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Review

The FEMA GRAS assessment of aromatic substituted secondary alcohols, ketones, and related esters used as flavor ingredients

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Abstract

This publication is the 11th 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. The list of GRAS substances has now grown to more than 2100 substances. 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. In this monograph, a detailed interpretation is presented on the renal carcinogenic potential of the aromatic secondary alcohol *alpha*-methylbenzyl alcohol, aromatic ketone benzophenone, and corresponding alcohol benzhydrol.

The relevance of these effects to the flavor use of these substances is also discussed. The group of aromatic substituted secondary alcohols, ketones, and related esters was reaffirmed as GRAS (GRASr) based, in part, on 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.

Abbreviations: ABS, chromosomal aberration; ALDH, alcohol dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the curve; *B. subtilis*, *Bacillus subtilis*; bw, body weight; CHO, Chinese hamster ovary; D. melanogaster, Drosophila melanogaster; DNA, deoxyribonucleic acid; *E. coli*, *Escherichia coli*; F, Female; FDA, United States Food and Drug Administration; FEMA, The Flavor and Extract Manufacturers Association of the United States; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; GRAS, Generally Recognized as Safe; GRASa, GRAS affirmed; GRASr, GRAS reaffirmed; ip, intraperitoneal; LD_{50}, median lethal dose; LOAEL, Lowest-observed adverse effect level; M, Male; NAS, National Academy of Science; NOAEL, No-observed-adverse effect level; NR, Not reported; NTP, National Toxicology Program; PCE, polychromatic erythrocytes; PFC, plaque-forming cell; ppm, parts per million; SRBC, sheep red blood cell; *S. typhimurium*, *Salmonella typhimurium*; SCE, sister chromatid exchanges; SLR, scientific literature review; UDS, unscheduled DNA synthesis.

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Contents

1. Chemical identity

This summary presents the key scientific data relevant to the safety evaluation of 38 aromatic substituted secondary alcohols, ketones, and related esters and their intended use as flavoring ingredients (Table 1). All members of this group are aromatic substituted secondary alcohols, ketones and related esters.

2. Exposure

2.1. Flavor use and natural occurrence

The total annual production volume of the 38 aromatic secondary alcohols, ketones, and related esters is approximately 17,003 kg in the USA (Lucas et al., 1999; NAS, 1972, 1982, 1987). Approximately 80% of the total annual production volume in the USA arises from the use of 4 ingredients that also occur naturally in plants as products of the shikimic acid pathway. Two are structurally related substances, *alpha*-methylbenzyl acetate (No. 3) and acetophenone (No. 8), together with raspberry ketone [4-(*p*-methoxyphenyl)-2-butanone (No. 19)] and the ethyl ester of benzoylacetic acid [ethyl benzoylacetate (No. 30)]. The *per capita* intake[1] of each flavoring ingredient is reported in Table 1.

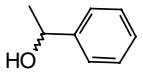
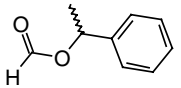
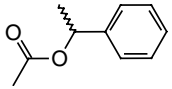
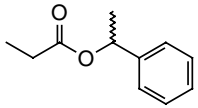
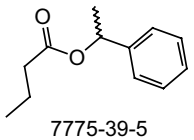
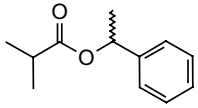
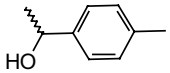
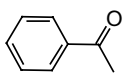
As expected, a number of these aromatic secondary alcohols and ketones are present in food. Seventeen (17) of the aromatic substituted secondary alcohols, ketones, and related esters considered here have been reported to occur naturally in foods (Nijssen et al., 2005). For instance, *alpha*-methylbenzyl alcohol (No. 1) has been detected in cheeses, fruits, and teas. The corresponding ketone acetophenone (No. 8) is a natural component of berries, seafood, beef, and nuts.

Quantitative natural occurrence data and consumption ratios[2] are available for three flavoring ingredients (*alpha*-methylbenzyl alcohol (No. 1); 4-methylacetophenone

[1] Intake (μg/person/d) calculated as follows: [(annual volume, kg) × (1×10^9 μg/kg)/(population × survey correction factor × 365 d)], where population (10%, "eaters only") = 26×10^6 for the 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), NAS (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.

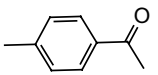
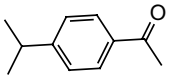
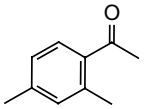
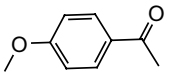
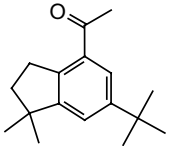
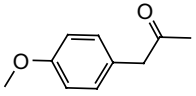
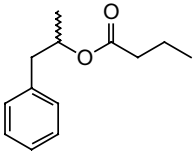
[2] The consumption ratio is calculated as follows: (annual consumption via food, kg)/(most recent reported volume as a flavoring substance, kg).

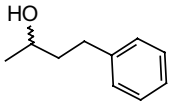
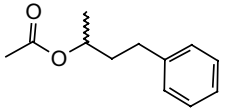
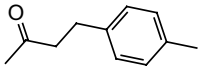
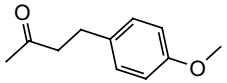
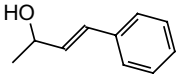
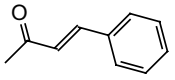
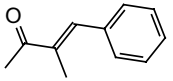
Table 1
Identity and exposure data for aromatic substituted secondary alcohols, ketones, and related esters used as flavoring ingredients

Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume, kg	Daily per capita intake ("eaters only")[a]		Annual volume in naturally occurring in food, kg[b]	Consumption ratio[c]
				μg/d	μg/kg bw/d		
1. *alpha*-(±)-Methylbenzyl alcohol	2685	98-85-1  7775-38-4	553	73	1	365	0.7
2. *alpha*-(±)-Methylbenzyl formate	2688	 93-92-5	2[d]	0.4	0.007	–	NA
3. *alpha*-(±)-Methylbenzyl acetate	2684	 120-45-6	4990	657	11	+	NA
4. *alpha*-(±)-Methylbenzyl propionate	2689	 3460-44-4	204	27	0.4	–	NA
5. *alpha*-(±)-Methylbenzyl butyrate	2686	 7775-39-5	0.1	0.01	0.0002	–	NA
6. *alpha*-(±)-Methylbenzyl isobutyrate	2687	 536-50-5	7	0.9	0.01	–	NA
7. *p, alpha*-(±)-Dimethylbenzyl alcohol	3139	 98-86-2	3[e]	0.5	0.008	–	NA
8. Acetophenone	2009		1334	176	3	+	NA

(*continued on next page*)

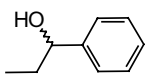
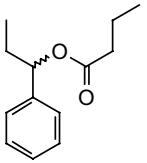
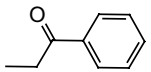
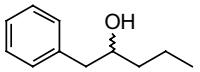
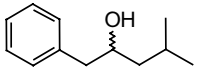
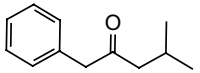
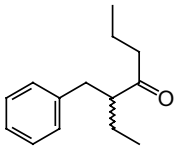
Table 1 (*continued*)

