetate in plasma is evidence that benzyl acetate is rapidly hydrolyzed to benzyl alcohol, which is then rapidly oxidized to benzoic acid in vivo (Yuan et al., 1995).

Benzyl acetate was readily hydrolyzed in pig liver homogenate (Heymann, 1980). The plasma half-lives ($t_{1/2}$) for the in vitro hydrolysis of a series of four alkyl benzoates [including methyl benzoate (No. 19), ethyl benzoate (No. 20) and propyl benzoate (No. 21)] and two aryl benzoates in 80% human blood plasma decreased from 210 min for ethyl benzoate to 24 min for butyl benzoate and 19 and 15 min for phenyl benzoate and benzyl benzoate, respectively (Nielson and Bundgaard, 1987).

An in vitro hydrolysis study found that benzyl phenylacetate (No. 12) was 90% hydrolyzed within 1 h and completely hydrolyzed within 2 h of incubation with a 2% pancreatin solution (Leegwater and van Straten, 1974).

Benzaldehyde related acetals readily hydrolyze to their component alcohols and benzaldehyde under acidic conditions. Benzaldehyde propylene glycol acetal (No. 16) was 97% hydrolyzed after incubation for 5 h with simulated gastric juice and intestinal fluid in vitro compared to blank incubation (Morgareidge, 1962).

3.2. Absorption, distribution and excretion

The benzyl derivatives are rapidly absorbed through the gut, metabolized primarily in the liver, and excreted in the urine as glycine conjugates of benzoic acid derivatives (Davison, 1971; Abdo et al., 1985; Temellini, 1993). Recent studies on the effect of dose, species, sex and mode of administration on the absorption, distribution and excretion of these substances are described in detail below.

Groups of 50 male Fischer 344 rats and 50 male B6C3F$_1$ mice were administered ^{14}C-benzyl acetate (No. 3) orally at levels of 5–500 mg/kg bw for rats and 10–1000 mg/kg bw for mice for five days per week for a period of two weeks. Urine and feces were collected separately over a period of 24 h following dosing. Carbon dioxide (CO_2) and other volatile substances were collected at 2, 4, 5, and 24 h following each dose. At termination, blood, liver, muscle, adipose, skin, lung, kidney, and stomach were collected and analyzed for

[1] Intake (μg/kg) calculated as follows: [[(annual volume, kg) × (1×10^9 μg/kg)]/[population × correction factor × 365 days]], where population (10%, "eaters only") = 26×10^6 for USA; where correction factor = 0.8 for the Lucas et al. and 0.6 for NAS USA surveys, and represents the assumption that only 80% and 60%, respectively, of the flavor volume was reported in the surveys (Lucas et al., 1999; NAS, 1970, 1982, 1987). For agents that were not surveyed the anticipated volume was used with a correction factor of 0.6. Intake (μg/kg bw per day) calculated as follows: [(μg per day)/body weight], where body weight = 60 kg. Slight variations may occur from rounding off.

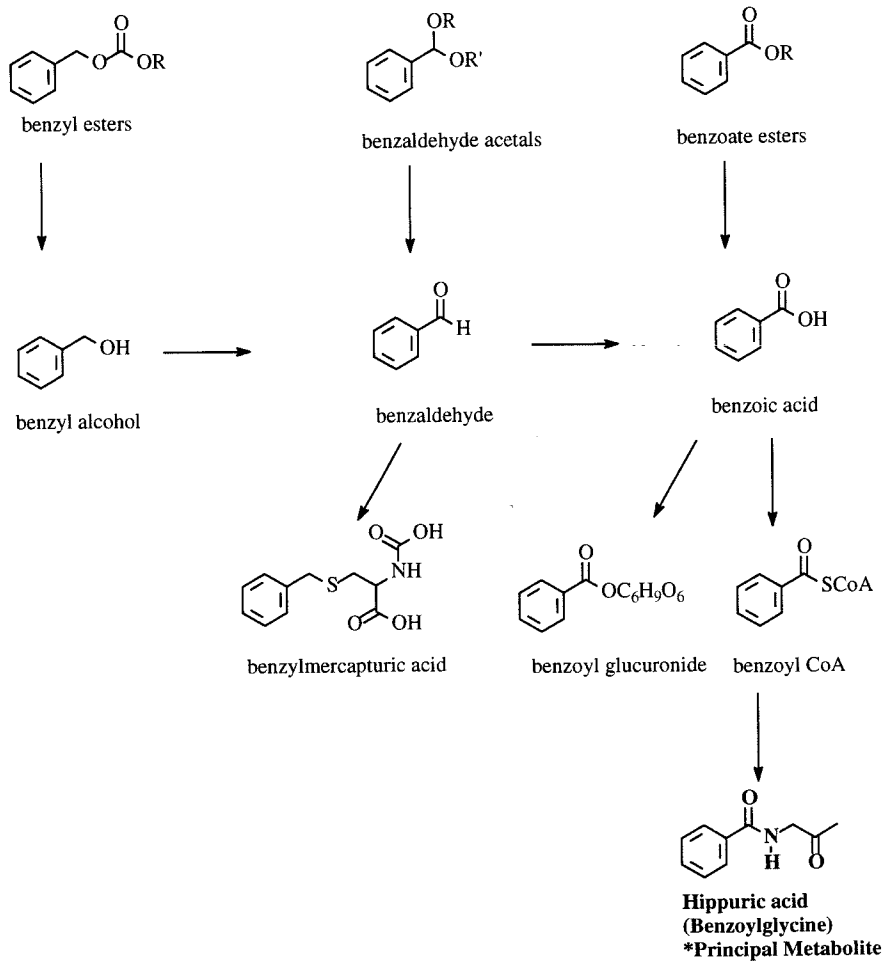


Fig. 1. Metabolism of benzyl derivatives.

radioactivity. The compound was readily absorbed from the gastrointestinal tract of both species, and approximately 90% and 0.3–1.3% of the total dose was recovered as hippuric acid in the urine and feces, respectively, within 24 h. No benzyl acetate-derived radioactivity was detected in any tissue analyzed at 24 h. The clearance pattern was not affected by repeated dosing 5 days/week for 2 weeks at 500 mg/kg bw in rats or 1000 mg/kg bw in mice. Such complete clearance indicates that benzyl acetate is readily absorbed and excreted (Abdo et al., 1985).

Following gavage administration of ^{14}C-benzyl acetate to groups of three or more male Fischer 344 rats at doses of 5, 250 or 500 mg/kg bw as the substance alone, in corn oil, or propylene glycol, 70–89% of the dose was excreted in the urine within 24 h. Approximately 4% radioactivity was detected in the feces after 72 h. The elimination of benzyl acetate and metabolites, regardless of vehicle, was largely complete after 3 days. Only negligible residues were found in tissues. Although no benzyl acetate was detected in the plasma or urine, small amounts of benzyl alcohol were detected in the plasma. At 500 mg/kg bw, unconjugated benzoic acid was the major plasma metabolite while at 5 mg/kg, hippuric acid was the major urinary metabolite. The proportion of the glucuronic acid conjugate of benzoic acid increased with increasing dose, while low levels (1.0–3.6%) of benzoic acid and benzylmercapturic acid were not affected by dose or vehicle (Chidgey and Caldwell, 1986).

Fischer 344 rats and C57Bl/6N mice were given a single oral dose of ^{14}C benzyl acetate at doses of 5 or 500 mg/kg bw (rats) or 10 mg/kg bw (mice) to determine the effects of age on disposition of benzyl acetate (McMahon et al., 1989). Age groups studied were 3–4, 9, and 25-month-old rats and 2, 13, and 25-month-old mice. In rats, approximately 80% of radioactivity was recovered in the urine in the first 24 h for all age groups. The major urinary metabolite was hippuric acid and a minor urinary metabolite was benzylmercapturic acid in rats. There was no age difference in the percentage of ^{14}C benzyl acetate excreted as hippuric acid, but the amount of excreted benzylmercapturic acid increased slightly in the 25-month-old rats. The percentage of

radioactivity excreted in the feces was slightly decreased in the 25-month-old group.

In mice, hippuric acid was the major urinary metabolite, constituting 93–96% of the total dose. Less radioactivity was excreted in the urine of 25-month-old mice than in the younger groups. Fecal excretion was a minor route and the amount was similar for all age groups. The authors concluded that formation of hippuric acid is not affected by age, but aging does affect the minor routes of metabolism and excretion of benzyl acetate in rats and mice (McMahon et al., 1989).

In a study of the influence of gavage and dietary administration on the toxicokinetics of benzyl acetate, plasma levels of benzyl alcohol, benzoic acid and hippuric acid were measured at 24 h intervals after groups of 6 F344 rats were given 500 mg/kg bw of benzyl acetate by gavage in corn oil, and groups of 12 B6C3F$_1$ mice were given 1,000 mg/kg bw of benzyl acetate by the same route. Groups of 10 rats and 10 mice were fed diets containing 2700 ppm and 10,800 ppm, respectively, of benzyl acetate for 7 days. The calculated daily dose levels were reported to be approximately 648 mg/kg bw in rats and 900 mg/kg bw in mice. Benzyl acetate was undetectable in the plasma after gavage or dietary administration. After gavage administration, benzyl alcohol could not be detected in the plasma after 5 min in mice and 10 min in rats. Peak plasma levels of benzoic acid and hippuric acid were reached within 3 h of gavage administration. Compared to the gavage mode of administration, peak plasma concentrations of benzoic acid were 40-fold less in rats and 300-fold less in mice after dietary administration. Plasma concentrations of hippuric acid were similar regardless of the mode of administration (Yuan et al., 1995).

Following administration of 375 mg ^{14}C benzoic acid (No. 18)/kg bw to rats (oral) and mice (ip), 88–89% of the radioactivity was recovered in the urine within 24 h and 91–94% after 72 h, and only 1–6% was present in the feces (Nutley, 1990).

In a study on the effects of benzyl alcohol in neonates, 4 full-term and 9 pre-term infants received intravenous (iv) or intramuscular (im) doses of 0.007–0.222 μmol benzyl alcohol (No. 1)/kg bw in medication. Maximum serum concentration levels of benzoic acid in pre-term infants were approximately 10 times those in full-term infants. Larger percentages of benzyl alcohol doses were found as benzoic acid {75.2% (iv) and 83.8%(im) vs. 38.1%(iv) and 57.7%(im)} and less as hippuric acid {64.2%(iv) and 26.3%(im) vs. 82.3%(iv) and 85.2%(im)} in pre-term infants compared to term infants. The study indicates that glycine conjugation is deficient in pre-term compared to full-term infants (LeBel et al., 1988).

After administration of oral doses of 40, 80, and 160 mg/kg bw of sodium benzoate to humans, the clearance of benzoic acid increased disproportionately to the dose while the clearance for hippuric acid was proportional to dose. Peak plasma concentrations of benzoic acid increased with increasing dose, while peak hippuric acid concentrations did not change. The data suggest that the conjugation with glycine to form hippuric acid is a saturable process in humans (Kubota et al., 1988; Kubota and Ishizaki, 1991).

Based on these studies, benzyl derivatives are expected to be rapidly absorbed, and rapidly metabolized to benzoic acid. The acid is then conjugated with glycine and excreted mainly in the urine. At high dose levels, the glycine conjugation pathway may be saturated; in which case, free benzoic acid is excreted unchanged.

3.3. Metabolism

3.3.1. Metabolism of benzyl alcohol, benzaldehyde, benzoic acid and related esters

Benzyl esters and acetals are hydrolyzed to benzyl alcohol and benzaldehyde, respectively (see Fig. 1). The alcohol and aldehyde are rapidly oxidized to benzoic acid while benzoate esters are hydrolyzed to benzoic acid. The benzoic acid derivatives are excreted primarily as the glycine conjugate. Benzoic acid is readily conjugated with glycine in liver (321 nmol/min/g) and kidney homogenate (254 nmol/min/g) (Temellini, 1993). At high dose levels formation of the glycine conjugate is glycine limited. When glycine is depleted, free benzoic acid may be excreted unchanged or as the glucuronic acid conjugate (Bray et al., 1951; Diack and Lewis, 1928; Williams, 1959).

Following oral, subcutaneous, or ip administration of ^{14}C benzyl acetate to mice (10–1000 mg/kg bw) and rats (5–500 mg/kg bw), hippuric acid was the major urinary metabolite. Minor amounts of benzyl alcohol, benzoic acid and benzylmercapturic acid were also detected in the urine (Abdo et al., 1985; Chidgey and Caldwell, 1986; Yuan et al., 1995; Clapp and Young, 1970; McMahon et al., 1989).

Using specific enzyme inhibitors, a pathway was proposed for the metabolic conversion of benzyl acetate to benzylmercapturic acid in male Fischer rats. Five hundred (500) mg/kg bw of ^{14}C benzyl acetate was administered to groups of 3–5 male Fischer rats by gavage, alone or in conjunction with ip injections of 200 mg/kg bw of pyrazole (an alcohol dehydrogenase inhibitor), 10 mg/kg bw of pentachlorophenol (a sulfotransferase inhibitor) or both. Within 24 h of administration of benzyl acetate alone, 92% of the dose was recovered in the urine, 3% in the feces and <1% in the carcasses. The combination treatment of benzyl acetate and inhibitor delayed the excretion of ^{14}C in urine compared to treatment with benzyl acetate alone. The pyrazole only group had an 11-fold increase in the amount of benzylmercapturic acid excreted in the first 24 h with a halving of the percentage of benzoyl glucuronide eliminated in the

urine. The elimination of hippuric acid was unaffected in the pyrazole treated group as compared to controls. The amount of benzylmercapturic acid was significantly increased in the combination group, but not to the same levels as the pyrazole only group. Benzyl sulphate has been shown to be converted to benzylmercapturic acid in rats (Clapp and Young, 1970) and S-benzylglutathione is formed in the presence of rat liver cytosol (Gillam, 1971). Benzyl alcohol is converted to benzylmercapturic acid in rats involving an obligatory benzylsulphate intermediate (Van Doorn et al., 1981). In the case of benzyl acetate, these results suggest that benzylmercapturic acid forms via the sulfate ester of benzyl alcohol (Chidgey et al., 1986).

Less than 5 min after single intraperitoneal doses of 770–1100 mg/kg bw of benzyl alcohol given to CD1 mice, benzyl alcohol and benzaldehyde were detected in the plasma. Animals pretreated with an alcohol dehydrogenase inhibitor (pyrazole) resulted in a 200% increase in plasma benzyl alcohol levels, while pretreatment with an aldehyde inhibitor (disulfiram) resulted in a 368% increase in plasma benzaldehyde levels. These data suggest that benzaldehyde and benzyl alcohol are interconvertible in the liver (McCloskey et al., 1986).

The principal pathway of metabolism of benzaldehyde (No. 13) includes oxidation to yield benzoic acid, which is subsequently conjugated with glycine and excreted as hippuric acid (Bray et al., 1951; NTP, 1990a,b). In the rabbit, approximately 83% of single doses of 350 or 750 mg/kg bw of benzaldehyde is excreted in the urine of both dose groups. The aldehyde is oxidized mainly to benzoic acid and excreted predominantly as hippuric acid (~68%). Other urinary metabolites detected are benzoylglucuronic acid (10%), benzoyl glucuronide (3%), free benzoic acid (1.5%), and trace amounts of benzylmercapturic acid (Laham et al., 1988).

To a minor extent, benzaldehyde is reduced to benzyl alcohol, which as the sulfate conjugate may react with glutathione to form benzylmercapturic acid (Laham and Potvin, 1987). Groups of Sprague–Dawley rats (5/group/sex) were given single oral doses of 400, 750, or 1000 mg/kg bw of pure benzaldehyde by gavage daily for 13 consecutive days. Twenty-four (24) h urinary excretion of benzylmercaputric acid was measured after the 2nd, 8th, and 13th dose. Although females in the mid- and high-dose groups exhibited a slight decrease in excretion of benzylmercapturic acid after the 8th dose, all groups showed increased urinary levels of the conjugated acid after 13 doses. An increase in dose from 400 to 1000 mg/kg bw per day resulted in a 7–8-fold increase in excreted benzylmercapturic acid.