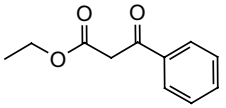
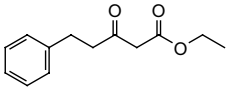
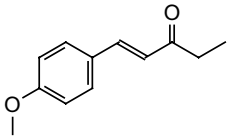
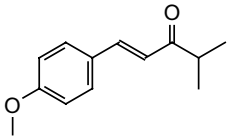
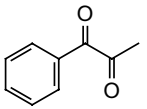
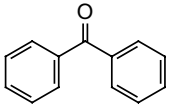
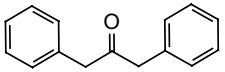
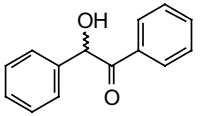
Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume, kg	Daily per capita intake ("eaters only")[a]		Annual volume in naturally occurring in food, kg[b]	Consumption ratio[c]
				μg/d	μg/kg bw/d		
9. 4-Methylacetophenone	2677	122-00-9 	286	38	0.6	15	0.1
10. *p*-Isopropylacetophenone	2927	645-13-6 	1[f]	0.2	0.004	+	NA
11. 2,4-Dimethylacetophenone	2387	89-47-7 	0.05	0.006	0.0001	+	NA
12. Acetanisole	2005	100-06-1 	644	85	1	+	NA
13. 4-Acetyl-6-*t*-butyl-1,1-dimethylindan	3653	13171-00-1 	114	15	0.2	–	NA
14. 1-(*p*-Methoxyphenyl)-2-propanone	2674	122-84-9 	848	112	2	+	NA
15. *alpha*-Methylphenethyl butyrate	3197	68922-11-2 	1[d]	0.2	0.003	–	NA

16. (±)-4-Phenyl-2-butanol	2879	2344-70-9 	3[e]	0.5	0.008	+	NA
17. (±)-4-Phenyl-2-butyl acetate	2882	10415-88-0 	34[e]	6	0.1	–	NA
18. 4-(*p*-Tolyl)-2-butanone	3074	7774-79-0 	1[e]	0.2	0.004	–	NA
19. 4-(*p*-Methoxyphenyl)-2-butanone	2672	104-20-1 	6441	848	14	+	NA
20. 4-Phenyl-3-buten-2-ol	2880	17488-65-2 	1	0.1	0.002	–	NA
21. 4-Phenyl-3-buten-2-one	2881	122-57-6 	54	7	0.1	–	NA
22. 3-Methyl-4-phenyl-3-butene-2-one	2734	1901-26-4 	0.5[g]	0.08	0.001	–	NA

(*continued on next page*)

Table 1 (*continued*)

Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume, kg	Daily per capita intake ("eaters only")[a]		Annual volume in naturally occurring in food, kg[b]	Consumption ratio[c]
				μg/d	μg/kg bw/d		
23. (±)-1-Phenyl-1-propanol	2884	93-54-9 	1	0.1	0.002	+	NA
24. *alpha*-(±)-Ethylbenzyl butyrate	2424	10031-86-4 	1[e]	0.2	0.004	+	NA
25. Propiophenone	3469	93-55-0 	0.2[d]	0.04	0.0007	+	NA
26. *alpha*-(±)-Propylphenethyl alcohol	2953	705-73-7 	4[e]	0.7	0.01	+	NA
27. *alpha*-(±)-Isobutylphenethyl alcohol	2208	7779-78-4 	20	3	0.04	–	NA
28. 4-Methyl-1-phenyl-2-pentanone	2740	5349-62-2 	2	0.3	0.005	–	NA
29. (±)-3-Benzyl-4-heptanone	2146	7492-37-7 	5	0.6	0.01	–	NA

30. Ethyl benzoylacetate	2423	94-02-0 	1039	137	2	–	NA
31. Ethyl 2-acetyl-3-phenylpropionate	2416	620-79-1 	1	0.2	0.003	–	NA
32. 1-(*p*-Methoxyphenyl)-1-penten-3-one	2673	104-27-8 	848	112	2	–	NA
33. 1-(4-Methoxyphenyl)-4-methyl-1-penten-3-one	3760	103-13-9 	1[f]	0.2	0.003	–	NA
34. 1-Phenyl-1,2-propanedione	3226	579-07-7 	1	0.2	0.003	+	NA
35. Benzophenone	2134	119-61-9 	86	11	0.2	1	0.01
36. 1,3-Diphenyl-2-propanone	2397	102-04-5 	0.5[g]	0.08	0.001	–	NA
37. (±)-Benzoin	2132	119-53-9 	159	21	0.3	–	NA

(*continued on next page*)

Table 1 (*continued*)

Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume, kg	Daily per capita intake ("eaters only")[a]		Annual volume in naturally occurring in food, kg[b]	Consumption ratio[c]
				μg/d	μg/kg bw/d		
38. Methyl *beta*-naphthyl ketone	2723	92-08-3	367	48	0.8	+	NA

[a] Intake (μg/person/d) calculated as follows: [(annual production volume, kg) × (1×10^9 μg/kg)/(population × survey correction factor × 365 d)], where population (10%, "eaters only") = 26×10^6 for the 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), NAS (1982, 1987). Intake (μg/kg bw/d) calculated as follows: [(μg/person per day)/body weight], where body weight = 60 kg. Slight variations may occur from rounding.

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

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

[d] NAS (1982).

[e] NAS (1970).

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

[g] NAS (1987).

(No. 9); and benzophenone (No. 35)). These data indicate that exposure to these ingredients occurs predominantly from intake as a flavoring agent as consumption ratios are less than one with the exception of *alpha*-methylbenzyl alcohol (No. 1) where intake from foods is approximately equivalent to that from intake as a flavoring ingredient (Stofberg and Kirschman, 1985; Stofberg and Grundschober, 1987).

3. Hydrolysis, absorption, distribution, and excretion

3.1. Hydrolysis of esters

Hydrolysis of esters occurs in all animals leading to the corresponding alcohols and carboxylic acids. Aromatic esters are hydrolyzed *in vivo* through the catalytic activity of carboxylesterases, the most important of which are the A-esterases (Heymann, 1980). Carboxylesterases are found in the endoplasmic reticulum of most mammalian tissues (Hosokawa et al., 2001) though they occur predominantly in hepatocytes (Heymann, 1980; Graffner-Nordberg et al., 1998; Hosokawa et al., 2001).

Hydrolysis of *alpha*-methylbenzyl esters yields *alpha*-methylbenzyl alcohol and simple aliphatic carboxylic acids. *In vitro* hydrolysis (Leegwater and vanStraten, 1974) of structurally related benzyl esters (benzyl acetate, benzyl 2-methylbutanoate, benzyl cinnamate, and benzyl phenylacetate) in simulated intestinal fluid containing pancreatin indicates that significant ester hydrolysis is expected prior to absorption. After absorption, rapid *in vivo* hydrolysis is also expected in the blood and liver. Benzyl acetate was readily hydrolyzed in pig liver homogenate (Heymann, 1980). The plasma half-lives ($t_{1/2}$) for the hydrolysis of a series of 4 alkyl benzoates (including methyl benzoate, ethyl benzoate and propyl benzoate) and 2 aryl benzoates in 80% human blood plasma were in the range from 15 to 210 min (Nielson and Bundgaard, 1987).

3.2. Absorption, distribution and excretion

Simple aromatic ketones and aromatic secondary alcohols have been shown to be rapidly absorbed from the gut, metabolized by the liver and excreted primarily in the urine, and to a very minor extent, in the feces. Recent pharmacokinetic data suggest that aromatic ketones administered orally undergo essentially complete first-pass hepatic clearance in both mice and rats. The absence of *trans*-4-phenyl-3-buten-2-one in systemic blood after oral dosing, and the observation that the systemic clearance of *trans*-4-phenyl-3-buten-2-one is approximately equivalent to hepatic clearance support this conclusion (Sauer et al., 1997a,b).

In earlier studies (Quick, 1928; Thierfelder and Daiber, 1923; Smith et al., 1954a,b), acetophenone or *alpha*-methylbenzyl alcohol is absorbed, metabolized and excreted as polar metabolites within 24 h. Approximately half of the 450 mg/kg bw oral dose of acetophenone or 460 mg/kg bw

dose of *alpha*-methylbenzyl alcohol administered to rabbits by gavage was excreted within the urine 24 h after administration (Smith et al., 1954a). Likewise, approximately half of a 500 mg/kg bw (4000 mg/8 kg bw) dose of acetophenone added to the food of dogs was accounted for in the first 24-h and following 12-h urine samples (Quick, 1928).

In a recent study (Sauer et al., 1997a), male F344/N rats (3/group) were given single oral doses of 200 mg/kg bw of ^{14}C-ring labelled *trans*-4-phenyl-3-buten-2-one by gavage. Periodic collection of urine (6, 12, 24, and 48 h), feces (24 and 48 h), blood (0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 12, 24, and 48 h after dosing) and tissues (48 h) revealed that >70% of the radiolabel was excreted in the urine within 6 h and >96.6% within 48 h. After 48 h, only 4.8% of radioactivity was measured in the feces, while <0.2% was retained in the tissue. No parent ketone could be detected in the blood at any time during the experiment. Intravenous administration of 20 mg/kg bw of the labelled ketone gave a strikingly similar pattern of absorption and excretion. Blood concentrations of the ketone were below limits of detection after 60 min. Essentially 100% of the radioactivity was accounted for in the urine and feces 48 h after dosing. The short disposition half-life (17.7 min), relatively small volume of distribution (0.89 l/kg bw), and high systemic clearance (69.8 ml/kg min, approximately equivalent to hepatic clearance) suggest that the ketone undergoes essentially complete first-pass hepatic clearance (Sauer et al., 1997a).

In a parallel study (Sauer et al., 1997b), female B6C3F1 mice (3/group) were given single oral doses of 200 mg/kg bw of ^{14}C-ring labelled *trans*-4-phenyl-3-buten-2-one by gavage using the protocol described above. Greater than 84% of the radiolabel was excreted in the urine within 6 h and >94% within 48 h. After 48 h, 1.2% of radioactivity was measured in the feces and 0.3% in exhaled air. Unlike rats, parent ketone was detected in the blood, albeit accounting for only 2.6% of the total dose. Following intravenous administration (20 mg/kg bw), blood ketone levels were below limits of detection after 30 min. The disposition half-life (8.9 min), volume of distribution (3.3 l/kg bw), and high systemic clearance (540 ml/kg min) indicate that the ketone was cleared more rapidly from the blood of mice than that of rats. The larger apparent volume of distribution in mice suggests that one or more tissues accumulate the parent ketone to a greater extent in mice than in rats. The appearance of the parent ketone in the blood of mice could be due to the higher rate of intestinal absorption compared to that of rats (Sauer et al., 1997b).

The toxicokinetics of benzophenone in male and female F344 rats and B6C3F1 mice has been investigated (Dix et al., 1997). Plasma benzophenone concentrations were measured following single intravenous doses (2.5 mg/kg bw for rats and 15 mg/kg bw for mice), and single oral gavage doses (2.5, 5 or 10 mg/kg bw for rats or 15, 30 or 60 mg/kg bw for mice) of benzophenone. Plasma benzophenone was also followed in mice and rats maintained on diets containing either 312 or 1200 ppm benzophenone for 7–8 d. Results of the studies indicate that oral bioavailability of benzophenone is higher (100%) in rats than in mice (50%). After oral gavage, mice cleared benzophenone more rapidly than rats suggesting a more extensive first-pass metabolism in mice. When administered the same concentration in the feed, mice received significantly more (3–4 times more) benzophenone (mg/kg bw/d) than rats, but plasma concentrations of benzophenone were significantly higher (3–4 times) in rats than mice, again suggesting that mice clear benzophenone more rapidly than rats.

3.3. Metabolism

3.3.1. alpha-Methylbenzyl alcohol (No. 1) and acetophenone (No. 8)

Simple aromatic-substituted ketones and their corresponding secondary alcohols [*alpha*-Methylbenzyl alcohol (No. 1) and acetophenone (No. 8)] are readily interconvertible *in vivo,* and, therefore, similar excretion products are formed from both. Reduction of acetophenone to *alpha*-methylbenzyl alcohol and oxidation of *alpha*-methylbenzyl alcohol to acetophenone has been reported to occur in rat hepatocytes (Hopkins et al., 1972; Maylin et al., 1973). The reduction and oxidation steps have been shown to be stereoselective *in vitro* and *in vivo* (Sullivan et al., 1976; Culp and McMahon, 1968; Callaghan et al., 1973). The alcohol is mainly conjugated with glucuronic acid and excreted in the urine, while the ketone undergoes *alpha*-oxidation and subsequent oxidative decarboxylation to yield benzoic acid that is excreted mainly in the urine as hippuric acid (benzoylglycine). Little or no oxidation of the aromatic ring has been observed. Studies indicate that the formation and excretion of the glucuronic acid conjugate of *alpha*-methylbenzyl alcohol is the major excretion pathway in animals.

Acetophenone administered to rabbits *via* a variety of different routes or to dogs in the diet is primarily reduced to *alpha*-methylbenzyl alcohol. In other studies, *alpha*-methylbenzyl alcohol is, in part, oxidized to acetophenone in rats or a metabolite of acetophenone in rabbits (El Masry et al., 1956; Kiese and Lenk, 1974). (S)-(−)-*alpha*-methylbenzyl alcohol is formed when acetophenone is incubated with carbonyl reductase isolated from rabbit kidney (Culp and McMahon, 1968). Conversely, incubation of (+)- or (+/−)-*alpha*-methylbenzyl alcohol in rat liver cytosolic preparations containing $NADP^+$ resulted in stereospecific oxidation of the (−)-isomer only to yield acetophenone (Callaghan et al., 1973).