Administration of 375 mg ^{14}C- benzoic acid/kg bw to mice (ip) and rats (oral) resulted in excretion of hippuric acid (70.2–84.2%), benzoyl glucuronide (0.7–1.8%), benzoic acid (0.4–12.8%), and 3-hydroxy-3-phenyl propionic acid (0.1–0.2%) (Nutley, 1990).

^{14}C-Benzoic acid was administered orally at doses in the range from 1 to 400 mg/kg bw to various species including primates, pigs, rabbits, rodents, cats, dogs, hedgehogs, bats, birds, and reptiles. Hippuric acid was the primary urinary metabolite in most species. The ornithine conjugate of benzoic acid, ornithic acid, was the major urinary metabolite excreted within 24 h in chickens and reptiles. Benzoyl glucuronide was predominant in the fruit bat. In humans, >99% ^{14}C was excreted as hippuric acid within 24 h (Bridges et al., 1970).

Male volunteers were given oral doses of 2000–5000 mg sodium benzoate. The 5000 mg dose group was given a 5000 mg dose of glycinel h later and 2000 mg doses every 2 h thereafter. Benzoate was excreted mainly as hippuric acid. No free benzoic acid was detected. Minor amounts of benzoylglucuronide were detected, with more formed at the 5000 mg dose than at the 2000 mg dose. Co-administration of glycine with benzoate increased the rate of hippuric acid excretion, indicating that at high dose levels, glycine is rate limiting for formation of hippuric acid (Amsel and Levy, 1969).

3.3.2. Metabolism of substituted benzyl alcohols, benzaldehydes and benzoic acid derivatives

Aromatic ring substitution has little or no influence on the principal pathway of metabolism. Oxidation of the alcohol or aldehyde to the benzoic acid derivative may be accompanied by minor amounts of side-chain oxidation or *O*-dealkylation of a ring alkoxy group.

Large doses (2 g) of cuminaldehyde (*p*-isopropylbenzaldehyde, No. 37) were administered orally to male rabbits. Urine was collected for 3 days post-treatment. Cuminaldehyde undergoes a combination of oxidation of the aldehyde function and the oxidation of the alkyl-side chain to yield 9-hydroxycuminic acid, 8-hydroxycuminic acid. Cumyl alcohol and 2-carboxyphenylpropionic acid were minor urinary metabolites (Ishida et al., 1989).

3.3.3. Summary of metabolic data

In summary, benzyl and benzoate esters and benzaldehyde acetals are readily hydrolyzed to the corresponding parent alcohol, aldehyde, or acid. Following hydrolysis, benzyl alcohol is sequentially oxidized to benzaldehyde and then benzoic acid, followed by excretion as hippuric acid. To a minor extent, benzyl alcohol may conjugate with glutathione, benzaldehyde may be reduced to benzyl alcohol, and benzoic acid may conjugate with glucuronic acid. The latter conjugation pathway may become more important as high levels of benzoic acid saturate the glycine (hippurate) conjugation pathway. At very high levels of exposure, free

benzoic acid may sequester significant quantities of acetyl CoA.

4. Toxicological studies

4.1. Acute toxicity

Oral LD_{50} values have been reported for 30 of the 37 substances in this group (see Table 2). LD_{50} values in rats are in the range from 1020 mg/kg bw for *p*-isopropylbenzyl alcohol (No. 32) to 12,300 mg/kg bw for hexyl benzoate (No. 22), indicating that the oral acute toxicity of the benzyl derivatives is very low (Graham and Kuizenga, 1945; Draize et al., 1948; Smyth et al., 1951, 1954; Jenner et al., 1964; Taylor et al., 1964; Sporn et al., 1967; Kravets-Bekker and Ivanova, 1970; Owen and Meyer, 1971; Shelanski and Moldovan, 1971; Weir and Wong, 1971; Levenstein, 1973, 1974; Moreno, 1973, 1974, 1977, 1979, 1980; DeGroot et al., 1974; Lewis and Palanker, 1979; Costello and Moore, 1984; Ciba-Geigy, 1991; Proctor and Gamble, 1992). In mice the oral LD_{50} values are in the range from 1580 mg/kg bw for benzyl alcohol (No. 1) to 9408 mg/kg bw for linalyl benzoate (No. 27) indicating that the acute oral toxicity of benzyl derivatives is very low (Jenner et al., 1964; Hoffmann-LaRoche, 1967; Kravets-Bekker and Ivanova, 1970; Sado, 1973; Shell, 1982; Schafer and Bowles, 1985). In rabbits, guinea pigs and cats the oral LD50 values are in the range from 1000 mg/kg bw for benzaldehyde (No. 13) to greater than 5000 mg/kg bw for linalyl benzoate (No. 27) indicating that the acute oral toxicity of benzyl derivatives is very low (Graham and Kuizenga, 1945; Draize et al., 1948; Jenner et al., 1964; Kravets-Bekker and Ivanova, 1970; Moreno, 1973).

4.2. Short-term and long-term studies of toxicity

Short-term toxicological studies have been reported for nine (9) representative benzyl derivatives. Short-term studies for benzyl alcohol, one benzyl ester, three benzoate esters, and two alkyl-substituted benzaldehyde derivatives are summarized in Table 2 and described in detail below.

4.2.1. Mice

4.2.1.1. Benzyl alcohol (No. 1). Groups of 10 male and 10 female B6C3F$_1$ mice were administered 0 (control), 50, 100, 200, 400, or 800 mg/kg bw benzyl alcohol daily by gavage 5 days per week for a period of 13 weeks. No compound related histopathology was observed. Deaths occurred in most groups, however, all but one was attributed to the gavage procedure. Mean body weights of males and females were reduced by 5% and 8%, respectively, at the 800 mg/kg bw per day dose level and body weights of females were reduced 5% at the 400 mg/kg bw per day. At the highest dose level, staggering was seen in both male and female mice during the first 2 weeks of the study. The dose level of 100 mg/kg bw per day produced no adverse effects (NTP, 1989).

4.2.1.2. Benzyl acetate (No. 3). Benzyl acetate was administered to groups of 10 male B6C3F$_1$ mice in corn oil daily by gavage at doses of 0 (control), 62.5, 125, 250, 500, or 1,000 mg/kg bw 5 days per week for a period of 13 weeks. Groups of 10 female mice were administered benzyl acetate by gavage at doses of 0 (control), 125, 250, 500, 1,000, or 2,000 mg/kg bw on the same schedule. Eight of the 10 female mice that received 2,000 mg/kg bw died; however, one of these deaths was due to gavage error. Compound-related clinical signs, including trembling, inactivity, labored breathing, and depressed body temperature, were reported in the high-dose mice. Gross examination after necropsy and histopathological examination failed to reveal any effects related to administration of the test substance (NTP, 1986). Subsequent microscopic examination revealed hippocampal necrosis in one female at the 1000 mg/kg bw per day dose (NTP, 1993a). No adverse effects were observed at dose levels less than or equal to 500 mg/kg bw per day (NTP, 1986).

For a period of 13 weeks, 5 days per week, groups of 10 male and 10 female B6C3F$_1$ mice were maintained on diets containing 0, 3130, 6250, 12,500, 25,000, or 50,000 ppm benzyl acetate calculated to provide an average daily intake of 0 (control), 425, 1000, 2000, 3700, or 7900 mg benzyl acetate/kg bw for males and 650, 1280, 2980, 4300, or 9400 mg/kg bw for females (FDA, 1993). At all dose levels, statistically significant ($P < 0.01$) decreases in body weights were reported compared to the control group in both sexes. Absolute and relative organ weights were affected by lower terminal body weights. A non-statistically significant decrease in feed consumption was reported at all dose levels, which may have been due to poor palatability. Hematological examination and clinical chemistry determinations show normal values. In the 50,000 ppm group, brain lesions, consisting of hippocampal necrosis and cerebellar hemorrhage, were reported in 4 mice (1 male, 3 females). Hepatocellular necrosis also occurred in the male mouse with brain lesions (NTP, 1993a).

4.2.1.3. Benzaldehyde (No. 13). Groups of 10 male and 10 female B6C3F$_1$ mice were administered doses of 0 (control), 75, 150, 300, 600 or 1200 mg benzaldehyde/kg bw by gavage daily in corn oil 5 days per week for a period of 13 weeks. At the 1200 mg/kg bw per day dose, 9/10 males and 1/10 females died during the first week of the study. Mean body weight of males at the 600 mg/kg bw per day level were 9% less than that of controls. Mild to moderate renal tubule degeneration

Table 2
Acute, short- and long-term toxicity studies for benzyl derivatives used as flavor ingredients

Flavoring ingredient	Oral acute studies	Reference	Short- and long-term studies	Time (days)/ route	NOAEL (mg/kg bw)	Reference
	Oral LD_{50} mg/kg bw (Species)		Species, sex[a]			
Benzylalcohol	1040 (Rabbit)	Graham and Kuizenga (1945)	Rat, MF	91	100	NTP (1989)
Benzylalcohol	2979 (Rat)	Ciba-Geigy (1991)	Rat, MF	16	125	NTP (1989)
Benzylalcohol	2080 (Rat)	Graham and Kuizenga (1945)	Rat, MF	721	<200	NTP (1989)
Benzylalcohol	1230 (Rat)	Jenner et al. (1964)	Mouse, MF	91	100	NTP (1989)
Benzylalcohol	1570 (Rat)	Proctor and Gamble (1992)	Mouse, MF	16	250	NTP (1989)
Benzylalcohol	3100 (Rat)	Smyth et al. (1951)	Mouse, MF	721	200	NTP (1989)
Benzylalcohol	1580 (Mouse)	Jenner et al. (1964)				
Benzyl formate	<5000 (Rat)	Shelanski and Moldovan (1971)	Rat, MF	14	500	NTP (1986)
Benzyl acetate	2640 (Rabbit)	Graham and Kuizenga (1945)	Rat, MF	91	250	NTP (1986)
Benzyl acetate	2490 (Rat)	Jenner et al. (1964)	Rat, MF	91	460	NTP (1993a)
Benzyl acetate	3690 (Rat)	Graham and Kuizenga (1945)	Rat, MF	721	<130	NTP (1993a)
Benzyl acetate			Rat, MF	721	250	NTP (1986)
Benzyl acetate			Mouse, MF	14	1000	NTP (1986)
Benzyl acetate			Mouse, M	91	500	NTP (1986)
Benzyl acetate			Mouse, MF	721	<35	NTP (1993a)
Benzyl acetate			Mouse, MF	721	<500	NTP (1986)
Benzyl propionate	3300 (Rat)	Moreno (1973)				
Benzyl butyrate	1850 (Rat)	Moreno (1973)				
Benzyl butyrate	2330 (Rat)	Jenner et al. (1964)				
Benzyl isobutyrate	2850 (Rat)	Owen and Meyer (1971)				
Benzyl isovalerate	<5000 (Rat)	Moreno (1974)				
Benzyl *trans*-2-methyl-2-butenoate	>5000 (Rat)	Moreno (1979)				
Benzyl benzoate	2240 (Cat)	Graham and Kuizenga (1945)				
Benzyl benzoate	2016 (Rabbit)	Draize et al. (1948)				
Benzyl benzoate	1680 (Rabbit)	Graham and Kuizenga (1945)				
Benzyl benzoate	1120 (Guinea pig)	Draize et al. (1948)				
Benzyl benzoate	1904 (Rat)	Draize et al. (1948)				
Benzyl benzoate	2800 (Rat)	Graham and Kuizenga (1945)				
Benzyl benzoate	1568 (Mouse)	Draize et al. (1948)				
Benzyl phenylacetate	>5000 (Rat)	Owen and Meyer (1971)				
Benzaldehyde	1000 (Guinea pig)	Jenner et al. (1964)	Rat, MF	91	200	Kluwe et al. (1983)
Benzaldehyde	1300 (Rat)	Jenner et al. (1964)	Rat, MF	91	200	NTP (1990a, 1990b)
Benzaldehyde	2850 (Rat)	Sporn et al. (1967)	Rat, MF	189-196	>50	Hagan et al. (1967)
Benzaldehyde	1300 (Rat)	Taylor et al. (1964)	Rat, MF	721	<200	NTP (1990a, 1990b)
Benzaldehyde	1250 (Mouse)	Schafer and Bowles (1985)	Mouse, MF	91	300	Kluwe et al. (1983)
Benzaldehyde			Mouse, MF	91	300	NTP (1990a, 1990b)
Benzaldehyde			Mouse, MF	728	<200	NTP (1990a, 1990b)
Benzaldehyde dimethyl acetal	1220 (Rat)	Moreno (1977)				
Benzaldehyde glyceryl acetal	3749 (Rat)	Levenstein (1974)				

(*continued on next page*)

Table 2 (*continued*)

Flavoring ingredient	Oral acute studies	Reference	Short- and long-term studies	Time (days)/ route	NOAEL (mg/kg bw)	Reference
	Oral LD_{50} mg/kg bw (Species)		Species, sex[a]			
Benzaldehyde glyceryl acetal	2750 (Rat)	Moreno (1980)				
Benzaldehyde propylene glycol acetal	3000 (Rat)	Lewis and Palanker (1979)				
Benzoic acid	1250 (Mouse)	Schafer and Bowles (1985)	Rat, MF	540-730	<370	Sodemoto and Enomoto (1980)
Benzoic acid	1996 (Mouse)	Sado (1973)	Mouse, MF	90	80	Shtenberg and Ignat'ev (1970)
Benzoic acid	1950 (Mouse)	Shell (1982)	Mouse, MF	510	40	Shtenberg and Ignat'ev (1970)
Methyl benzoate	2170 (Rabbit)	Graham and Kuizenga (1945)	Rat	183	0.005	Kravets-Bekker and Ivanova (1970)
Methyl benzoate	4100 (Guinea pig)	Kravets-Bekker and Ivanova (1970)				
Methyl benzoate	2170 (Rat)	Graham and Kuizenga (1945)				
Methyl benzoate	1350 (Rat)	Jenner et al. (1964)				
Methyl benzoate	3500 (Rat)	Kravets-Bekker and Ivanova (1970)				
Methyl benzoate	3420 (Rat)	Smyth et al. (1954)				
Methyl benzoate	3330 (Mouse)	Jenner et al. (1964)				
Methyl benzoate	3000 (Mouse)	Kravets-Bekker and Ivanova (1970)				
Ethyl benzoate	2630 (Rabbit)	Graham and Kuizenga (1945)				
Ethyl benzoate	2100 (Rat)	Graham and Kuizenga (1945)				
Ethyl benzoate	6480 (Rat)	Smyth et al. (1954)				
Hexyl benzoate	12,300 (Rat)	Smyth et al. (1951)				
Isopropyl benzoate	3730 (Rat)	Smyth et al. (1951)				
Isobutyl benzoate	3685 (Rat)	Levenstein (1973)				
Isoamyl benzoate	6330 (Rat)	Weir and Wong (1971)				
cis-3-Hexenyl benzoate	>5000 (Rat)	Moreno (1976)				
Linalyl benzoate	>5000 (Rabbit)	Moreno (1973)				
Linalyl benzoate	>5000 (Rat)	Moreno (1973)				
Linalyl benzoate	9408 (Mouse)	Hoffmann-LaRoche (1967)				
Geranyl benzoate	>5000 (Rat)	Moreno (1973)				
Glyceryltribenzoate			Rat, MF	90	604	Carson (1972a)
Propylene glycol dibenzoate			Rat, MF	90	2541	Carson (1972b)
p-Isopropylbenzyl alcohol	1020 (Rat)	Moreno (1973)				
4-Ethylbenzaldehyde	1970 (Rat)	Costello and Moore (1984)				
Tolualdehydes (mixed *o*, *m*, *p*)	2250 (Rat)	Moreno (1973)	Rat, MF	90	36.1	Oser et al. (1965)
Tolualdehydes (mixed *o*, *m*, *p*)			Rat, MF	91	250	Brantom et al. (1972)
Tolualdehyde glyceryl acetal	3400 (Rat)	Moreno (1972)				
Cuminaldehyde	1390 (Rat)	Jenner et al. (1964)				
2,4-Dimethyl-benzaldehyde	<5000 (Rat)	DeGroot et al. (1974)	Rat, MF	14	1.75	DeGroot et al. (1974)

[a] M = Male; F = Female. If not listed, sex was not specified in the report.

was reported in all males at the 1200 mg/kg bw per day dose level and 1 male at the 600 mg/kg bw per day dose level. These lesions were not observed in lower dose levels in males or any dose level in females. The no adverse

effect level was reported to be 300 mg/kg bw per day (NTP, 1990a).