Approximately 28% of a single 244 mg/kg bw dose of α-methylbenzyl alcohol administered to In Chinchilla rabbits *via* stomach tube was excreted in the urine as hippuric acid within 24 h. Rabbits given single doses of 240 mg acetophenone/kg bw excreted 19% as hippuric acid (El Masry et al., 1956). Also in rabbits, approximately 50% of a single oral dose of 450 mg/kg bw *alpha*-methylbenzyl alcohol was excreted as the glucuronic acid conjugate in the urine within 24 h. Other urinary metabolites included hippuric

acid (30%) and mandelic acid (1–2%). Under similar conditions, acetophenone exhibits essentially the same metabolic fate. A 450 mg/kg bw oral dose of acetophenone was excreted in the 24-h urine as the glucuronic acid conjugate of *alpha*-methylbenzyl alcohol (47%) and, to a lesser extent, as hippuric acid (17%) (Smith et al., 1954a).

The metabolic fate of the alcohol or ketone is not significantly affected by either the mode of administration of the test material or the animal species tested. The major urinary metabolites remained the glucuronic acid conjugate of *alpha*-methylbenzyl alcohol (35%) and hippuric acid (24%) when rabbits were administered single subcutaneous doses of 500–1400 mg acetophenone/kg bw. Small amounts were excreted as mandelic acid or unchanged (Thierfelder and Daiber, 1923). When dogs were administered single oral doses of 500 mg acetophenone/kg bw, 35% was recovered in the urine as the glucuronic acid conjugate of *alpha*-methylbenzyl alcohol while 20% was excreted as hippuric acid. Much of the remainder was excreted unchanged (Quick, 1928).

Minor urinary metabolites in rabbits given large doses (5.36 g total dose) of acetophenone by intraperitoneal injection include *o*-hydroxyacetophenone, *m*-hydroxyacetophenone, and *p*-hydroxyacetophenone. These metabolites accounted for approximately 1% of the dose (Kiese and Lenk, 1974). In metabolic studies with ethyl benzene, the formation of optically active and racemic *alpha*-methylbenzyl alcohol (No. 1), acetophenone (No. 8), and *o*-hydroxyacetophenone suggest that, (1) chiral mandelic acid (2-phenyl-2-hydroxyacetic acid) forms from *alpha*-methylbenzyl alcohol *via* acetophenone, (2) benzoic acid also forms directly from acetophenone, and (3) *alpha*-hydroxyacetophenone is an intermediary metabolite in the formation of chiral mandelic acid and benzoic acid from acetophenone or *alpha*-methylbenzyl alcohol (Sullivan et al., 1976).

Single intraperitoneal doses of racemic labelled [^{3}H-C_1]-*alpha*-methylbenzyl alcohol given to rats (8/group) resulted in the excretion of mandelic acid in the urine. The isolated acid was the (−)-enantiomer but did not contain the tritium [^{3}H-] label, suggesting that the alcohol was oxidized to acetophenone prior to the formation of mandelic acid. Acetophenone is concluded to be the precursor of optically active mandelic acid given that either stereoisomer or the racemic *alpha*-methylbenzyl alcohol forms only the (−) enantiomer of mandelic acid. Formation of benzoic acid from acetophenone was confirmed when, in male rats (8/group), 30% of a single 100 mg/kg bw dose of [methyl-^{14}C]-acetophenone was exhaled as $^{14}CO_2$ within 30 h. The intermediacy of *alpha*-hydroxyacetophenone in the formation of benzoic acid and mandelic acid is supported by the observation that incubation of acetophenone in microsomes of rat hepatocytes yields mainly *alpha*-hydroxyacetophenone (Sullivan et al., 1976).

Based on these observations it is concluded that, in animals, *alpha*-methylbenzyl alcohol and acetophenone are interconvertible. *alpha*-Methylbenzyl alcohol is excreted in the urine predominantly as the glucuronic acid conjugate. To a lesser extent, acetophenone undergoes α-oxidation to yield *alpha*-hydroxyacetophenone. Subsequent stereoselective reduction of the ketone function and oxidation of the terminal alcohol yields mandelic acid. Oxidation of the secondary alcohol only yields the corresponding ketoacid which undergoes oxidative decarboxylation to yield benzoic acid which is then excreted as hippuric acid (see Fig. 1).

The metabolic reduction of benzophenone (diphenylketone) to yield the corresponding alcohol is consistent with a similar reduction step in acetophenone metabolism. Given that benzophenone has no oxidizable side chain, other metabolic options such as *alpha*-hydroxylation are unavailable. Therefore, the majority (46–61%) of a single dose of 364 mg benzophenone/kg bw administered to rabbits *via* stomach tube is excreted as the glucuronide conjugate of the corresponding alcohol diphenylmethanol (Robinson, 1958). Incubation of an 8 mM solution of benzophenone with rabbit liver homogenate and NADPH resulted in the formation of 20% diphenylmethanol in 1 h (Leibman, 1971).

3.3.2. Aromatic ketones–higher homologues

The side-chain length of the aromatic ketone or secondary alcohol does not significantly affect metabolism. The ketone and alcohol are interconvertible. In major metabolic pathways, the ketone is stereoselectively reduced to the corresponding alcohol that is subsequently excreted as the glucuronic acid conjugate. If the alkyl chain is even numbered, the ketone may undergo oxidation and cleavage to yield a phenylacetic acid derivative or, if the alkyl chain is odd numbered, oxidative cleavage yields mainly a benzoic acid derivative. The acids are excreted almost exclu-

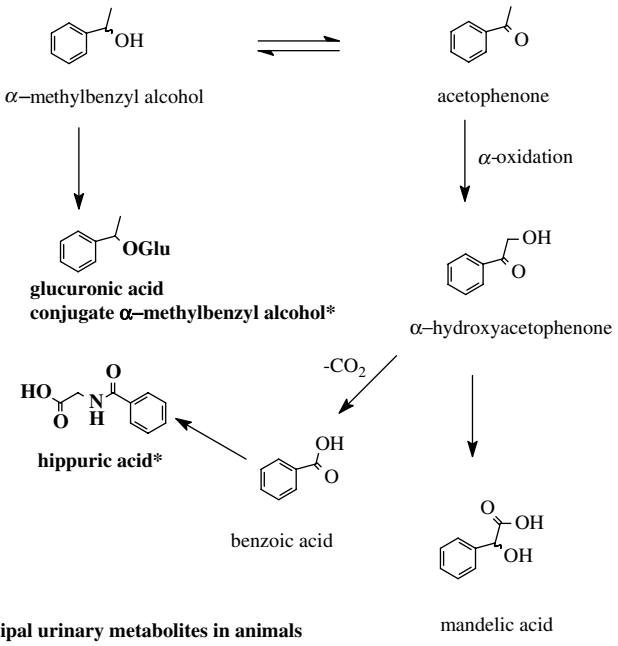


Fig. 1. Metabolism of acetophenone and *alpha*-methylbenzyl alcohol.

sively as glycine conjugates (i.e., phenylaceturic acid and hippuric acid).

1-Phenyl-1-propanol (No. 23) and propiophenone (No. 25) are interconvertible *in vitro*. Metabolic reduction of propiophenone produced 19% and 24% 1-phenyl-1-propanol in the NADPH and NADH-fortified male rat liver preparations, respectively. Male rabbit liver homogenate incubated with propiophenone and fortified with NADPH and NADH-generating systems produced 75% and 61% 1-phenyl-1-propanol, respectively. Hydroxylation of the *alpha*-methylene group to produce 2-hydroxypropiophenone and oxidation of the terminal carbon of propiophenone to yield a *beta*-ketoacid are minor metabolic pathways. In the latter pathway, the *beta*-ketoacid is subsequently decarboxylated to yield acetophenone (4–8%). A greater amount of propiophenone (17–18%) was produced in both rat liver preparations (Coutts et al., 1981). In a follow-up experiment, 93–97% of the 1-phenyl-1-propanol produced from propiophenone in the rat and rabbit preparations occurred as the (S)-(−)-enantiomer. The remainder occurred in the (R)-(+)-enantiomer. NADPH and NADH-generating systems were equally efficient reducing both species (Prelusky et al., 1982).

The presence of *alpha,beta*-unsaturation does not significantly alter the metabolic fate of aromatic ketones. The glycine conjugate of phenylacetic acid (65%) was the major urinary metabolite collected 48 h after male F344 rats were administered single doses of 200 mg/kg bw of 4-phenyl-3-buten-2-one (No. 21, even-numbered alkyl side-chain) *via* oral gavage. Minor urinary metabolites included hippuric acid (9.9%) and glutathione conjugates of the parent ketone (5.6%) and alcohol (2.2%). Presumably, hippuric acid is formed from hydration of the double bond, subsequent retro-aldol reaction to form benzaldehyde, and then oxidation to benzoic acid. The parent ketone could not be detected in the blood following dosing. The principal blood metabolite after intravenous administration of the same dose was the corresponding alcohol 4-phenyl-3-buten-2-ol, which represented 4.4% of the total dose (Sauer et al., 1997a). Essentially the entire administered dose was recovered within 48 h.

In a similar experiment in female B6C3F1 mice, principal urinary metabolites included phenylaceturic acid (35.1%) and hippuric acid (19%), the glutathione conjugate of the ketone (6.7%) and unchanged ketone (8.6%). The principal blood metabolite after intravenous administration of the same dose administered to rats was the corresponding alcohol and the hydrated ketone 4-hydroxy-4-phenyl-2-butanone, which represented 5.4% and 2.3% of the total dose, respectively. Only about 1.2% of the administered dose was present in the feces. Approximately 96% of the administered dose was recovered within 48 h (Sauer et al., 1997a).

3.3.3. Summary of metabolism

Aromatic ketones and their corresponding alcohols are interconvertible *in vivo*. The more polar alcohol is excreted in the urine primarily as conjugates of glucuronic acid. This is the major metabolic pathway in animals. The ketone may also undergo *alpha*-oxidation (hydroxylation) in the side chain to yield intermediary metabolites (e.g., *alpha*-hydroxyacetophenone) that undergo further oxidation and cleavage to yield aromatic carboxylic acids (phenylacetic acid or benzoic acid). These aromatic acids are excreted primarily as glycine conjugates (phenylaceturic acid or hippuric acid). Metabolic options are limited for substances such as benzophenone which is a diphenylketone. Lacking other reactive positions, the ketone is reduced to the corresponding alcohol that is then conjugated with glucuronic acid. Based on recent pharmacokinetic data for a representative aromatic ketone (Sauer et al., 1997a,b), it appears that orally administered ketones undergo essentially complete first-pass metabolism prior to systemic distribution.

4. Toxicological studies

4.1. Acute toxicity

Acute rat oral LD_{50} values have been reported for 17 of the 38 agents in this group. These values are in the range from 400 to greater than 10,000 mg/kg bw. However, the majority of these values are >1000 mg/kg bw, demonstrating that the oral acute toxicity of aromatic substituted secondary alcohols, ketones, and related esters is extremely low (Minner, 1977; Brown et al., 1955; Burdock and Ford, 1990; Calandra, 1971; Carpenter et al., 1974; Denine and Palanker, 1973; Fogleman and Margolin, 1970; Jenner et al., 1964; Levenstein, 1973, 1976; Levenstein and Wolven, 1972; Linet et al., 1962; Moreno, 1973, 1977; Posner, 1971; Reagan and Becci, 1984; Rohrbach and Robineau, 1958; Russell, 1973; Smyth and Carpenter, 1944, 1948; Smyth et al., 1969; Trubek Laboratories, 1964).

The available mouse acute oral LD_{50} values range from 500 to 3100 mg/kg bw (Caprino et al., 1976; Moreno, 1982; Damment, 1992; Rohrbach and Robineau, 1958; Schafer and Bowles, 1985) (Table 2).

4.2. Short-term studies of toxicity

Short-term toxicity studies have been performed for a representative number (12 of 38) of aromatic substituted secondary alcohols, ketones, and related esters (Brown et al., 1955; Burdock et al., 1991; Ford et al., 1983; Gaunt et al., 1974; Hagan et al., 1967; Kapp et al., 2003; NTP, 1980, 1990; Oser et al., 1965; Posternak et al., 1969; Trubek Laboratories, 1956, 1958). These studies include evaluation of toxic potential for the parent alcohol *alpha*-methylbenzyl alcohol (No. 1), its corresponding ketone (No. 8), the corresponding acetate ester (No. 3), one naphthyl ketone [methyl beta-naphthyl ketone (No. 38)], three aromatic secondary alcohols or related esters of chain length $>C_2$ [1-phenyl-1-propanol (No. 23), the butyric acid ester of 1-phenyl-2-propanol (No. 15), and *alpha*-isobutylphenethyl alcohol (No. 27)], two *p*-methoxyphenyl substituted

Table 2
Acute, short-term and long-term toxicity studies for aromatic substituted secondary alcohols, ketones and related esters used as flavor ingredients