4.2.1.4. Benzoic acid (No. 18). Groups of 50 male and 50 female crossbred white mice (strain not specified) were administered 80 mg benzoic acid/kg bw by oral intubation daily for 3 months. Weight gain of the test animals was reduced compared to control animals. This was not due to reduced feed intake, but it may have been due to stress factors such as food restriction, low temperature, swimming test, centrifugation, carbon tetrachloride detoxication test or kidney function testing (Shtenberg and Ignat'ev, 1970). Benzoic acid was administered orally to groups of 25 male and 25 female mice at 40 mg/kg bw daily for 17 months. Survival at 2.5 months was greater for the benzoic acid group (68%) than that for the control group of males (60%) or female rats (62%) (Shtenberg and Ignat'ev, 1970).

4.2.2. Rats

4.2.2.1. Benzyl alcohol (No. 1). Benzyl alcohol was administered to groups of 10 male and 10 female F344/N rats at doses of 0 (control), 50, 100, 200, 400 or 800 mg/kg bw in corn oil daily by gavage five days per week for a period of 13 weeks. After dosing, staggering, lethargy and labored breathing occurred in both sexes at 800 mg/kg bw. Eight (8) males and 2 females at 800 mg/kg bw per day dose died after treatment, with the deaths of 4 males and one female being attributed to gavage errors. Reduction in the relative weight gain of female rats in the 200 and the 400 mg/kg bw per day groups was observed. At the end of the study, body weights of males and females at the 800 mg/kg bw level were reduced by 7% and 5%, respectively. At the 800 mg/kg bw per day level, histopathologic examination revealed necrosis of the dentate gyrus of the hippocampus in all surviving male (9) and female (7) rats, skeletal muscle necrosis in 5 males, kidney nephrosis in 6 males, consisting of degeneration of the tubular epithelium. The renal lesions were non-specific and similar to age related renal disease. No adverse effects were reported at 100 mg/kg bw per day (NTP, 1989).

4.2.2.2. Benzaldehyde (No. 13). Groups of 10 male and 10 female F344/N rats were administered benzaldehyde five days per week for a period of 13 weeks by corn oil gavage at doses of 0 (control), 50, 100, 200, 400 or 800 mg/kg bw. Prior to the end of the study, 6/10 males and 3/10 females at 800 mg/kg bw per day, 1/10 female at the 400 mg/kg bw per day and one control female died. Surviving male rats at 800 mg/kg bw per day had body weights 26% lower than those of controls. At the highest dose, there was necrosis of the cerebellum and neurons of the hippocampus, forestomach hyperplasia and hyperkeratosis, liver degeneration (males only), and kidney tubular epithelial degeneration. No adverse effects were observed at dose levels of 200 mg/kg (NTP, 1990a,b).

Groups of 5 male and 5 female Osborne–Mendel rats were maintained on a diet calculated to provide an average daily intake of 50 mg benzaldehyde/kg of food for a period of 27–28 weeks (Hagan et al., 1967). Weekly measurement of body weight and food intake revealed no significant difference between test animals and controls. At termination, hematological examinations revealed normal values. At necropsy, no differences were reported between major organ weights of test and control animals. Gross examination and histopathological examinations failed to reveal any changes related to the administration of the test substance. No adverse effects were seen at the dietary level examined (50 mg/kg of food) which was calculated to be 600 μg benzaldehyde/kg bw per day (FDA, 1993).

4.2.2.3. Benzoic acid (No. 18). Male and female Fischer 344 rats were fed 0, 1, or 2% sodium benzoate in the diet (approximately 0, 370, or 735 mg/kg bw per day for males and 0, 445, or 880 mg/kg bw per day for females) for a period of 18–24 months (Sodemoto and Enomoto, 1980). Mortality of all groups was affected by hemorrhagic pneumonia; however, there was no reported difference in mortality, growth, or food intake between treated and control rats. There was also no significant difference in tumor response in treated as compared to control rats.

4.2.2.4. Benzyl acetate (No. 3). Groups of F344 male rats were maintained on diets providing 0 (control), 20,000, 35,000, or 50,000 ppm of benzyl acetate 5 days a week for 103 weeks. These dietary levels correspond to an average daily intakes of 0, 1000, 1750, 2500 mg/kg bw of benzyl acetate (FDA, 1993). No effects on mortality or body weights of rats were reported at dietary concentrations of 20,000 ppm. Compound-related neuronal necrosis was seen in the groups receiving 35,000 or 50,000 ppm benzyl acetate. When a glycine supplement was fed in conjunction with the highest dose, mortality and signs of toxicity were significantly reduced. The authors concluded that adequate levels of glycine were instrumental in reducing the toxicity of benzyl acetate (Abdo et al., 1998).

Groups of 10 male and 10 female F344/N rats were administered 0 (control), 62.5, 125, 250, 500 or 1000 mg benzyl acetate/kg bw in corn oil daily by gavage 5 days per week for a period of 13 weeks. Male and female rats receiving 1000 mg/kg bw per day and females receiving 500 mg/kg bw per day showed signs of ataxia, trembling and sluggishness. On day 86, 2 males and one female died at the highest dose. At 1000 mg/kg bw per day, males showed depressed mean body weight (12%). At necropsy, thickened stomach walls were reported in 2 of 8 surviving males and 4 of 9 surviving

females. No adverse effects were observed at 250 mg/kg bw per day (NTP, 1986). At 1000 mg/kg bw per day, histopathologic reexamination of brains revealed hippocampal necrosis in 8 males and 4 females, the severity being greater in males (NTP, 1993a).

For a period of 13 weeks, groups of 10 male and 10 female F344/N rats were maintained on diets containing 0, 3130, 6250, 12500, 25,000, or 50000 ppm of benzyl acetate. These dietary levels were calculated to provide an average daily intake of 0 (control), 230, 460, 900, 1750, or 3900 mg benzyl acetate/kg bw for males and 0 (control), 240, 480, 930, 1870, or 4500 mg benzyl acetate/kg bw for females (FDA, 1993). At the highest dose level, 9/10 rats of each sex died before completion of the study. Final mean body weights of males and females at the highest dietary level were less than half that of the control group. Males at 1750 mg/kg bw per day showed a 10% decrease in mean body weight compared to that of controls. Food consumption in the males at 1750 and 3900 mg/kg bw and females at 4500 mg/kg bw per day was reduced compared to controls. Serum cholesterol was significantly decreased in females at the three highest dose levels. Substance-related histopathology in males and females at the highest dose level included necrosis of neurons and glial cells in the cerebellum and hippocampus, renal tubule degeneration and regeneration, and degeneration and hyperplasia of the sarcolemmal nuclei in the skeletal muscles of the thigh and in the tongue. Indirect treatment-related effects secondary to the poor condition of the animals included bone marrow depletion, thymus atrophy, degeneration of splenic lymphoid follicles, zymogen granule depletion, and increased basophilia of the pancreatic acinar cells, erosion of the glandular stomach, secretory depletion in of the seminal vesicles and immature/hypoplastic uterus. At termination, examination of females of the control, 1870 and 4500 mg/kg bw per day dose groups showed an increase in the volume, surface, and numerical density of hepatic peroxisomes in treated groups compared to those of controls. At 900 mg/kg bw per day and higher dose levels, testicular changes, including aspermatogenesis and atrophy of the seminiferous tubules were seen. No adverse effects were observed at dosages of 480 mg/kg bw per day (NTP, 1993a).

In an earlier 4-month study, no pancreatic tumors were reported in male F344 and Lewis rats administered 500 mg/kg bw per day of benzyl acetate by gavage for 5 days a week (Longnecker et al., 1986).

4.2.2.5. Benzyl butyrate (No. 5). For a period of 12 weeks, rats (unspecified strain, 12/sex/group) were maintained on a diet containing a mixture of six aromatic esters at levels calculated to provide an average daily target intake of 0 or100 mg/kg bw per day. Six aromatic esters commonly used in foods (ethyl benzoate, 0.15 ppm; isobutyl benzoate, 25 ppm: benzyl acetate, 18.7 ppm; benzyl butyrate, 25 ppm; ethyl methylphenylglycidate, 25 ppm; and glycidate M-116, 25 ppm) were blended and incorporated in the diet in the ratio of practical use levels in foods. The blend was fed in a nutritionally adequate diet to weanling rats for the 12-week period at a level computed to be 100 times the assumed flavor intake for man (on a per kilogram of body weight basis). Throughout the experimental period the group receiving this aromatic ester blend exhibited normal body weight gain, food consumption, efficiency of food utilization, appearance, and behavior. At the end of the feeding period, blood and urine samples were taken from 3 males and 3 females. There was no significant difference between blood hemoglobin and urine glucose levels in test and control groups. Traces of albumin present in urine specimens from the control as well as the test group are not regarded as significant. At autopsy no abnormalities were observed relating to the test materials and the weights of livers and kidneys were within normal limits for both groups. These experimental data indicate that no toxic effect was manifest in rats as a result of feeding a blend of six aromatic esters in common use as flavoring agents for a 12-week period at a daily dosage level calculated to be 126 mg/kg bw per day (Oser, 1957).

4.2.2.6. Tolualdehydes—mixed o, m, p (No. 34). For a period of 90 days, groups of 15 male and 15 female weanling Sprague–Dawley rats, individually housed, were provided with a diet containing tolualdehyde at levels calculated to provide an average daily intake of 36.1 mg/kg bw. Substances were dissolved in acetone and blended into a basal laboratory diet to yield the required daily intake for the test substance. Control diet was the basal laboratory diet admixed with acetone. Dietary acetone was evaporated before presentation to the animals. Samples of the treatment diet were taken weekly for assessment of stability and concentration of the test material. Daily observations of appearance, behavior, appetite, elimination, gross signs of toxic effects, and mortality were similar among test and control animals. Weekly measurement of body weights and food consumption revealed no significant differences between test and control animals. Hematological examinations, blood chemical determinations, and urine analysis performed during weeks 6 and 12 on 8 males and 8 females revealed normal values. At necropsy, the weights of the liver and kidneys were recorded. Tissues from major organs of 8 male and 8 female rats were subsequently preserved in formalin. In addition, the livers and kidneys of the remaining 7 animals were examined for histopathological changes. There was no difference in absolute or relative organ weights between test and control animals or any evidence of gross pathology or histopathology. The dietary intake of

36.1 mg/kg bw per day produced no changes attributable to tolualdehyde (Oser et al., 1965) is greater than 1,000 times the daily *per capita* intake ("eaters only") of 19 μg tolualdehydes/kg bw from use as a flavoring agent in USA.

Groups of 15 male and 15 female CFE rats were administered 0 (control), 50, 250, or 500 mg tolualdehyde/kg bw in corn oil daily by oral intubation for a period of 13 weeks. Additional groups of 5 rats per sex were given 0, 250, or 500 mg/kg bw daily for 2 or 6 weeks by the same method. Weekly measurement of body weights and food and water intake revealed that the female group at 500 mg/kg bw per day exhibited significantly lower body weight after 2 weeks. No body weight changes were observed in any other group regardless of the study duration (2, 6, or 13 weeks). Hematological examination, blood chemical determinations, and urine analysis performed at 2, 6, or 13 weeks revealed a transient increase in erythrocyte count, hematocrit, and hemoglobin concentration in males, but only at 2 weeks. At necropsy at week 13, measurement of organ weights revealed a significant decrease (9–18%) in absolute or relative small intestine weight in all treatment groups and decreased relative pituitary weights in females at 500 mg/kg bw per day. The magnitude of the change in mean small intestine weights was not dose related. In a second study, groups of 30 female rats were administered 0 or 500 mg/kg bw in corn oil by gavage daily for 13 weeks. There was no significant difference in absolute or relative mean small intestine weights between the control and test group in the second study or between the control group in the second study and female dosed group in the first 13-week study. The authors noted that, for some unknown reason, the small intestine weights of the control animals in the first 13-week study were abnormally high. The organ weight changes were not associated with any evidence of gross and histopathological abnormalities. The authors reported a no observable adverse effect level of 250 mg/kg bw per day (Brantom et al., 1972).

4.2.2.7. Glyceryl tribenzoate (No. 29) and Propylene glycol dibenzoate (No. 30). For a period of 90 days, 4 groups each consisting of 15 male and 15 female weanling FDRL-weanling rats, individually housed, were maintained on a diet containing glyceryl tribenzoate or propylene glycol dibenzoate at levels calculated to provide an average daily intake of 120, 604 or 2571 mg/kg bw or 126, 633, or 2,541 mg/kg bw, respectively. Daily observations of appearance, behavior, appetite, elimination, gross signs of toxic effects, and mortality were similar among test and control animals. Weekly measurement of body weights and food consumption revealed a depressed growth rate and efficiency of food utilization in high dose males in the glyceryl tribenzoate study. No significant differences in these parameters were observed in the propylene glycol dibenzoate study. In either study, hematological examinations, blood chemical determinations, and urine analysis performed during weeks 6 and 12 on 8 males and 8 females from each group revealed normal values. At necropsy, the weights of the liver and kidneys were recorded. Tissues from major organs of 8 male and 8 female rats were subsequently preserved in formalin. Histopathologic examinations were made on hematoxylin and eosin-stained sections. In addition, the livers and kidneys of the remaining seven animals were examined for histopathological changes. There was no difference in absolute or relative organ weights between test and control animals or any evidence of gross pathology or histopathology in either study. Based on the depressed weight gain in high dose males, the no observable adverse effect level for glyceryl tribenzoate was reported to be 604 mg/kg bw per day (Carson, 1972a). This dose is greater than 100 times the daily *per capita* intake of 1 μg/kg bw from use of glyceryl tribenzoate as a flavoring substance in the USA. The NOAEL of 2541 mg/kg bw per day is greater than 100,000 times the daily *per capita* intake ("eaters only") of 0.2 μg propylene glycol dibenzoate/kg bw from use as a flavoring ingredient in the USA.

4.2.2.8. 2,4-Dimethylbenzaldehyde (No. 37). Groups of 5 male and 5 female rats were administered 0 (control), 0.175 or 1.75 mg 2,4-dimethylbenzaldehyde/kg bw by stomach tube daily, 6 days per week for a period of 14 days. At 1.75 mg/kg bw per day, male rats showed an increased relative liver weight. Female rats showed no significant difference in relative liver weight at the same dose level. Histopathological examination of the liver and kidneys revealed no abnormalities associated with the administration of dimethylbenzaldehyde (DeGroot et al., 1974).

4.3. Long-term studies of toxicity and carcinogenicity

The results of six long-term toxicity and carcinogenicity studies with 4 representative benzyl derivatives are summarized in Table 2 and described below.

4.3.1. National toxicology program studies

4.3.1.1. Benzyl alcohol (No. 1), benzaldehyde (No. 13), benzyl acetate (No. 3). The National Toxicology Program (NTP) conducted four long-term toxicity and carcinogenicity studies on three members of this group: benzyl alcohol, benzaldehyde and benzyl acetate (a gavage and a microencapsulation study). Based on extensive metabolic data that benzyl acetate is readily hydrolyzed to benzyl alcohol in vivo, and benzyl alcohol is rapidly oxidized to benzaldehyde in vivo, the results of all four 2-year bioassay studies are reviewed together.