	Flavoring ingredient	Oral acute studies		Short-term studies			
		Oral LD_{50} mg/kg bw (Species)	References	Species; Sex[a]	Time (days)/ Route	NOAEL (mg/kg bw)	References
1	*alpha*-Methylbenzyl alcohol	400 (Rat)	Smyth and Carpenter (1944)	Mouse; M, F	16/Gavage	500	NTP (1990)
1	*alpha*-Methylbenzyl alcohol	5000 (Rat)	Posner (1971)	Mouse; M, F	91/Gavage	750[b]	NTP (1990)
1	*alpha*-Methylbenzyl alcohol			Mouse; M, F	721/Gavage	<375	NTP (1990)
1	*alpha*-Methylbenzyl alcohol			Rat; M, F	16/Gavage	1000	NTP (1990)
1	*alpha*-Methylbenzyl alcohol			Rat; M, F	91/Gavage	187	NTP (1990)
1	*alpha*-Methylbenzyl alcohol			Rat; M, F	721/Gavage	<375	NTP (1990)
3	*alpha*-Methylbenzyl acetate			Rat; M, F	91/Gavage	15	Gaunt et al. (1974)
4	*alpha*-Methylbenzyl propionate	5200 (Rat)	Levenstein (1973)	Rat; M, F	119/Gavage	1000[b]	Hagan et al. (1967)
7	*alpha*-Dimethylbenzyl alcohol	2707 (Rat)	Linet et al. (1962)				
8	Acetophenone	3000 (Rat)	Smyth and Carpenter (1944)				
8	Acetophenone	3200 (Rat)	Jenner et al. (1964)				
8	Acetophenone	2549 (Rat)	Smyth et al. (1969)				
8	Acetophenone	900 (Rat)	Smyth and Carpenter (1948)				
8	Acetophenone	1780 (Mouse)	Damment (1992)				
9	4-Methylacetophenone	1400 (Rat)	Calandra (1971)				
12	Acetanisole	1720 (Rat)	Moreno (1973)				
13	4-Acetyl-6-*t*-butyl-1,1-diemthylindan	>5000	Denine and Palanker (1973)				
13	4-Acetyl-6-*t*-butyl-1,1-diemthylindan	3690 (Rat)	Minner (1977)				
14	1-(*p*-Methoxyphenyl)-2-propanone	3663 (Rat)	Levenstein (1976)				
15	*alpha*-Methylphenethyl butyrate			Rat; M, F	90/Food	3.09[b] (M), 3.46[b] (F)	Posternak et al. (1969)
19	4-(*p*-Methoxyphenyl)-2-butanone	>5000 (Rat)	Russell (1973)	Rat; M	14/Food	500	Trubek Laboratories (1956)
19	4-(*p*-Methoxyphenyl)-2-butanone			Rat; M, F	90/Food	114[b]	Trubek Laboratories (1958)
21	4-Phenyl-3-buten-2-one	5049 (Rat)	Levenstein and Wolven (1972)				
21	4-Phenyl-3-buten-2-one	5250 (Rat)	Trubek Laboratories (1964)				
22	3-Methyl-4-phenyl-3-buten- 2-one	5500 (M, Rat) 4100 (F, Rat)	Burdock and Ford (1990)				
23	1-Phenyl-1-propanol	2800 (Rat)	Brown et al. (1955)	Rat; M, F	120/Food	415[b] (M), 476[b] (F)	Brown et al. (1955)
23	1-Phenyl-1-propanol	2500 (Rat)	Rohrbach and Robineau (1958)				
23	1-Phenyl-1-propanol	500 (Mouse)	Rohrbach and Robineau (1958)				
25	Propiophenone	4533 (Rat)	Carpenter et al. (1974)				
27	*alpha*-Isobutylphenethyl alcohol			Rat; M, F	90/Oral	10	Ford et al. (1983)

29	3-Benzyl-4-heptanone	4400 (Rat)	Burdock and Ford (1990)				
29	3-Benzyl-4-heptanone	4441 (Rat)	Reagan and Becci (1984)				
32	1-(*p*-Methoxyphenyl)-1-penten-3-one	>5000 (Rat)	Moreno (1977)	Rat; M, F	90/Food	12.7[b] (M), 15.2[b] (F)	Oser et al. (1965)
34	1-Phenyl-1,2-propanedione			Rat; M, F	90/Food	17.53[b] (M), 17.26[b] (F)	Posternak et al. (1969)
35	Benzophenone	>10,000 (Rat)	Fogleman and Margolin (1970)	Rat; M, F	28/Food	<20	Burdock et al. (1991)
35	Benzophenone	2895 (Mouse)	Caprino et al. (1976)	Rat; M, F	90/Food	18.6[b] (M), 21.6[b] (F)	Burdock et al. (1991)
35	Benzophenone			Rat; M, F	735/Food	<15	NTP (2004)
35	Benzophenone			Mouse; M, F	735/Food	15 double check	NTP (2004)
37	Benzoin	1025 (Mouse)	Schafer and Bowles (1985)	Rat; M, F	14/Food	1000	NTP (1980)
37	Benzoin			Mouse; M, F	14/Food	2150	NTP (1980)
37	Benzoin			Rat; M, F	90	<50	NTP (1980)
37	Benzoin			Rat; M, F	90	12.5 (M), 50[b] (F)	NTP (1980)
37	Benzoin			Mouse; M, F	90	1500[b]	NTP (1980)
37	Benzoin			Rat; M, F	728	<12.5 (M), <25 (F)	NTP (1980)
37	Benzoin			Mouse; M, F	728	750	NTP (1980)
38	Methyl *beta*-napthyl ketone	3100 (Mouse)	Moreno (1982)	Rat; M, F	90	33.0[b] (M), 36.9[b] (F)	Oser et al. (1965)

[a] M = Male; F = Female. If not listed, sex was not specified in the report.
[b] This study was performed at either a single dose level or multiple dose levels that produced no adverse effects. Therefore this dose level is not a true NOAEL, but the highest dose tested that produced no adverse effects. The actual NOAEL would be higher.

ketones [4-(*p*-methoxyphenyl)-2-butanone (No. 19) and 1-(*p*-methoxyphenyl)-1-penten-3-one (No. 32)], two ketones containing two aromatic rings [benzophenone (No. 35) and benzoin (No. 37)], and one aromatic diketone [phenyl-1,2-propandione (No. 34)]. Results of these studies are summarized in Table 2 and described below. It is noteworthy that short- and long-term studies on *alpha*-methylbenzyl alcohol (No. 1), benzophenone (No. 35) and benzoin (No. 37) were performed by the National Toxicology Program. The results of these studies provided no observable adverse effect levels (NOAELs) that are at least 1000 times (most 100,000–1,000,000 times) the daily *per capita* intake (''eaters only'') of the respective substances for their intended use as flavoring ingredients.

4.2.1. Mice

4.2.1.1. α-Methylbenzyl alcohol (No. 1). Oral doses of 0, 125, 250, 500, 1000, or 2000 mg *alpha*-methylbenzyl alcohol/kg bw/d were administered to groups of 4 or 5 male and 5 female B6C3F$_1$ mice by corn oil gavage 5 d per week for 16 d. All 4 males and all 5 females receiving the highest dose and 3 of 4 males and 4 of 5 females receiving 1000 mg died prior to the end of the study. Histopathological evaluations did not reveal any abnormality (NTP, 1990).

In a follow-up 13-week study, groups of 10 male and 10 female B6C3F$_1$ mice were administered 0, 46.9, 93.8, 187.5, 375, or 750 mg *alpha*-methylbenzyl alcohol/kg bw/d *via* corn oil gavage 5 d per week for 13 weeks. Animals were observed twice daily and weighed weekly. Necropsies, including liver weight measurements, were performed on all test and control animals. Complete histologic examinations performed on all male and female mice assigned to the 750 mg/kg bw/d dose group revealed no evidence of lesions related to treatment with the test agent. No compound-related deaths occurred. Final body weights were comparable to controls (NTP, 1990).