Each of the three test compounds were administered in corn oil gavage five days per week to groups of 50 F344/N rats and B6C3F$_1$ mice for a period of two years at two dose levels. An additional study was conducted in which test groups of 60 rats or mice were maintained on diets containing benzyl acetate. Animals were observed once weekly for 12–13 weeks and at least monthly thereafter. All animals were subject to necropsy after death, or at the end of the study. Throughout these studies, mean body weights were comparable among all groups, except for rats and mice maintained on the high dose level of benzyl acetate in the feed, which was reduced (5–16%) due to decreased feed consumption. Increased mortality seen in some of the groups was attributable to the gavage procedure, reflux and aspiration of the gavage material into the lungs, or administration errors resulting in direct disposition of material into the lungs (NTP, 1986, 1989, 1990a,b, 1993b).

4.3.1.2. Results and discussion

4.3.1.2.1. Benzyl alcohol (No. 1) and benzaldehyde (No. 13). According to the NTP, "under conditions of the 2 year gavage study, there was no evidence of carcinogenic activity of benzyl alcohol in male or female F344/N rats or B6C3F1 mice receiving either 200 or 400 mg/kg bw."

NTP also concluded "under conditions of the 2 year gavage studies, there was no evidence of carcinogenic activity for male or female F344/N rats receiving 200 or 400 mg/kg bw/d benzaldehyde. However, there was some evidence of carcinogenic activity of benzaldehyde in B6C3F$_1$ mice at 300 or 600 mg/kg bw, as indicated by increased incidences of squamous cell papillomas and hyperplasia of the forestomach." (NTP, 1989, 1990a).

The occurrence of squamous cell papillomas and forestomach hyperplasia in rodents is common in NTP bioassay gavage studies in which a high concentration of an irritating material in corn oil is delivered daily by tube into the forestomach for two years. High concentrations of aldehydes (i.e. malonaldehyde, furfural, and benzaldehyde) (NTP, 1988, 1990a,b) and other irritating substances (i.e. dihydrocoumarin and coumarin) (NTP, 1992, 1993b) delivered in corn oil by gavage are consistently associated with these phenomena in the forestomach of rodents. Squamous cell papillomas are benign lesions of surfaces covered with squamous epithelium. A majority of papillomas arise as a result of chronic irritation, or less frequently from infection from some strains of viruses (Smith and Ford, 1993). Additionally, forestomach hyperplasia and papillary proliferation in these studies did not progress to squamous cell carcinomas.

The relevance of the appearance of forestomach tumors in rodents to potential carcinogenic targets in humans has been the subject of much investigation (Grice, 1988; Wester and Kroes, 1988; Clayson et al., 1990). Although it has been suggested that the mucosa of the rodent forestomach is similar to that of the human esophagus, this is clearly not the case. The rodent forestomach has a storage function and contains mucosa of keratinizing squamous epithelium that is constantly exposed to the strong acid medium of the gastric contents. Conversely, the esophagus has no storage capacity and contains non-keratinizing squamous epithelium that is extremely sensitive to the adverse effects of strong acid medium. The esophagus has no significant contact with food contents in that it is a muscle that exerts a motive action on food contents propelling them from the pharynx to the stomach.

Apparently, the combination of daily introduction of a dosing tube into the forestomach and delivery of high concentrations of an irritating test material in corn oil, which itself is a mild irritant and mitogen, was the likely source of the papillomas in the rodent forestomach. This conclusion is supported by the observation that the occurrence of squamous cell papillomas and forestomach hyperplasia in gavage administration of a test material in corn oil for 2 years (NTP, 1986) disappear when the same substance is administered at similar intake levels in the diet (NTP, 1993a). Therefore, the appearance of these benign lesions in the 2-year rodent bioassay has no relevance to humans, given that human exposure occurs when low levels of benzaldehyde are consumed in the diet.

4.3.1.2.2. Benzyl acetate (No. 3). In the first 2-year bioassay, F344/N rats and B6C3F1 mice received benzyl acetate in corn oil by gavage daily, five days per week, for a period of 103 weeks at doses of 0 (control), 250, or 500 mg/kg bw and 0 (control), 500 or 1000 mg/kg bw, respectively. The NTP concluded: "Under conditions of the gavage studies, benzyl acetate administration was associated with increased incidence of acinar cell adenoma of the exocrine pancreas in male F344/N rats. No evidence of carcinogenicity was found for female F344/N rats. For male and female B6C3F1 mice there was evidence of carcinogenicity, in that benzyl acetate caused an increased incidence of hepatocellular neoplasms particularly adenoma, and squamous cell neoplasms of the forestomach." (NTP, 1986).

In the subsequent benzyl acetate dietary study, F344/N rats and B6C3F1 mice were maintained on diets containing 0 (control), 3000, 6000, or 12,000 ppm and 0 (control), 330, 1000, or 3000 ppm, respectively, for 103 weeks. This corresponds to 0, 130, 260, or 510 mg/kg bw and 0, 145, 290, or 575 mg/kg bw for male and female rats, respectively, and 0, 35, 110 or 345 mg/kg bw and 0, 40, 130 or 375 mg/kg bw for male and female mice, respectively. In this study NTP concluded: "under conditions of these 2-year feed studies, there was no evidence of carcinogenic activity of benzyl acetate in male or female F344/N rats receiving 3000, 6000, or 12,000 ppm. There was no evidence of carcinogenic

activity of benzyl acetate in male or female B6C3F1 mice receiving 330, 1000, or 3000 ppm." (NTP, 1993a).

Upon completion of the second study, the NTP concluded that the increased incidence of pancreatic acinar cell neoplasms specific to male rats reported in the earlier study was probably due to the use of corn oil as a vehicle in the gavage study. Administration of high levels of fat to experimental animals has been shown to enhance the development of spontaneous and chemical-induced neoplasms (NTP, 1994a,b). More specifically, administration of corn oil and other high-fat vehicles by gavage daily at doses of 2.5, 5, or 10 ml/kg bw for 2 years was associated with an increased incidence of proliferative lesions of the exocrine pancreas (NTP, 1994a).

The increased incidence of hepatocellular adenomas observed in the gavage study was not observed in the dietary study. The NTP proposed that the difference was due to the different mode of administration and the resulting higher plasma levels of benzyl acetate metabolites (benzoic acid) in the gavage study (NTP, 1993a). In a comparative toxicokinetic study of gavage and dietary mode of administration discussed earlier (Yuan et al., 1995), peak plasma concentrations of benzoic acid were higher in the gavage study than those in the dietary study. Although daily dietary intake level and gavage dose levels were similar, gavage administration saturated the benzoic acid elimination pathway. Hippuric acid plasma concentrations were similar indicating depletion of the glycine pool in the gavage study with concomitant increases in free plasma benzoic acid. The authors suggested that higher plasma levels of benzyl acetate and free benzoic acid in the gavage study may be, in part, associated with the different toxicological outcomes of the gavage and dietary studies. The conclusion that high levels of free benzoic acid is associated with hepatocellular adenomas is highly unlikely, given the fact that chronic administration of high dietary levels of benzoic acid (greater than 1% in the diet) failed to induce hepatocellular neoplasms in lifetime mice studies (Toth, 1984). However, there is evidence that the incidence of hepatocellular adenomas in the gavage study were the result of chronic exposure to high levels of the corn oil vehicle (Haseman et al., 1985).

Statistical analysis of liver adenomas is suspect because the vehicle control animals had an aberrantly low incidence when compared to historical controls. Control values for liver adenomas in NTP bioassays vary from 20% to as high as 42%. In this study, the liver adenoma rates were zero. If these historical controls are adopted for comparison, the statistical significance of the adenoma finding in the high dose animals disappears or becomes marginal ($p = 0.038$ for females, and $p = 0.078$ for males). Procedural concerns include the fact that the diets were not tested for contaminants and there are interpretive problems with corn oil gavage and tumor occurrence (NTP, 1986; Bernard, 1983).

In conclusion, evidence of carcinogenicity in the gavage benzaldehyde and benzyl acetate studies are associated with the repeated gavage administration of high dose levels of test substance in a corn oil vehicle or a statistical anomaly. The above observations strongly suggest that the tumors found in the gavage NTP studies have no relevance to human carcinogenic risk.

4.4. Genotoxicity studies

Genotoxicity testing has been performed on 13 representative compounds in this group. The vast majority of standardized in vitro genotoxicity assays Ames (AMS), Mouse Lymphoma (MLA), Sister Chromatid Exchange (SCE), Chromosomal Aberration (ABS), and Unscheduled DNA Synthesis (UDS) show no evidence of genotoxicity. Equivocal results have been reported mainly for aromatic aldehydes in the MLA and ABS assays. None of the 13 in vivo assays produced any evidence of genotoxicity for these benzyl derivatives. The results of these tests are presented in Table 3 and are summarized below.

4.4.1. In vitro

The benzyl derivatives were non-mutagenic in all standard plate incorporation and/or pre-incubation Ames assays using *Salmonella typhimurium* strains TA92, TA94, TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA2637, when tested at concentrations ranging up to the level of cytotoxicity or at ICH/OECD-recommended maximum test concentrations, both in the absence and presence of metabolic activation (S9 fraction) (FDA, 1975; McCann et al., 1975; Milvy and Garro, 1976; Cotruvo et al., 1977; Anderson and Styles, 1978; Sasaki and Endo, 1978; Rockwell and Raw, 1979; Florin et al., 1980; Rapson et al., 1980; Kasamaki et al., 1982; Haworth et al., 1983; Ball et al., 1984; Ishidate et al., 1984; Marnett et al., 1985; Nohmi et al., 1985; Mortelmans et al., 1986; Rogan et al., 1986; Schunk et al., 1986; Zeiger et al., 1988; Aeschbacher et al., 1989; Heck et al., 1989; NTP, 1989, 1990a,b; Vamvakas et al., 1989; Dillon et al., 1992; Zeiger et al., 1992;). Additional Ames tests on metabolites isolated from the urine of rats administered benzaldehyde (No. 13), isopropylbenzyl alcohol (No. 32), or cuminaldehyde (No. 36) by oral gavage also were negative in *S. typhimurium* strains TA98 and TA100, both with and without metabolic activation (Rockwell and Raw, 1979). A mutation assay in *S. typhimurium* strain TA1535/pSK1002, using *umu* gene expression as an endpoint, produced negative results with benzoic acid (No. 18) (Nakamura et al., 1987). Mutation or DNA repair assays using *Escherichia coli* strains WP2 *uvrA*, PQ37, or Sd-4-73

Table 3

#	Substance name	Test system in vitro	Test object	Maximum concentration of substance	Result	Reference
In vitro genotoxicity studies on benzyl derivatives						
1	Benzyl alcohol	Ames test[1]	*Salmonella typhimurium* TA92, TA94, TA98, TA100, TA1535, and TA1537	10,000 μg/plate	Negative[2]	Ishidate et al. (1984)
1	Benzyl alcohol	Ames test[3]	*S. typhimurium* TA100	1,000 μg/plate	Negative[4]	Ball et al. (1984)
1	Benzyl alcohol	Ames test[3]	*S. typhimurium* TA98, TA100	Dose not reported	Negative[4]	Rogan et al. (1986)
1	Benzyl alcohol	Ames test[1]	*S. typhimurium* TA98, TA100, TA1535, TA1537	6,666 μg/plate	Negative[2]	Mortelmans et al. (1986)
1	Benzyl alcohol	Ames test[3]	*S. typhimurium* TA98, TA100, TA1535, and TA1537	3 μmole/plate	Negative[2]	Florin et al. (1980)
1	Benzyl alcohol	Ames test[3]	*S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538	50,000 μg/plate	Negative[2]	Heck et al. (1989)
1	Benzyl alcohol	Ames test[3]	*S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538	5 μl/plate	Negative[4]	Milvy and Garro (1976)
1	Benzyl alcohol	Ames test[1]	*S. typhimurium* TA98, TA100, TA1535, and TA1537	6,666 g/plate	Negative[2,5]	NTP (1989)
1	Benzyl alcohol	Mutation	*Escherichia coli* WP2 uvrA	8.0 mg/plate	Negative[6,7]	Yoo (1986)
1	Benzyl alcohol	Rec assay	*B. subtilis* H17 and M45	21 μg/disk	Negative[6]	Oda et al. (1979)
1	Benzyl alcohol	Rec assay	*B. subtilis* H17 and M45	10 μg/disk	Positive[6,8]	Kuroda et al. (1984a)
1	Benzyl alcohol	Rec assay	*B. subtilis* H17 and M45	20 μl/disk	Positive[6,7]	Yoo (1986)
1	Benzyl alcohol	Chromosome aberration	Chinese hamster fibroblast cells	1.0 mg/ml	Negative[4,9]	Ishidate et al. (1984)
1	Benzyl alcohol	Chromosome aberration	Chinese hamster ovary cells	5,000 μg/ml	Equivocal[2,10,11]	Anderson et al. (1990)
1	Benzyl alcohol	Chromosome aberration	Chinese hamster ovary cells	5,000 μg/ml	Positive[2,12]	NTP (1989)
1	Benzyl alcohol	Sister chromatid exchange (SCE)	Chinese hamster ovary cells	5,000 μg/ml	Weakly Positive[13]	NTP (1989)
1	Benzyl alcohol	SCE	Chinese hamster ovary cells	5,000 μg/ml	Weak Positive[2,11,14]	Anderson et al. (1990)
1	Benzyl alcohol	Mutation	L5178Y mouse lymphoma cells	5,000 g/ml	Questionable[15,11]	McGregor et al. (1988); Myhr et al., 1990
1	Benzyl alcohol	Mutation	L5178Y Mouse Lymphoma Cells	4,500 μg/ml	Positive[2,16]	NTP (1989)
1	Benzyl alcohol	Mutation	*E. coli* WP2 uvrA	Dose not reported	Negative[17]	Kuroda et al. (1984b)
1	Benzyl alcohol	Cytotoxicity	Human alveolar tumor cells	0.5 mM	Negative	Waters et al. (1982)
1	Benzyl alcohol	DNA damage	Human alveolar tumor cells	0.5 mM	Negative	Waters et al. (1982)
1	Benzyl alcohol	DNA damage	Rat hepatocytes	10 mM	Negative[18]	Storer et al. (1996)
1	Benzyl alcohol	DNA damage	*E. coli* P3478	50 μl/disk	Negative[2]	Fluck et al. (1976)
2	Benzyl formate	Rec assay	*B. subtilis* H17 and M45	20 μl/disk	Positive[6,7]	Yoo (1986)
2	Benzyl formate	Mutation	*E. coli* WP2 uvrA	4.0 mg/plate	Negative[6,7]	Yoo (1986)
3	Benzyl acetate	Ames test[1]	*S. typhimurium* TA98, TA100, TA1535, and TA1537	10 mg/plate	Negative[2]	Mortelmans et al. (1986)
3	Benzyl acetate	Ames test[1,3]	*S. typhimurium* TA98, TA100	5,000 μg/plate	Negative[2,19]	Schunk et al. (1986)
3	Benzyl acetate	Ames test[20]	*S. typhimurium* TA98, TA100, TA1535, and TA1537	3 μmol/plate	Negative[2]	Florin et al. (1980)
3	Benzyl acetate	Rec assay	*B. subtilis* H17 and M45	21 μg/disk	Negative[2,6]	Oda et al. (1979)