4.2.1.2. Benzophenone (No. 35). Benzophenone was provided to groups of B6C3F1mice (10/sex/dose) in the feed at concentrations of 0, 1250, 2500, 5000, 10,000, or 20,000 ppm for 14 weeks. These concentrations in the feed are estimated by the study authors to provide a daily intake of 0, 200, 400, 800, 1600, or 3300 mg/kg bw for males and 0, 270, 540, 1000, 1900, or 4200 mg/kg bw for females (NTP, 2000). All animals were observed twice daily and clinical signs were recorded twice weekly. Body weights were measured weekly. Necropsies were performed on all animals, including those who died or were killed moribund during the study. Major organs and tissues were evaluated for the presence of gross lesions. Histological examinations were performed on tissues prepared from all control, 10,000 ppm, 20,000 ppm and test animals that died during the study. Due to the potential photoionizing properties of benzophenone, complete histopathology examination of the eyes including the lens, retina, and other ocular structures was performed. Microsomal P450 content of the liver was also measured.

One male in the 1250 ppm group was accidentally killed on day 26. Four males in the 20,000 ppm group died in week 1 and 1 male and 1 female in the 20,000 ppm group died in week 2. Two males were removed from the study at week 10 and three at week 11 plus 3 females were removed at week 12 in the 20,000 ppm dietary level groups due to significantly reduced body weight as compared to controls. Body weights of the mice in the 5000 and 10,000 ppm groups were significantly reduced and surviving females in the 20,000 ppm group lost weight. Clinical observations included lethargy for both males and females in the 20,000 ppm groups. Food consumption for the 20,000 ppm groups was reduced compared to controls. The authors attribute this to the lack of palatability of the diet. No hematology or clinical chemistry was performed at week 14 for the 20,000 ppm males. The males in the 5000 and 10,000 ppm groups showed anemia with minimal decreases in hematocrit values, hemoglobin levels and erythrocyte counts. 20,000, 10,000 and 5000 ppm females showed erythrocytosis. The authors found this consistent with hemoconcentration caused by dehydration supported by increases in albumin and total protein concentrations. Total bile salt concentrations and sorbitol dehydrogenase activities were increased in all treated females and the 2500 ppm or greater male groups. The kidney weights of males exposed to 2500 ppm or greater and the liver weights of all males exposed were significantly increased. The absolute and relative liver weights of all exposed females except the 20,000 ppm group were significantly increased. Females in the 2500 and 10,000 ppm groups showed a significant increase in absolute kidney weights and females exposed to 2500 ppm or greater showed a significant increase in relative kidney weights. The absolute and relative thymus weights of females at the 20,000 ppm exposure level and absolute thymus weights at the 5000 and 10,000 ppm levels were significantly less than controls. Other organ weight differences were considered due to the overall lower body weights of the exposed mice. No significant changes in the ocular structures or tissues were observed.

There were no exposure-related gross lesions at necropsy. Significant histopathology findings were limited to centrilobular hypertrophy of hepatocytes which corresponded to an increase in liver weights at all exposure levels in both sexes. No microscopic finding supported the increased kidney weights. Males in the 10,000 ppm exposure showed significantly greater hepatic CYP450 activity. Ethoxyresorufin deethylase activities and pentoxyresorufin dealkylase activities were significantly increased for all exposed male groups compared to controls. Based on these observations, the no adverse effect level is 1250 ppm or 200 mg/kg per day.

4.2.1.3. Benzoin (No. 37). Groups of 5 male and 5 female B6C3F$_1$ mice were administered 0, 215, 464, 1000, 2150, or 4640 mg benzoin/kg bw/d *via* the diet for a period of 14 d. Daily observation and weekly measurement of body weight revealed no difference between test and control groups. At necropsy, enlarged lymph nodes and spleens were reported in males at the highest dose and enlarged lymph nodes in females at the highest dose (NTP, 1980).

In a subsequent study, groups of 10 male and 10 female B6C3F$_1$ mice were administered 0, 620, 1250, 2500, 5000, and 10,000 ppm benzoin in the diet for a period of 90 d. Dietary concentrations were calculated (FDA, 1993) to provide corresponding average daily intake levels of 0, 93, 188, 375, 750, and 1500 mg/kg bw, respectively. Animals were observed daily for mortality and body weights, and food consumption, appearance, and behavior were recorded weekly. Test feed was replaced with control feed for one week following the 90-d test period. All surviving animals were then killed and necropsied. Microscopic examinations were performed on major organs and tissues, as well as gross lesions. No evidence of any compound-related effect was observed at any dose level (NTP, 1980). The no observable adverse effect level for dietary administration of benzoin to mice for 90 d is 10,000 ppm or 1500 mg/kg bw per day.

4.2.2. Rats

4.2.2.1. α-Methylbenzyl alcohol (No. 1). Dose levels of 0, 125, 250, 500, 1000, or 2000 mg *alpha*-methylbenzyl alcohol/kg bw/d were administered to F344 rats (5 M & 5 F) by corn oil gavage 5 d per week for a period of 16 d. Two males and 4 females receiving 2000 mg *alpha*-methylbenzyl alcohol/kg bw/d died before the end of the study. Significantly reduced final body weights (males, 21%; females, 15%) and hemorrhagic gastrointestinal tracts (males, 2/5; females, 1/5) were also observed in the 2000 mg/kg bw group. Histopathological evaluations performed on 2 males and 2 females dosed at 1000 mg/kg bw revealed no evidence of lesions related to administration of the test agent (NTP, 1990).

In a subsequent study, groups of 10 male and 10 female F344/N rats were administered 0, 93, 187, 375, 750, or 1500 mg *alpha*-methylbenzyl alcohol/kg bw/d by corn oil gavage 5 d per week for a period of 13 weeks. One male and 3 females receiving the highest dose died prior to the end of the study. Weekly measurement of body weights revealed a significant reduction in body weights of males (12%) and females (7%) in the highest dose group. At necropsies, relative liver weights for males receiving the 3 highest doses and for all female dose groups were greater than controls. Histopathologic examinations performed on all male and female rats in the 750 and 1500 mg/kg bw/d group, and males receiving 375 mg/kg bw/d, revealed minimal to mildly elevated levels of hemosiderin in spleen macrophages of male (9/10) and female (6/10) rats receiving 1500/kg bw/d and male (10/10) rats receiving 750 mg/kg bw/d (NTP, 1990). The dose level of 187 mg/kg bw per day that resulted in no adverse effects in rats is at least 100,000 times the daily *per capita* intake (“eaters only”) of 1 μg/kg bw/d for the intended use of *alpha*-methylbenzyl alcohol as a flavoring substance.

4.2.2.2. α-Methylbenzyl acetate (No. 3). Dose levels of 0, 15, 50, or 150 mg *alpha*-methylbenzyl acetate/kg bw were administered to groups of rats (15 M & 15 F) by gavage daily for a period of 13 weeks. Daily observations showed no changes in the appearance or behavior of the treated or control rats. Weekly measurements of body weight, and food and water consumption revealed a statistically significant increase in food and water intake in the 150 mg/kg bw dose group of males. Urine analysis of this group showed an increase in the number of unspecified cells excreted at week 6, but not at week 13.

At necropsy, absolute and relative kidney and liver weights of the males in the 150 mg/kg bw group were higher than that of the controls, though no histopathological abnormalities were observed. Kidney and liver weights were slightly increased in the 50 mg/kg bw group, but the increase was not statistically significant.