3	Benzyl acetate	Rec assay	*B. subtilis* H17 and M45	20 µl/disk	Positive[6,7]	Yoo (1986)
3	Benzyl acetate	Mutation	*E. coli* WP2 uvrA	2.0 mg/plate	Negative[6,7]	Yoo (1986)
3	Benzyl acetate	Mutation	Mouse lymphoma L5178Y cells and Human lymphoblast TK6 cells	Mouse cells = 500 µg/ml Human cells = 1,500 µg/ml	Positive[2,12]	Caspary et al. (1988)
3	Benzyl acetate	Mutation	Mouse lymphoma L5178Y cells	1,600 µL/mL	Positive[2,18]	McGregor et al. (1988)
3	Benzyl acetate	Mutation	Mouse lymphoma L5178Y cells	Not reported	Positive[2,12]	Rudd et al. (1983)
3	Benzyl acetate	Chromosome aberration	Chinese hamster ovary cells	5,000 µg/ml	Negative[2]	Galloway et al. (1987)
3	Benzyl acetate	Chromosome aberration	Chinese hamster lung fibroblast cells	2.4 mg/ml	Negative[2,18]	Matsouka et al. (1996)
3	Benzyl acetate	SCE	Chinese hamster ovary cells	5,000 µg/ml	Negative[2]	Galloway et al. (1987)
3	Benzyl acetate	Unscheduled DNA synthesis	Rat hepatocytes	Not reported	Negative[17]	Mirsalis et al. (1983)
4	Benzyl propionate	Rec assay	*B. subtilis* H17 and M45	21 µg/disk	Negative[4,6]	Oda et al. (1979)
11	Benzyl benzoate	Ames test[20]	*S. typhimurium* TA98, TA100, TA1535, and TA1537	3 µmol/plate	Negative[2]	Florin et al. (1980)
11	Benzyl benzoate	Ames test[1,3]	*S. typhimurium* TA98, TA100	5,000 µg/plate	Negative[2,19]	Schunk et al. (1986)
13	Benzaldehyde	Ames test[3]	*S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538	37,500 µg/plate	Negative [2]	Heck et al. (1989)
13	Benzaldehyde	Ames test[3]	*S. typhimurium* TA98, TA100	300 µl/plate	Negative[2,21]	Rockwell and Raw (1979)
13	Benzaldehyde	Ames assay[3]	*S. typhimurium* TA98 and TA100	100 µl/plate	Negative[22]	Rockwell and Raw (1979)
13	Benzaldehyde	Ames test[20]	*S. typhimurium* TA98, TA100, TA2637	2.0 mg/plte	Negative[2,6]	Nohmi et al. (1985)
13	Benzaldehyde	Ames test[20]	*S. typhimurium* TA98, TA100, TA1535, and TA1537	3.0 µmol/plate	Negative[2]	Florin et al. (1980)
13	Benzaldehyde	Ames test[1]	*S. typhimurium* TA98, TA100, TA1535, and TA1537	1,000 µg/plate	Negative[2]	Haworth et al. (1983)
13	Benzaldehyde	Ames test[20]	*S. typhimurium* TA100, TA102 and TA104	3,333 µg/plate	Negative[2]	NTP (1990a, 1990b)
13	Benzaldehyde	Ames test[20]	*S. typhimurium* TA100	1.0 mg/plate	Negative[7]	Rapson et al. (1980)
13	Benzaldehyde	Ames test[1]	*S. typhimurium* TA98, TA100	Not reported	Negative[2]	Sasaki and Endo (1978)
13	Benzaldehyde	Ames test[1]	*S. typhimurium* TA100, TA102 and TA104	Not reported	Negative[2]	Dillon et al. (1992)
13	Benzaldehyde	Ames test[1]	*S. typhimurium* TA100	2,000 nmol/plate	Negative[2]	Vamvakas et al. (1989)
13	Benzaldehyde	Ames test[20]	*S. typhimurium* TA98, TA100	500 µg/plate	Negative[2]	Kasamaki et al. (1982)
13	Benzaldehyde	Rec assay	*B. subtilis* H17 and M45	21 µg/disk	Negative[6,7]	Oda et al. (1979)
13	Benzaldehyde	Rec assay	*B. subtilis* H17 and M45	Not reported	Positive[2,12]	Matsui et al. (1989)
13	Benzaldehyde	Unscheduled DNA synthesis (UDS)	Rat hepatocytes	251 µg/ml	Negative[2]	Heck et al. (1989)
13	Benzaldehyde	Mutation	Mouse L5178Y lymphoma cells	600 µg/ml	Positive[12]	Heck et al. (1989)
13	Benzaldehyde	Mutation	Mouse L5178Y lymphoma cells	800 µg/ml	Positive[4,23]	McGregor et al. (1991)
13	Benzaldehyde	Chromosomal aberrations	Chinese hamster cells	1.2 mg/ml	Positive[6,16,24,25]	Sofuni et al. (1985)

Table 3 (*continued*)

#	Substance name	Test system in vitro	Test object	Maximum concentration of substance	Result	Reference
In vitro genotoxicity studies on benzyl derivatives						
13	Benzaldehyde	Chromosomal aberrations	Chinese hamster ovary cells	1,600 μg/ml	Negative[2]	Galloway et al. (1987)
13	Benzaldehyde	Chromosomal aberrations	Chinese hamster cells	50 nM	Positive[2]	Kasamaki et al. (1982)
13	Benzaldehyde	SCE	Chinese hamster ovary cells	1,600 μg/ml	Positive[2]	Galloway et al. (1987)
13	Benzaldehyde	SCE	Chinese hamster ovary cells	1,000 μM	Negative[2,18]	Sasaki et al. (1989)
13	Benzaldehyde	SCE	Human lymphocytes	2.0 mM	Positive[4]	Jansson et al. (1988)
18	Benzoic acid	Ames test[3]	*S. typhimurium* TA98, TA100, TA1535, and TA1538	2,500 μg/plate	Negative[2]	Anderson and Styles (1978)
18	Benzoic acid	Ames test[20]	*S. typhimurium* TA98, TA100, TA1535, and TA1536	3.6 μg/plate	Negative[2]	Cotruvo et al. (1977)
18	Benzoic acid	Ames test[1]	*S. typhimurium* TA97; TA98, TA100, TA1535, and TA1537	10 mg/plate	Negative[2]	Zeiger et al. (1988)
18	Benzoic acid	Ames test[20]	*S. typhimurium* TA100	1.0 mg/plate	Negative[7]	Rapson et al. (1980)
18	Benzoic acid	Ames test[3]	*S. typhimurium* TA98, TA100, TA1535, and TA1537	1.0 mg/plate	Negative[22]	McCann et al. (1975)
18	Benzoic acid	Ames test[1]	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, and TA1537	10.0 mg/plate	Negative[2]	Ishidate et al. (1984)
18	Benzoic acid	Ames test[3]	*S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538	100 μg/plate	Negative[4]	Milvy and Garro (1976)
18	Benzoic acid	Ames test[3]	*S. typhimurium* TA1535, TA1537, and TA1538	0.5%	Negative[2]	FDA (1975)
18	Benzoic acid	Mutation	*S. typhimurium* TA1535/pSK1002	1.67 mg/ml	Negative[2,26]	Nakamura et al. (1987)
18	Benzoic acid	Rec assay	*B. subtilis* H17 and H45	Not reported	Positive[17]	Nonaka (1989)
18	Benzoic acid	Mutation	Saccharomyces cerevisiae D3	0.18%	Negative[2]	Cotruvo et al. (1977)
18	Benzoic acid	Mutation	S. cerevisiae D4	0.15%	Negative[2]	FDA (1975)
18	Benzoic acid	Indirect DNA repair	*E. coli* PQ37	400 μg/ml	Negative[27]	Glosnicka and Dziadziuszko (1986)
18	Benzoic acid	Chromosome aberration test	Chinese hamster fibroblast cells	1.5 mg/ml	Weak Positive[4,28]	Ishidate et al. (1984)
18	Benzoic acid	SCE	Human lymphocytes	2.0 mM	Negative[4]	Jansson et al. (1988)
19	Methyl benzoate	Ames test[1]	*S. typhimurium* TA97, TA98; TA100, TA1535, and TA1537	6,666 μg/plate	Negative[2]	Zeiger et al. (1992)
19	Methyl benzoate	Mutation	*E. coli* Sd-4-73	Dose not reported	Negative[4]	Szybalski (1958)
25	Isoamyl benzoate	Mutation	*E. coli* Sd-4-73	Dose not reported	Negative[4]	Szybalski (1958)
32	Isopropylbenzyl alcohol	Ames assay[3]	*S. typhimurium* TA98 and TA100	100 μl/plate	Negative[22]	Rockwell and Raw (1979)
32	Isopropylbenzyl alcohol	Ames assay[3]	*S. typhimurium* TA98 and TA100	300 μl/plate	Negative[2,29]	Rockwell and Raw (1979)
34	Tolualdehydes (mixed *o*, *m*, *p*)	Ames test[1]	*S. typhimurium* TA104	0.8 μmoles/plate	Negative[2]	Marnett et al. (1985)

34	Tolualdehydes (mixed *o*, *m*, *p*)	Ames test[20]	*S. typhimurium* TA98, TA100, TA1535, and TA1537	3 μmol/plate	Negative[2]	Florin et al. (1980)
34	Tolualdehydes (mixed *o*, *m*, *p*)	Ames test[3]	*S. typhimurium* TA98, TA100, TA1535, TA 1537, and TA1538	18,750 μg/plate	Negative[2]	Heck et al. (1989)
34	Tolualdehydes (mixed *o*, *m*, *p*)	Ames test[3]	*S. typhimurium* TA98, TA100 and TA102	0.8 mmol/plate	Negative[2]	Aeschbacher et al. (1989)
34	Tolualdehydes (mixed *o*, *m*, *p*)	Ames test[1]	*S. typhimurium* TA97, TA100, TA1535, and TA1537	666 μg/plate	Negative[2]	Zeiger et al. (1988)
34	Tolualdehydes (mixed *o*, *m*, *p*)	UDS	Rat hepatocytes	1,000 μg/ml	Negative[2]	Heck et al. (1989)
34	Tolualdehydes (mixed *o*, *m*, *p*)	Mouse Lymphoma Mutation Assay	L5178Y mouse lymphoma cells	300 g/ml	Negative[2]	Heck et al. (1989)
36	Cuminaldehyde	Ames assay[3]	*S. typhimurium* TA98 and TA100	100 μl/plate	Negative[22]	Rockwell and Raw (1979)
36	Cuminaldehyde	Ames assay[3]	*S. typhimurium* TA98 and TA100	300 μl/plate	Negative[2,30]	Rockwell and Raw (1979)
36	Cuminaldehyde	SCE	Chinese hamster ovary cells	333 μM		
In vitro antimutagenicity studies on benzyl derivatives						
1	Benzyl alcohol	Antimutagenesis	*E. coli* WP2 uvrA	2.5 mg/ml	Positive Antimutagenic Effect [6,31]	Yoo (1986)
1	Benzyl alcohol	Antimutagenesis	*E. coli* WP2 uvrA	1,000 μg/ml	Negative Antimutagenic Effect[6,32]	Yoo (1986)
1	Benzyl alcohol	Antimutagenesis	*E. coli* WP2 uvrA	Dose not reported	Positive Antimutagenic Effect[31]	Kuroda et al. (1984b)
2	Benzaldehyde	Antimutagenesis	*E. coli* WP2 uvrA	300 μg/ml	Negative[33] Antimutagenic Effect	(Ohta et al., 1983)
2	Benzyl formate	Antimutagenesis	*E. coli* WP2 uvrA	8.0 mg/ml	Negative Antimutagenic Effect[6,31]	Yoo (1986)
3	Benzyl acetate	Antimutagenesis	*E. coli* WP2 uvrA	8.0 mg/plate	Negative Antimutagenic Effect[6,31]	Yoo (1986)
13	Benzaldehyde	Antimutagenesis	*E. coli* PQ37	500 μg/ml	Negative[34] Antimutagenic effect	(Ohta et al., 1986a)
20	Ethyl benzoate	Antimutagenesis	*E. coli* PQ37	500 μg/ml	Negative Antimutagenic Effect[34]	(Ohta et al., 1986a)
20	Ethyl benzoate	Antimutagenesis	*S. typhimurium* TA98	200 μg/ml	Negative Antimutagenic Effect[35]	(Ohta et al., 1986b)
20	Ethyl benzoate	Antimutagenesis	*E. coli* WP2s	200 μg/ml	Negative antimutagenic effect	(Ohta et al., 1986b)

Table 3 (*continued*)

#	Substance name	Test system in vitro	Test object	Maximum concentration of substance	Result	Reference
In vitro genotoxicity studies on benzyl derivatives						
		Test system in vivo				
In vivo genotoxicity studies on benzyl derivatives						
1	Benzyl alcohol	Sex-linked recessive lethal mutations (SLRL)	*Drosophila melanogaster*	5000 ppm	Negative[36]	Foureman et al. (1994)
1	Benzyl alcohol	SLRL	*Drosophila melanogaster*	8000 ppm	Negative[37]	Foureman et al. (1994)
1	Benzyl alcohol	Micronucleus test	Mouse bone marrow cells	200 mg/kg bw	Negative[38]	Hayashi et al. (1988)
1	Benzyl alcohol	Replicative DNA synthesis test	Mouse hepatocytes	Not reported	Positive[39]	Yoshikawa (1996)
3	Benzyl acetate	SLRL	*Drosophila melanogaster*	300 ppm	Negative[36]	NTP (1993a); Foureman et al. (1994)
3	Benzyl acetate	SLRL	*Drosophila melanogaster*	20,000 ppm	Negative[37]	NTP (1993a); Foureman et al. (1994)
3	Benzyl acetate	SCE	Mouse bone marrow cells	1,700 mg/kg bw	Negative[38]	NTP (1993a)
3	Benzyl acetate	Chromosome aberration	Mouse bone marrow cells	1,700 mg/kg bw	Negative[38]	NTP (1993a)
3	Benzyl acetate	Micronucleus test	Mouse bone marrow cells	1,250 mg/kg bw	Negative[38]	NTP (1993a); Shelby et al. (1993)
3	Benzyl acetate	Micronucleus test	Mouse erythrocytes	50,000 ppm	Negative[38]	NTP (1993a)
3	Benzyl acetate	UDS	Rat hepatocytes	Not reported	Negative[17,40]	Mirsalis et al. (1983)
3	Benzyl acetate	UDS	Rat hepatocytes	1,000 mg/kg bw	Negative[40]	Mirsalis et al. (1989)
3	Benzyl acetate	UDS	Rat pancreatic cells	1,000 mg/kg bw	Negative[40]	Steinmetz and Mirsalis (1984)
3	Benzyl acetate	DNA damage	Rat pancreatic cells	500 mg/kg bw	Negative[40]	Longnecker et al. (1990)
3	Benzyl acetate	DNA damage	Rat pancreatic cells	0.9%	Negative[36]	Longnecker et al. (1990)
13	Benzaldehyde	SLRL	*Drosophila melanogaster*	1,150 ppm	Negative[36]	Woodruff et al. (1985)
13	Benzaldehyde	SLRL	*Drosophila melanogaster*	2,500 ppm	Negative[37]	Woodruff et al. (1985)

[1] Preincubation method.
[2] Assay was performed with and without metabolic activation.
[3] Plate incorporation method.
[4] Assay was performed without metabolic activation.
[5] Cytotoxicity was reported at the highest concentration tested.
[6] Japanese article. English summary.
[7] Not reported if assay was performed with or without metabolic activation.
[8] Inhibition of growth was reported when assay was performed without metabolic activation.
[9] Cells were exposed for 48 h.[29] Assay of urine samples from rats given isopropylbenzyl alcohol by oral gavage.
[10] Positive results were not reproducible.
[11] No dose–response observed.
[12] Positive results reported only with metabolic activation.
[13] Dose response at concentrations from 500 to 1250 μg/ml (without metabolic activation).
[14] No dose response observed. Increase in SCE at single doses.
[15] Positive and negative responses could not be reproduced.
[16] Positive result reported only in absence of metabolic activation.
[17] Abstract; methods and test concentrations were not reported.
[18] Cytotoxicity was observed at the maximum dose tested.
[19] Cytotoxicity observed at 3 maximum concentrations.

[20] Mutagenesis induced by 2-amino-3-methyl-imidazo[3,4-*f*]quinoline (IQ), 2-amino-3, 4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3, 8-dimethylimidazo[4.5-*f*]quinoxaline HCl (MeIQx), 3-amino-4-dimethy-5 H-pyrido-[4,3-*b*]indole (Trp-P-1), 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine HCl (PhIP).
[21] Assay of urine samples from rats given benzaldehyde by oral gavage.
[22] Assay was performed with metabolic activation.
[23] Significant increases in mutant fraction were close to toxic doses.
[24] Weak positive results were observed with metabolic activation.
[25] Cytotoxicity was observed at the two maximum concentrations tested.
[26] Assay measured induction of *umu* gene expression as indicator of genotoxicity.
[27] Genotoxicity measured as ability to induce β-galactosidase.
[28] Total incidence of cells with aberrations was 5.0–9.0%.
[29] Assay of urine samples from rats given isopropylbenzyl alcohol by oral gavage.
[30] Assay of urine samples from rats given cuminaldehyde by gavage.
[31] Mutagenesis induced by furylfuramide (AF-2).
[32] Mutagenesis induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG).
[33] Mutagenesis induced by 4-nitroquinoline 1-oxide and UV irradiation.
[34] Mutagenesis induced by UV irradiation.
[35] Mutagenesis induced by 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole or 2-amino-3-methyl-imidazo[4,5-*f*]quinoline.
[36] Feeding study.
[37] Dose administered by injection.
[38] Administered by intraperitoneal injection.
[39] Route of administration not reported.
[40] Administered by oral gavage.

with eight different benzyl derivatives (see Table 3) (Szybalski, 1958; Kuroda et al., 1984b; Glosnicka and Dziadziuszko, 1986; Yoo, 1986), and *Saccharomyces cerevisiae* strains D3 or D4 with benzoic acid (FDA, 1975; Cotruvo et al., 1977) also showed no evidence of genotoxicity. In a cytogenetics assay in *E. coli* strain P3478, benzyl alcohol did not show any activity (Fluck et al., 1976).

Equivocal results were obtained with the benzyl derivatives in the Rec DNA repair assay using *Bacillus subtilis* strains H17 and M45 (Oda et al., 1979; Sekizawa and Shibamoto, 1982; Kuroda et al., 1984a; Yoo, 1986; Matsui et al., 1989; Nonaka, 1989). Positive results only were reported for benzyl formate, while negative results were reported for benzyl propionate (No. 4) (Oda et al., 1979; Yoo, 1986). Positive and negative results for Rec assays using the same substance were apparently due to laboratory-specific factors. Yoo (1986) reported only positive findings and Oda et al. (1979) reported only negative results for the same compounds.

In vitro assays in isolated mammalian cells produced both negative and positive results. In addition to benzyl acetate, benzaldehyde exhibited evidence of mutagenicity in the forward mutation assay with L5178Y mouse lymphoma cells (MLA), both with and without metabolic activation (Rudd et al., 1983; Caspary et al., 1988; McGregor et al., 1988, 1991; Heck et al., 1989; Myhr et al., 1990). Questionable results were reported for benzyl alcohol in the MLA (McGregor et al., 1988). Tolualdehydes (mixed) (No. 34) were negative in the MLA (Heck et al., 1989).

In cytogenetic tests, equivocal results were reported for the benzyl derivatives in the ABS assay. In the ABS assay, performed in Chinese hamster ovary and fibroblast cell lines, both positive and negative results were reported with benzyl alcohol and benzaldehyde, with the positive result usually occurring at the higher culture concentration (Kasamaki et al., 1982; Ishidate et al., 1984; Sofuni et al., 1985; Galloway et al., 1987; Anderson et al., 1990). Negative results in two separate ABS assays were reported for benzyl acetate (Galloway et al., 1987; Matsouka et al., 1996). Weak positive results were reported for benzoic acid in the ABS assay (Ishidate et al., 1984). The positive results in the ABS assays were generally obtained independently of the presence or absence of metabolic activation.

The authors of the MLA and ABS assays (Heck et al., 1989) have emphasized that the positive results in the MLA and ABS assays may be artifacts resulting from changes in culture pH and osmolality. Treatment with high dose levels of substances (e.g., reactive aldehydes and carboxylic acids) with the potential to alter acidity or osmolality may induce a significant increase in mutant frequencies or aberrations in these assays. Often the results are inconsistent with the results of other genotoxicity assays (i.e., AMS and UDS) (Heck et al., 1989).

In the SCE assay, equivocal results were reported for benzyl alcohol and benzaldehyde, in Chinese hamster ovary cell lines and in human lymphocytes (Galloway et al., 1987; Sasaki et al., 1989; Jansson et al., 1988; NTP, 1989; Anderson et al., 1990). Negative results were obtained in this assay for benzyl acetate, benzoic acid, and cuminaldehyde (No. 36) (Galloway et al., 1987; Sasaki et al., 1989; Jansson et al., 1988).

DNA damage was not observed in either rat or human cells exposed to benzyl alcohol (Waters et al., 1982; Storer et al., 1996). No unscheduled DNA synthesis (UDS) was observed in rat hepatocytes exposed to benzaldehyde, benzyl acetate, or mixed tolualdehydes (Mirsalis et al., 1983; Heck et al., 1989).

The standardized in vitro genotoxicity assays {AMS, MLA, SCE, ABS, and UDS} show no evidence of genotoxicity. Equivocal results have been reported for mainly aromatic aldehydes in the MLA and ABS assays and may be due artifacts under experimental conditions.

4.4.2. In vivo

None of the benzyl derivatives showed any evidence of genotoxicity in well-recognized in vivo assays (mouse micronucleus, sex-linked recessive lethal, and in vivo–in vitro UDS assays). In mammals, compounds were administered orally, by gavage, or by ip injection at doses that were significant fractions of the reported lethal dose levels. Benzyl acetate was the most frequently tested compound, producing negative findings in SCE, CA, micronuclei, and UDS in both rats and mice (Mirsalis et al., 1983, 1989; NTP, 1993a,b; Shelby et al., 1993; Steinmetz and Mirsalis, 1984). The micronucleus test also was negative with benzyl alcohol (Hayashi et al., 1988). Positive results were reported for benzyl alcohol in mice (dose not specified) in the Replicative DNA Synthesis Assay (Yoshikawa, 1996).

In the (sub-mammalian) sex-linked recessive lethal mutation assay in fruit flies (*Drosophila melanogaster*), negative results were obtained with benzyl alcohol, benzaldehyde, and benzyl acetate, either after feeding or administration by injection (Woodruff et al., 1985; NTP, 1993a; Foureman et al., 1994).

4.4.3. Conclusion

Based on the mainly negative results in the standardized battery {i.e., AMS (−), UDS (−), SCE (±), ABS (±), and MLA (±)} of in vitro genotoxicity assays and the uniformly negative results in well-recognized in vivo genotoxicity assays, it is concluded that the group of benzyl derivatives is not genotoxic in vivo.

4.5. Other relevant studies

4.5.1. Reproduction

4.5.1.1. Benzyl alcohol (No. 1). Fifty female CD-1 mice were administered 550 mg/kg bw per day benzyl alcohol by gavage on days 6–15 of gestation. A control group of 50 mice received corn oil only. Body weight, clinical observations, and mortality were recorded daily throughout treatment and up to 3 days postpartum. All parameters tested, including gestation index, average number of live pups/litter, postnatal survival, and pup body weight, were statistically similar for the treated and control animals (York et al., 1986).

To examine the reproductive hazard of benzyl alcohol in CD-1 mice, 750 mg/kg bw per day of benzyl alcohol was administered by gavage to 50 mice on gestation days 6–13. A control group of 50 animals were given distilled water only. Maternal body weight gain and mortality, mating, gestation, numbers of live and dead pups per litter, total litter weight on days 1 and 2 postpartum, litter weight change between days 1 and 3 postpartum, and pup survival on days 1 and 3 postpartum were recorded. There was no significant difference in maternal body weight measured on days 4 and 7 of gestation between treated and control animals. However, statistically significant decreases were observed in treated females on gestation day 18 and day 3 postpartum. Maternal body weight gain during days 7–18 of gestation was also significantly lower than that of controls. Significant differences were also observed in pup body weight and weight gain, including mean pup weight per litter, mean litter weight change, and mean pup weight change (between day 1 and 3 postpartum). No differences were observed in the mating or gestation indices, the total number of resorptions, the number of live pups per litter, or in pup survival. Eighteen deaths were reported during the treatment period and they were all attributed to the treatment. One more death was reported the day after treatment was terminated (Hardin et al., 1987). It was reported in a review of this study that clinical signs of maternal toxicity were observed, including hunched posture, tremors, inactivity, prostration, hypothermia, ataxia, dyspnoea, swollen or cyanotic abdomen, and piloerection (York et al., 1986).

4.5.2. Benzyl acetate (No. 3)

Sperm morphology and vaginal cytology examinations (SMVCE) were developed by the National Toxicology Program, and used as a screen for reproductive toxicants. These examinations include evaluations of motility, concentration and head morphology of sperm from the caudia epididymis, and male reproductive organ weight data. At the end of the benzyl acetate 13-week dietary study (NTP, 1993a), 10 male and female mice from each dose group (0, 3, 130, 6250, 12,500, 25,000, and 50,000 ppm) were utilized for the SMVCE study. There was no effect on sperm motility, density or percent abnormality in any of the doses tested. There was no statistically significant change in the weights of the epididymis, the caudia epididymis, or the testis. The estrous cycle of the high dose female group was sig-

nificantly increased relative to the control group. There was also a significant decrease in body weight among animals in this group (Morrissey et al., 1988).

Morrissey et al. (1988) performed similar examinations on rats at the end of the benzyl acetate 13-week study (0, 3, 130, 6250, 12,500, 25,000, and 50,000 ppm) (NTP, 1993a). Benzyl acetate had no effect on any of the parameters measured.

Benzaldehyde (No. 13). Benzaldehyde (approximately 5 mg/kg bw) was administered by gavage to 10 breeding age rats every other day for a period of 32 weeks. Ten control animals received only the vehicle oil. Two pregnancies per rat were studied, one at 75 days and one at 180 days. The parameters examined included the number of pregnant females, number of off spring born, pup body weights at days 7 and 21 postpartum, and pup vitality. There was no statistically significant difference between treatment and control groups. It was reported that fewer females in the treated group became pregnant, however, no data or statistical analyses were performed, and the authors concluded that treatment did not cause a significant change in any of the parameters measured (Sporn et al., 1967).

4.6. Teratology

4.6.1. Benzyl acetate (No. 3)

Groups of 20 or more pregnant Wistar rats were administered benzyl acetate by gastric intubation at doses of 0 (control), 10, 100, 500, or 1,000 mg/kg bw from day 6–15 of pregnancy. On day 20 of pregnancy (term), they were terminated and the fetuses were examined for intrauterine death, and internal, external and skeletal malformations. Maternal parameters were comparable for all groups. At the highest dose level, fetal body weight was decreased. In addition, skeletal and internal malformations were observed at 1000 mg/kg bw per day. The authors reported that the skeletal malformations may be related to the significant decrease in fetal body weight. No increase in intrauterine death or external variations was noted at any dose level. No adverse effects were seen at or below 500 mg/kg bw per day. The results of this study show no evidence of teratogenic effects for benzyl acetate (Ishiguro et al., 1993).

4.6.2. Benzyl benzoate (No. 11)

Benzyl benzoate was fed to pregnant Wistar rats at concentrations of 0.4 and 1.0% during gestation days 0–21. These dietary levels correspond to the average daily intake of 200 and 500 mg/kg bw (FDA, 1993). There were no effects reported on the fetus relating to external, skeletal, or visceral anomalies (Morita et al., 1980).

4.6.3. Benzoic acid (No. 18)

Sodium benzoate administered by oral intubation to pregnant rats or rabbits during gestation days 6–15 or 6–18 at doses reaching 175 mg/kg bw per day or 250 mg/kg bw per day, respectively, produced no effects on maternal or fetal rats (Morgareidge, 1972). In another teratology study, sodium benzoate fed during gestation only produced adverse effects on the fetus at maternally toxic concentrations in the diet of 4% (1600 mg/kg bw/d) or higher (Onodera et al., 1978).

5. Recognition of GRASr status

The group of benzyl 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 benzyl 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-observed-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 benzyl derivatives as natural components of traditional foods is greater than their intake as intentionally added flavoring substances.

References

Abdo, K.M., Huff, J.E., Haseman, J.K., Boorman, G.A., Eustis, S.L., Matthews, H.B., Burka, L.T., Prejean, J.D., Thompson, R.B., 1985. Benzyl acetate carcinogenicity, metabolism and disposition in Fischer 344 rats and B6C3F$_1$ mice. Toxicology 37, 159–170.

Abdo, K.M., Wenk, M.L., Harry, G.J., Mahler, J., Goehl, T.J., Irwin, R.D., 1998. Glycine modulates the toxicity of benzyl acetate in F344 rats. Toxicologic Pathology 26, 395–402.

Aeschbacher, H.U., Wolleb, U., Loliger, J., Spadone, J.C., Liardon, R., 1989. Contribution of coffee aroma constituents to the mutagenicity of coffee. Food and Chemical Toxicology 27, 227–232.

Amsel, L.P., Levy, G., 1969. Drug biotransformation interactions in man II: a pharmacokinetic study of the simultaneous conjugation of benzoic and salicylic acids with glycine. Journal of Pharmaceutical Sciences 58, 321.

Anders, M.W., 1989. Biotransformation and bioactivation of xenobiotics by the kidney. In: Hutson, D.H., Caldwell, J., Paulson, G.D. (Eds.), Intermediary Xenobiotic Metabolism in Animals. Taylor and Francis, New York, pp. 81–97.

Anderson, D., Styles, J.A., 1978. The bacterial mutation test. British Journal of Cancer 37, 924–930.

Anderson, B.E., Zeiger, E., Shelby, M.D., Resnick, M.A., Gulati, D.K., Ivett, J.L., Loveday, K.S., 1990. Chromosome aberration

and sister chromatid exchange test results with 42 chemicals. Environmental and Molecular Mutagenesis 16 (Suppl. 18), 55–137.

Ball, J.C., Foxall-Van Aken, S., Jensen, T.E., 1984. Mutagenicity studies of *p*-substituted benzyl derivatives in the Ames Salmonella plate-incorporation assay. Mutation Research 138, 145–151.

Bernard, B.K., 1983. Response of the fragrance and flavor industries to the NTP draft report on benzyl acetate. The Flavor and Extract Manufacturers Association, Washington, DC, USA.

Brantom, P.G., Gaunt, I.F., Grasso, P., Lansdown, A.B.G., Gangolli, S.D., 1972. Short-term toxicity of tolualdehyde in rats. Food and Cosmetics Toxicology 10, 637–647.

Bray, H.G., Thorpe, W.V., White, K., 1951. Kinetic studies of the metabolism of foreign organic compounds. The formation of benzoic acid from benzamide, toluene, benzyl alcohol and benzaldehyde and its conjugation with glycine and glucuronic acid in the rabbit. The Biochemical Journal 48, 88–96.

Bridges, J.W., French, M.R., Smith, R.L., Williams, R.T., 1970. The fate of benzoic acid in various species. The Biochemical Journal 118, 47–51.

Carson, S., 1972a. 90-Day studies with glyceryl tribenzoate in rats. Unpublished Report to the Flavor and Extracts Manufacturers Association, Washington, DC, USA.

Carson, S., 1972b. 90-Day studies with propylene glycol dibenzoate in rats. Unpublished Report to the Flavor and Extracts Manufacturers Association, Washington, DC, USA.

Caspary, W.J., Langenbach, R., Penman, B.W., Crespi, C., Myhr, B.C., Mitchell, A.D., 1988. The mutagenic activity of selected compounds at the TK locus: rodent vs. human cells. Mutation Research 196, 61–81.

Chidgey, M.A.J., Caldwell, J., 1986. Studies on benzyl acetate. I. Effect of dose size and vehicle on the plasma pharmacokinetics and metabolism of [methylene-^{14}C] benzyl acetate in the rat. Food and Chemical Toxicology 24, 1257–1265.

Chidgey, M.A.J., Kennedy, J.F., Caldwell, J., 1986. Studies on benzyl acetate. II. Use of specific metabolic inhibitors to define the pathway leading to the formation of benzylmercaptic acid in the rat. Food and Chemical Toxicology 24, 1267–1272.

Ciba-Geigy, 1991. Acute oral toxicity study in rats with TK12186. Submission to the US Environmental Protection Agency. EPA/Office of Toxic Substances Document No. 8EHQ-1191-1557; OTS 0534655.

Clapp, J.J., Young, L., 1970. Formation of mercaptic acids in rats after the administration of aralkyl esters. The Biochemical Journal 118, 765–771.

Clayson, D.B., Iverson, F., Nera, E.A., Lok, E., 1990. The significance of induced forestomach tumors. Annual Reviews of Pharmacology and Toxicology 30, 441–463.

Costello, B.A., Moore, G.R., 1984. Acute oral toxicity LD50 study of 4-ethylbenzaldehyde in rats. Unpublished Report to the Flavor and Extracts Manufacturers Association, Washington, DC, USA.

Cotruvo, J.A., Simmon, V.F., Spanggord, R.J., 1977. Investigation of mutagenic effects of products of ozonation reactions in water. Annals of the New York Academy of Sciences 298, 124–140.

Davison, C., 1971. Salicylate metabolism in man. Journal of Pharmacology and Experimental Therapeutics 179, 249–268.

DeGroot, A.P., Spanjers, M.Th., van der Heijden, C.A., 1974. Acute and sub-acute oral toxicity studies in rats with five flavor compounds. Unpublished Report to the Flavor and Extracts Manufacturers Association, Washington, DC, USA.

Diack, S.L., Lewis, H.B., 1928. Studies in the synthesis of hippuric acid in the animal organism. Journal of Biological Chemistry 77, 89–95.

Dillon, D.M., McGregor, D.B., Combes, R.D., Zeiger, E., 1992. Detection of mutagenicity in *Salmonella* of some aldehydes and peroxides. Environmental and Molecular Mutagenesis 19, 15.

Draize, J.H., Alvarez, E., Whitesell, M.F., Woodard, G., Hagan, E.C., Nelson, A.A., 1948. Toxicological investigations of compounds proposed for use as insect repellents. Journal of Pharmacology and Experimental Therapeutics 93, 26–39.

Food and Drug Administration (FDA), 1975. Mutagenic Evaluation of Compound FDA 73-70, Benzoic acid, Certified A.C.S. Food and Drug Administration (FDA), Litton Bionetics, Inc. Washington, DC.

Food and Drug Administration (FDA), 1993. Priority-based assessment of food additives (PAFA) database. Center for Food Safety and Applied Nutrition. p. 58.

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

Fluck, E.R., Poirier, L.A., Ruelius, H.W., 1976. Evaluation of a DNA polymerase-deficient mutant of *E. coli* for the rapid detection of carcinogens. Chemico-Biological Interactions 15, 219–231.

Foureman, P., Mason, J.M., Valencia, R., Zimmering, S., 1994. Chemial mutagenesis testing in Drosophila: X. Results of 70 coded chemicals tested for the National Toxicology Program. Environmental and Molecular Mutagenesis 23, 208–227.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., Zeiger, E., 1987. Chromosome aberrations and sister chromatid exchange in Chinese hamster ovary cells: evaluations of 108 chemicals. Environmental and Molecular Mutagenesis 10 (Suppl. 10), 1–35.

Gillam, B., 1971. The reaction of aralkyl sulphate esters with glutathione catalysed by rat liver preparations. Biochemistry Journal 121, 667.

Glosnicka, R., Dziadziuszko, H., 1986. Mutagenic action of styrene and its metabolites. II. Genotoxic activity of styrene, styrene oxide, styrene glycol and benzoic acid tested with the SOS chromotest. Bulletin of the Institute of Maritime and Tropical Medicine in Gdynia 37, 3–4.

Graham, B.E., Kuizenga, M.H., 1945. Toxicity studies on benzyl benzoate and related benzyl compounds. The Journal of Pharmacology and Experimental Therapeutics 84, 358–362.

Grice, H.C., 1988. Safety evaluation of butylated hydroxyanisole from the perspective of effects on forestomach and oesophageal squamous epithelium. Food and Chemical Toxicology 26, 717–723.

Hagan, E.C., Hansen, W.H., Fitzhugh, O.G., Jenner, P.M., Jones, W.I., Taylor, J.M., Long, E.L., Nelson, A.A., Brouwer, J.B., 1967. Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. Food and Cosmetics Toxicology 5, 141–157.

Hardin, B.D., Schuler, R.L., Burg, J.R., Booth, G.M., Hazelden, K.P., MacKenzie, K.M., Piccirillo, V.J., Smith, K.N., 1987. Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratogenesis, Carcinogenesis, and Mutagenesis 7, 29–48.

Haseman, K., Huff, J.E., Rao, G.N., Arnold, J.E., Boorman, G.A., McConnell, E.E., 1985. Neoplasms observed in untreated and corn oil gavage control groups of (F344/N rats and C57BL/6N X C3H/HeN)F_1 (B6C3F_1) mice. Journal of the National Cancer Institute 75, 975–984.

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., Zeiger, E., 1983. *Salmonella* mutagenicity test results for 250 chemicals. Environmental Mutagenesis 5 (Suppl. 1), 3–142.

Hayashi, M., Kishi, M., Sofuni, T., Ishidate Jr., M., 1988. Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. Food and Chemical Toxicology 26, 487–500.

Heck, J.D., Vollmuth, T.A., Cifone, M.A., Jagannath, D.R., Myhr, B., Curren, R.D., 1989. An evaluation of food flavoring ingredients in a genetic toxicity screening battery. Toxicology 9, 257 (Abstract# 1031).

Heymann, E., 1980. Carboxylesterases and amidases. In: Jakoby, W.B., Bend, J.R., Caldwell, J. (Eds.), Enzymatic Basis of Detoxication, second ed. Academic Press, New York, pp. 291–323.

Hoffmann-LaRoche, 1967. Acute toxicity, eye and skin irritation tests of fragrance materials. Unpublished Report to the Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Ishida, T., Toyota, M., Asakawa, Y., 1989. Terpenoid biotransformation in mammals. V. Metabolism of (+)-citronellal, (±)-7-hydroxycitronellal, citral, (−)-perillaldehyde, (−)-myrtenal, cuminaldehyde, thujone, and (±)-carvone in rabbits. Xenobiotica 19, 843–855.

Ishidate Jr., M., Sofuni, T., Yoshikawa, D., Hayashi, M., Nohmi, T., Sawada, M., Marsouka, A., 1984. Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology 22, 623–636.

Ishiguro, S., Miyamoto, A., Obi, T., Nishio, A., 1993. Teratological studies on benzyl acetate in pregnant rats. Bulletin of the Faculty of Agriculture, Kagoshima University 43, 25–31.

Jansson, T., Curvall, M., Hedin, A., Enzell, C.R., 1988. In vitro studies of the biological effects of cigarette smoke condensate: III. Induction of SCE by some phenolic and related constituents derived from cigarette smoke: a study of structure–activity relationships. Mutation Research 206, 17–24.

Jenner, P.M., Hagan, E.C., Taylor, J.M., Cook, E.L., Fitzhugh, O.G., 1964. Food flavorings and compounds of related structure I. Acute oral toxicity. Food and Cosmetics Toxicology 2, 327–343.

Joint FAO/WHO Expert Committee on Food Additives (JECFA), 1980. Evaluation of certain food additives. The twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). World Health Organization Technical Report Series No. 648. World Health Organization, Geneva. <interrefhttp://whqlibdoc.who.int/trs/WHO_TRS_648.pdfurlhttp://whqlibdoc.who.int/trs/WHO_TRS_648.pdf>.

Joint FAO/WHO Expert Committee on Food Additives (JECFA), 1997. Evaluation of certain food additives and contaminants. The forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). World Health Organization Technical Report Series No. 868. World Health Organization, Geneva. <http://whqlibdoc.who.int/trs/WHO_TRS_868.pdf>.

Kasamaki, A., Takahashi, H., Tsumura, N., Niwa, J., Fujita, T., Urasawa, S., 1982. Genotoxicity of flavoring agents. Mutation Research 105, 387–392.

Kluwe, W.M., Montgomery, C.A., Giles, H.D., Prejean, J.D., 1983. Encephalopathy in rats and nephropathy in rats and mice after subchronic oral exposure to benzaldehyde. Food and Chemical Toxicology 21, 245–250.

Kravets-Bekker, A.A., Ivanova, O.P., 1970. Sanitary-toxicological characteristics of methyl benzoate and potassium benzoate. Farmacevtski Vestnik 2, 125–129.

Kubota, K., Ishizaki, T., 1991. Dose-dependent pharmacokinetics of benzoic acid following oral administration of sodium benzoate to humans. European Journal of Clinical Pharmacology 41, 363–368.

Kubota, K., Horai, Y., Kushida, K., Ishizaki, T., 1988. Determination of benzoic acid in human plasma and urine by high-performance liquid chromatography. Journal of Chromatography 425, 75–76.

Kuroda, K., Tanaka, S., Yoo, Y.S., Ishibashi, T., 1984a. Rec-assay of food additives. Nihon Koshu Eisei Zasshi. Japanese Society of Public Health 31, 277–281.

Kuroda, K., Yoo, Y.S., Ishibashi, T., 1984b. Antimutagenic activity of food additives. Mutation Research 130, 369.

Laham, S., Potvin, M., 1987. Biological conversion of benzaldehyde to benzylmercaptic acid in the Sprague-Dawley rat. Drug and Chemical Toxicology 10, 209–225.

Laham, S., Potvin, M., Robinet, M., 1988. Metabolism of benzaldehyde in New Zealand white rabbits. Chemosphere 17, 517–524.

LeBel, M., Ferron, L., Masson, M., Pichette, J., Carrier, C., 1988. Benzyl alcohol metabolism and elimination in neonates. Developmental Pharmacology and Therapeutics 11, 347–356.

Leegwater, DC., van Straten, S., 1974. In vitro study on the hydrolysis of twenty-six organic esters by pancreatin. Unpublished CIVO/TNO Report No. 4319, Flavor and Extract Manufacturers Association, Washington, DC, USA.

Levenstein, I., 1973. Acute toxicity study in rats and rabbits. Unpublished Report, Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Levenstein, I., 1974. Acute toxicity study in rats and rabbits. Unpublished Report, Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Lewis, C.A., Palanker, A.L., 1979. Acute toxicity studies in rats and rabbits. Unpublished Report, Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Longnecker, D.S., Roebuck, B.D., Curphey, T.J., Lhoste, E., Coon, C.I., MacMillan, D., 1986. Effects of corn oil and benzyl acetate on number and size of azaserine-induced foci in the pancreas of LEW and F344 rats. Environmental Health Perspectives 68, 197–201.

Longnecker, D.S., Roebuck, B.D., Curphey, T.J., MacMillan, D.L., 1990. Evaluation of promotion of pancreatic carcinogenesis in rats by benzyl acetate. Food and Chemical Toxicology 28, 665–668.

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

Maarse, H., Visscher, C.A., Willemsens, L.C., Boelens, M.H., 1999. Volatile Components in Food—Qualitative and Quantitative Data. Centraal Instituut Voor Voedingsonderzioek TNO. Zeist, The Netherlands.

Marnett, L.J., Hurd, H.K., Hollstein, M.C., Levin, D.E., Esterbauer, H., Ames, B.N., 1985. Naturally-occurring carbonyl compounds are mutagens in *Salmonella* tester strain TA104. Mutation Research 148, 25–34.

Matsouka, A., Yamakage, K., Kusakabe, H., Wakuri, S., Asakura, M., Noguchi, T., Sugiyama, T., Shimada, H., Nakayama, S., Kasahara, Y., Takahashi, Y., Miura, K.F., Hatanaka, M., Ishidate Jr., M., Morita, T., Watanabe, K., Hara, M., Odawara, K., Tanaka, N., Hayashi, M., Sofuni, T., 1996. Re-evaluation of chromosomal aberration induction on nine mouse lymphoma assay 'unique positive' NTP carcinogens. Mutation Research 369, 243–252.

Matsui, S., Yamamoto, R., Yamada, H., 1989. The *Bacillus subtilis*/microsome rec-assay for the detection of DNA damaging substances which may occur in chlorinated and ozonated waters. Water Science Technology: A Journal of the International Association on Water Pollution Research 21, 857–887.

McCann, J., Choi, E., Yamasaki, E., Ames, B.N., 1975. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. Proceedings of the National Academy of Sciences of the United States of America 79, 5135–5139.

McCloskey, S.E., Gershanik, J.J., Lertora, J.J.L., White, L., George, W.J., 1986. Toxicity of benzyl alcohol in adult and neonatal mice. Journal of Pharmaceutical Sciences 75, 702–705.

McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., Riach, C., Caspary, W.J., 1988. Responses of the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. Environmental and Molecular Mutagenesis 12, 85–154, Erratum, 12(3), 345.

McGregor, D.B., Brown, A.G., Howgate, S., McBride, D., Riach, C., Caspary, W.J., 1991. Responses of the L5178Y mouse lymphoma cell forward mutation assay. V: 27 coded chemicals. Environmental and Molecular Mutagenesis 17, 196–219.

McMahon, T.F., Diliberto, J.J., Birnbaum, L.S., 1989. Age related changes in disposition of benzyl acetate (BA): a model compound for glycine conjugation (Abstracts of the 28th Annual Meeting of the Society of Toxicology). Toxicology 9, 88.

Milvy, P., Garro, A.J., 1976. Mutagenic activity of styrene oxide (1,2 epoxyethyl benzene), a presumed styrene metabolite. Mutation Research 40, 15–18.

Mirsalis, J., Tyson, K., Beck, J., Loh, E., Steinmetz, K., Contreras, C., Austera, L., Martin, S., Spalding, J., 1983. Induction of

unscheduled DNA synthesis (UDS) in hepatocytes following in vitro and in vivo treatment. Environmental Mutagenesis 5, 482, Abstract #Ef-5.

Mirsalis, J.C., Tyson, C.K., Steinmetz, K.L., Loh, E.K., Hamilton, C.M., Bakke, J.P., Spalding, J.W., 1989. Measurement of unscheduled DNA synthesis and s-phase synthesis in rodent hepatocytes following in vivo treatment: testing of 24 compounds. Environmental and Molecular Mutagenesis 14, 155–164.

Moreno, O.M., 1972. Acute toxicity studies in mice, rats and rabbits. Unpublished Report to the Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Moreno, O.M., 1973. Acute toxicity studies on rats and rabbits. Unpublished Report, Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Moreno, O.M., 1974. Acute toxicity studies. Unpublished Report, Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Moreno, O.M., 1976. Acute toxicity studies in rats, mice, rabbits and guinea pigs. Unpublished Report to the Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Moreno, O.M., 1977. Acute toxicity studies in rats, rabbits and guinea pigs. Unpublished Report, Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Moreno, O.M., 1979. Acute toxicity studies in rats, rabbits and guinea pigs. Unpublished Report, Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Moreno, O.M., 1980. Acute toxicity studies. Unpublished Report, Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Morgareidge, K., 1962. In vitro digestion of four acetals. Unpublished Report, Flavor and Extracts Manufacturers Association, Washington, DC, USA.

Morgareidge, K., 1972. Teratologic evaluation of FDA 71-37 (sodium benzoate). Food and Drug Research Labs, Inc. Report to the US Food and Drug Administration PB-221 777.

Morita, S., Yamada, A., Ohgaki, S., Noda, T., Taniguchi, S., 1980. Safety evaluation of chemicals for use in household products. I. Teratological studies on benzyl benzoate and 2-(morpholinothio)benzothiazole in rats. Annual Report of the Osaka City Institute of Public Health and Environmental Sciences 43, pp. 90–97.

Morrissey, R.E., Schwetz, B.A., Lamb IV, J.C., Ross, M.D., Teague, J.L., Morris, R.W., 1988. Evaluation of rodent sperm, vaginal cytology, and reproductive organ weight data from National Toxicology Program 13-week studies. Fundamental and Applied Toxicology 11, 343–358.

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., Zeiger, E., 1986. *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. Environmental Mutagenesis 8, 1–119.

Myhr, B., McGregor, D., Bowers, L., Riach, C., Brown, A.G., Edwards, I., McBride, D., Martin, R., Caspary, W.J., 1990. L5178Y mouse lymphoma cell mutation assay results with 41 compounds. Environmental and Molecular Mutagenesis 16, 138–167.

Nakamura, S., Oda, Y., Shimada, T., Oki, I., Sugimoto, K., 1987. SOS-inducing activity of chemical carcinogens and mutagens in *Salmonella typhimurium* TA1535/pSK1002: Examination with 151 chemicals. Mutation Research 192, 239–246.

National Academy of Sciences (NAS) 1970. 1970 poundage and technical effects update of substances added to food. Committee on Food Additives Survey Data, Food and Nutrition Board, Institute of Medicine, Washington, DC.

National Academy of Sciences (NAS), 1982. 1982 poundage and technical effects update of substances added to food. Committee on Food Additives Survey Data, Food and Nutrition Board, Institute of Medicine, Washington, DC.

National Academy of Sciences (NAS), 1987. 1987 poundage and technical effects update of substances added to food. Committee on Food Additives Survey Data, Food and Nutrition Board, Institute of Medicine, Washington, DC.

National Toxicology Program (NTP), 1986. Toxicology and carcinogenesis studies of benzyl acetate in F 344/N rats and B6C3F$_1$ mice (gavage studies). NTP-TR-250; PB-86-2506. The National Toxicology Program, Research Triangle, NC.

National Toxicology Program (NTP), 1988. Toxicology and carcinogenesis studies of malonaldehyde, sodium salt (3-hydroxy-2-propeanal, sodium salt) in F344/N rats and B6C3F$_1$ mice (gavage studies). NTP-TR-331; NIH Publication No. 89-2587. The National Toxicology Program, Research Triangle, NC.

National Toxicology Program (NTP), 1989. Toxicology and carcinogenesis studies of benzyl alcohol in F 344/N rats and B6C3F$_1$ mice (gavage studies). NTP-TR-343; NIH Publication No. 89-2599. The National Toxicology Program, Research Triangle, NC.

National Toxicology Program (NTP), 1990a. Toxicology and carcinogenesis studies of benzaldehyde in F 344/N rats and B6C3F$_1$ mice (gavage studies). NTP-TR-378; NIH Publication No. 90-2833. The National Toxicology Program, Research Triangle, NC.

National Toxicology Program (NTP), 1990b. Toxicology and carcinogenesis studies of furfural in F344/N rats and B6C3F$_1$ mice (gavage studies). NTP-TR-382: NIH Publication No. 90-2837. The National Toxicology Program, Research Triangle, NC.

National Toxicology Program (NTP), 1992. Toxicology and carcinogenesis studies of coumarin in F344/N rats and B6C3F1 mice (gavage studies). NTP-TR-422: NIH Publication No. 92-31.3153. The National Toxicology Program, Research Triangle, NC.

National Toxicology Program (NTP), 1993a. Toxicology and carcinogenesis studies of benzyl acetate in F 344/N rats and B6C3F$_1$ mice (feed studies). NTP-TR-431; NIH Publication No. 92-3162. The National Toxicology Program, Research Triangle, NC.

National Toxicology Program (NTP), 1993b. Toxicology and carcinogenesis studies of 3,4-dihydrocoumarin in F344/N rats and B6C3F1 mice (gavage studies). NTP-TR-423: NIH Publication No. 93-3154. The National Toxicology Program, Research Triangle, NC.

National Toxicology Program (NTP), 1994a. Comparative Toxicology studies of corn oil, safflower oil and tricarpylin in male F344/N rats as vehicles for gavage. NTP-TR-426: NIH Publication No. 94-3157. The National Toxicology Program, Research Triangle, NC.

National Toxicology Program (NTP). 1994b, Effect of dietary restriction on toxicology and carcinogenesis studies in F344/N rats and B6C3F1 mice. NTP-TR-460: NIH Publication No. 97-3376. The National Toxicology Program, Research Triangle, NC.

Nielson, N.M., Bundgaard, H., 1987. Prodrugs as drugs delivery systems. 68. Chemical and plasma-catalyzed hydrolysis of various esters of benzoic acid: A reference system for designing prodrug esters carboxylic acid agents. International Journal of Pharmceutics 39, 75–85.

Nohmi, T., Miyata, R., Yoshikawa, K., Ishidate Jr., M., 1985. Mutagenicity tests on organic chemical contaminants in city water and related compounds. I. Bacterial mutagenicity tests. Eisei Shikenjo Hokoku Bulletin of the National Institute of Hygiene Science 103, 60–64.

Nonaka, M., 1989. DNA repair tests on food additives. Environmental and Molecular Mutagenesis 14 (Suppl. 15), 143, Abstract #414.

Nutley, B.P., 1990. Investigations into the metabolism of cinnamic acid, cinnamyl alcohol and cinnamaldehyde in relation to their safety evaluation. A thesis submitted for the degree of Doctor of Philosophy in the University of London Department of Pharmacology.

Oda, Y., Hamano, Y., Inoue, K., Yamamoto, H., Nihara, T., Kunita, N., 1979. Mutagenicity of food flavors in bacteria. Osaka-Furitsu Koshu Eisei Kenkyu Hokoku. Shokuhin Eisei Hen 91, 325.

Ohta, T., Watanabe, K., Moriya, M., Shirasu, Y., Kada, T., 1983. Anti-mutagenic effects of coumarin and umbelliferone on mutagenesis induced by 4-nitroquinoline 1-oxide or UV irradiation in *E. coli*. Mutation Research 117, 135–138.

Ohta, T., Watanabe, M., Tsukamoto, R., Shirasu, T., Kada, T., 1986a. Antimutagenic effects of 5-fluorourcil and 5-fluorodeoxyuridine on UV induced mutagenesis in *Escherichia coli*. Mutation Research 173 (1), 19–24.

Ohta, T., Watanabe, M., Watanabe, K., Shirasu, Y., Kada, T., 1986b. Inhibitory effects of flavourings on mutagenesis induced by chemicals in bacteria. Food and Chemical Toxicology 24 (1), 51–54.

Onodera, H., Ogiu, T., Matsuoka, C., Furuta, K., Takeuchi, M., Oono, Y., Kubota, T., Miyahara, M., Maekawa, A., Odashima, S., 1978. Studies on the effect of sodium benzoate on the fetuses and offspring of Wistar rats. Bulletin of the National Institute of Hygiene and Science 96, 47–55.

Oser, B.L., 1957. Toxicological screening of components of food flavors Class V. aromatic esters. Food Research Laboratories, Inc. Unpublished Report to the Flavor and Extracts Manufacturers Association, Washington, DC, USA.

Oser, B.L., Carson, S., Oser, M., 1965. Toxicological tests on flavouring matters. Food and Cosmetics Toxicology 3, 563–569.

Owen, G., Meyer, F.J., 1971. Acute oral and dermal toxicity studies. Unpublished Report to the Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Proctor and Gamble, 1992. Submission to the Environmental Protection Agency. Unpublished Report.

Rapson, W.H., Nazar, M.A., Butsky, V.V., 1980. Mutagenicity produced by aqueous chlorination of organic compounds. Bulletin of Environmental Contamination and Toxicology 24, 590–596.

Rockwell, P., Raw, I., 1979. A mutagenic screening of various herbs, spices and food additives. Nutrition and Cancer 1, 10–15.

Rogan, E.G., Cavalieri, E.L., Walker, B.A., Balasubramanian, R., Wislocki, P.G., Roth, R.W., Saugier, R.K., 1986. Mutagenicity of benzylic acetate, sulfates, and bromides of polycyclic aromatic hydrocarbons. Chemico-Biological Interactions 58, 253–275.

Rudd, C.J., Mitchell, A.D., Spalding, J., 1983. L5178Y mouse lymphoma cell mutagenesis assay of coded chemicals incorporating analyses of the colony size distributions. Environmental Mutagenesis 5, 419, Abstract # Cd-19.

Sado, I., 1973. Synergistic toxicity of official permissible preservative food additives. Nippon Eiseigaku Zasshi 28, 463–476.

Sasaki, Y., Endo, R., 1978. Mutagenicity of aldehydes in *Salmonella*. Mutation Research 54, 251–252.

Sasaki, Y.F., Imanishi, H., Ohta, T., Shirasu, Y., 1989. Modifying effects of components of plant essence on the induction of sister chromatid exchanges in cultured Chinese hamster ovary cells. Mutation Research 226, 103–110.

Schafer Jr., E.W., Bowles Jr., W.A., 1985. Acute oral toxicity and repellency of 933 chemicals to house and deer mice. Archives of Environmental Contamination and Toxicology 14, 111–129.

Schunk, H.H., Shibamoto, T., Tan, H.K., Wei, C-I., 1986. Biological and chemical studies on photochemical products obtained from euronol, benzyl acetate and benzyl benzoate. In: Lawrence, B.M., Mookherjee, B.D., Willis, B.J. (Eds.), Flavors and Fragrances: A World Perspective. Proceedings of the 10th International Congress of Essential Oils, Fragrance and Flavors, Washington, DC, USA, 16-029 November, 1986, pp. 1045–1068.

Sekizawa, J., Shibamoto, T., 1982. Genotoxicity of safrole-related chemicals in microbial test systems. Mutation Research 101, 127–140.

Shelanski, M., Moldovan, M., 1971. Acute toxicity in rats. Unpublished Report, Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Shelby, M.D., Erexson, G.L., Hook, G.J., Tice, R.R., 1993. Evaluation of a three-exposure mouse bone marrow micronucleus protocol: results with 49 chemicals. Environmental and Molecular Mutagenesis 21, 160–179.

Shell Oil Company, 1982. Study of the comparative toxicity of benzoic acid and p-t-butylbenzoic acid. Submission to the US Environmental Protection Agency. EPA/Office of Toxic Substances Document No. 8EHQ-0682-04405; 88-8200380; OTS0505458.

Shtenberg, A.J., Ignat'ev, A.D., 1970. Toxicological evaluation of some combinations of food preservatives. Food and Cosmetics Toxicology 8, 369–380.

Smith, R.L., Ford, R.A., 1993. Recent progress in the consideration of flavoring ingredients under the Food Additives Amendment. 16. GRAS substances. Food Technology 47 (6), 104–117.

Smyth Jr., H.F., Carpenter, C.P., Weil, C.S., 1951. Range finding toxicity data: list IV. Archives of Industrial Hygiene and Occupational Medicine 4, 119–122.

Smyth Jr., H.F., Carpenter, C.P., Weil, C.S., Pozzani, U.C., 1954. Range finding toxicity data: list V. Archives of Industrial Hygiene and Occupational Medicine 4, 119–122.

Sodemoto, Y., Enomoto, M., 1980. Report of carcinogenesis bioassay of sodium benzoate in rats: absence of carcinogenicity of sodium benzoate in rats. Journal of Environmental Pathology and Toxicology 4, 87–95.

Sofuni, M.D., Hayashi, M., Matsouka, A., Sawada, M., Hatanaka, M., Ishidate Jr., M., 1985. Mutagenicity tests on organic chemical contaminants in city water and related compounds. II. Chromosome aberration tests in cultured mammalian cells. Eisei Shikenjo Hokoku Bulletin of the National Institute of Hygiene Science 103, 64–75.

Sporn, A., Dinu, I., Stanciu, V., 1967. Cercetari cu privire la toxicitatea aldehidei benzoice. Igiena 16, 23–24.

Steinmetz, K.L., Mirsalis, J.C., 1984. Measurement of DNA repair in primary cultures of rat pancreatic cells following in vivo treatment. Environmental Mutagenesis 6, 446.

Stofberg, J., Grundschober, F., 1987. Consumption ratio and food predominance of flavoring materials. Perfumer and Flavorist 12, 27.

Stofberg, J., Kirschman, J.C., 1985. The consumption ratio of flavoring materials: a mechanism for setting priorities for safety evaluation. Food and Chemical Toxicology 23, 857–860.

Storer, R.D., McKelvey, T.M., Kraynak, A.R., Elia, M.C., Barnum, J.E., Harmon, L.S., Nichols, W.W., DeLuca, J.G., 1996. Revalidation of the in vitro alkaline elution/rat hepatocyte assay for DNA damage: improved criteria for assessment of cytotoxicity and genotoxicity and results for 81 compounds. Mutation Research 368, 59–101.

Szybalski, W., 1958. Special microbial systems. II. Observations on chemical mutagenesis in microorganisms. Annals of the New York Academy of Sciences, 475–489.

Taylor, J.M., Jenner, P.M., Jones, W.I., 1964. A comparison of the toxicity of some allyl, propenyl, and propyl compounds in the rat. Toxicology and Applied Pharmacology 6, 378–387.

Temellini, A., 1993. Conjugation of benzoic acid with glycine in human liver and kidney: a study on the interindividual variability. Fifth North American ISSX Meeting, Tucson, AZ, October 17–21, 1993, p. 4.

Toth, B., 1984. Lack of tumorigenicity of sodium benzoate in mice. Fundamental and Applied Toxicology 4, 494–496.

Vamvakas, S., Dekant, W., Anders, M.W., 1989. Mutagenicity of benzyl *S*-haloalkyl and *S*-haloalkenyl sulfides in the Ames test. Biochemical Pharmacology 38, 935–939.

Van Doorn, R., Leijdekkers, Ch.-M., Bos, R.P., Brouns, R.M.E., Henderson, P.Th., 1981. Alcohol and sulphate intermediates in the metabolism of toluene and xylenes to mercapturic acids. Journal of Applied Toxicology 1, 236–242.

Waters, R., Mirzayans, R., Meredith, J., Mallalah, G., Danford, N., Parry, J.M., 1982. Correlations in mammalian cells between types

of DNA damage, rates of DNA repair and the biological consequence. Progress in Mutation Research (DNA Repair, Chromosome Alterations Chromatin Structure) 4, 247–259.

Wester, P.W., Kroes, R., 1988. Forestomach carcinogens: pathology and relevance to man. Toxicologic Pathology 16, 165–171.

Weir, R.J., Wong, L.C.K., 1971. Acute oral and dermal toxicity studies in rabbits. Unpublished Report, Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Williams, R.T., 1959. Detoxication Mechanisms, second ed. Chapman & Hall, Ltd., London.

Woodruff, R.C., Mason, J.M., Valencia, R., Zimmering, S., 1985. Chemical mutagenesis testing in *Drosophila*. V. Results of 53 coded compounds tested for the National Toxicology Program. Environmental Mutagenesis 7, 677–702.

Yoo, Y.S., 1986. Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs. Osaka-Shiritsu Daigaku Igaki Zasshi 4, 267–288.

York, R.G., Barnwell, P., Bailes, W., 1986. Final Report. Screening of priority chemicals for reproductive hazards. Environmental Health Research and Testing, Inc. No. ETOX-1002.

Yoshikawa, K., 1996. Anomalous nonidentity between *Salmonella* genotoxicants and rodent carcinogens: Nongenotoxic carcinogens and genotoxic noncarcinogens. Environmental Health Perspectives 104, 40–46.

Yuan, J.H., Goehl, T.J., Abdo, K., Clark, J., Espinosa, O., Bugge, C., Garcia, D., 1995. Effects of gavage versus dosed feed administration on the toxicokinetics of benzyl acetate in rats and mice. Food and Chemical Toxicology 33, 151–158.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., 1988. *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. Environmental and Molecular Mutagenesis 11 (Suppl. 12), 1–158.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., 1992. *Salmonella* mutagenicity tests: V. Results from the testing of 311 chemicals. Environmental and Molecular Mutagenesis 19 (Suppl. 21), 2–141.