The authors concluded that the no-untoward effect level for *alpha*-methylbenzyl acetate was 15 mg/kg bw/d. The gavage dose of 15 mg/kg is >1000 times the estimated per capita intake (“eaters only”) of 11ug/kg bw per day from use of *alpha*-methylbenzyl acetate as a flavoring substance (Gaunt et al., 1974).

4.2.2.3. Acetophenone (No. 8). Sprague-Dawley rats (10/sex/dose) were administered 0, 75, 225 or 750 mg/kg bw per day of acetophenone via gavage for 28 d. There was no mortality from acetophenone administration. Mean forelimb grip strength and motor activity were reduced for the 750 mg/kg bw per day group of male rats. Pre- and post-dose salivation was noted for the mid- and high-dose male group. Body weight and food consumption were reduced for the high dose male and female groups. Hematology and clinical chemistry values were comparable between test and control animals. However it was noted that high-dose males showed increased cholesterol levels. The authors of this abstract determined the NOAEL to be 75 mg/kg bw per day for systemic toxicity and the NOAEL for neurological effects to be 225 mg/kg bw per day (Kapp et al., 2003).

Weanling Osborne-Mendel rats (5 M & 5 F) were maintained on a diet containing 1000, 2500, or 10,000 ppm acetophenone/kg bw/d for a period of 17 weeks. Dietary concentrations were calculated (FDA, 1993) to provide corresponding average daily intake levels of 0, 100, 250, and 1000 mg/kg bw/d, respectively. Body weight, food intake, and general condition of each test animal were recorded weekly. Necropsies were performed at study termination, and all tissues were examined macroscopically. Histopathological exams and organ weight measurements were performed for the liver, kidneys, spleen, heart, and testes. No effects were reported at any intake level (Hagan et al., 1967). The dietary intake of 1000 mg acetophenone/kg bw per day that produced no adverse effects is >100,000 times the estimated daily *per capita* intake (“eaters only”)[1] of 3 μg/kg bw/d of acetophenone from use as a flavoring substance.

4.2.2.4. α-Methylphenethyl butyrate (No. 15) and 1-Phenyl-1,2-propanedione (No. 34). Groups of 10–16 male and 10–16 female rats were maintained on a diet containing *alpha*-methylphenethyl butyrate at a level calculated to provide daily intakes of approximately 3.09 and 3.46 mg/kg bw/d, respectively, for 90 d. Body weights, food consumption, and efficiency of food utilization were recorded weekly. Hematological examinations were conducted on 50% of animals at week 7 of the study and again on all animals at week 12. A significant change in leukocyte count at week 7 was not considered by the authors to be of toxicological significance, and was not present at the end of the study. Neither measurements of growth, clinical chemistry, organ weights, nor histopathology of the major organs and tissues (23) provided any evidence of adverse toxicological effects (Posternak et al., 1969). The intake level of 3.09 (males) and 3.46 (females) mg/kg bw/d that produced no adverse effects is >1,000,000 times the estimated daily per capita intake (“eaters only”)[1] of 0.002 μg/kg bw/d from use of *alpha*-methylphenethyl butyrate as a flavoring substance.

In a second 90-d study based on the same test protocol, rats (8 M & 8 F) were fed 1-phenyl-1,2-propanedione in the diet at levels calculated to provide average daily intakes that were reported as 17.53 and 17.26 mg/kg bw for males and females, respectively. Neither measurements of growth, hematology, clinical chemistry, organ weights, nor histopathology revealed any adverse toxicological effects (Posternak et al., 1969). The dose levels of 17.53 (males) and 17.26 (females) mg/kg bw/d that produced no adverse effects are >1,000,000 times the estimated daily per capita intake (“eaters only”)[1] of 0.002 μg/kg bw/d from use of 1-phenyl-1,2-propanedione as a flavoring substance.

4.2.2.5. 4-(p-Methoxyphenyl)-2-butanone (No. 19). Albino rats (6M/group) were administered diets containing 0, 0.5, 1.0, or 2.0% 4-(*p*-methoxyphenyl)-2-butanone for a period of 2 weeks. Dietary concentrations were calculated (FDA, 1993) to provide corresponding average daily intake levels of 0, 500, 1000, and 2000 mg/kg bw, respectively. Behavior and appearance of all test rats were comparable to controls. Body weight gains for the groups receiving 1000 and 2000 mg/kg bw 4-(*p*-methoxyphenyl)-2-butanone were significantly less than controls. The authors note the likelihood that the test compound reduced the palatability of the diet. Gross pathology examinations performed at necropsy gave normal results (Trubek Laboratories, 1956).

In a follow-up study, rats of unspecified strain (10 M & 10 F) were given diets containing 4-(*p*-methoxyphenyl)-2-butanone at concentrations resulting in average daily intakes of 56 or 114 mg/kg bw/d for a period of 90 d. Body weight, food consumption, and efficiency of food utilization were recorded weekly, and animals were examined for physical appearance and behavior more frequently. Urine and blood samples collected from 3 animals in each group at week 12 revealed normal levels of urinary glucose and blood hemoglobin in all groups. Liver and kidney

weights were recorded at necropsy. There were no statistically significant differences between control and test animals at either dietary level for measurements of body weight, general appearance and behavior, hematology, urinalysis or histopathology (Trubek Laboratories, 1958). The intake level of 114 mg/kg bw/d that produced no adverse effects is >1000 times the estimated daily per capita intake ("eaters only")[1] of 14 μg/kg bw/d from use as a flavoring substance.

4.2.2.6. 1-Phenyl-1-propanol (No. 23). 1-Phenyl-1-propanol was administered in the diet to rats (5 M & 5 F) for a period of 4 months at average daily intake levels of 476 mg/kg bw in females and 415 mg/kg bw in males. Results of organ and body weight measurements and histological tissue studies revealed no adverse effects of 1-phenyl-1-propanol administration. No other details were given (Brown et al., 1955). The intake levels of 415 (males) and 476 (females) mg/kg bw/d that produced no adverse effects are >1,000,000 times the estimated daily per capita intake ("eaters only")[1] of 0.002 μg/kg bw/d from use of 1-phenyl-1-propanol as a flavoring substance.

4.2.2.7. α-Isobutylphenethyl alcohol (No. 27). Groups of rats (15 M & 15 F) were administered *alpha*-isobutylphenethyl alcohol in the diet at concentrations calculated to provide an average daily intake of 0, 10, 40 or 160 mg/kg bw/d for 90 d. Body weights and food and water intakes were recorded twice weekly. Blood and urine analyses were performed at week 6 and at study termination. Food intake was generally lower in test groups than in controls. A decrease in serum glucose was observed in mid-dose males, but this effect was of questionable toxicological significance. At 160 mg/kg bw/d, the following changes were statistically significant: a reduction in weight gain (possibly due to reduced palatability) in both sexes, mild proteinuria in females, increased relative liver weight in males, increased relative cecal weights in both sexes, increased relative spleen weight in females, a reduction in serum glucose in both sexes, and a lower reticulocyte count in both sexes. Significantly increased relative spleen weights were also observed in males and females receiving 40 mg/kg bw/d. There was no evidence of histopathology in either liver or spleen tissues of any treated animals. Other organs also showed no evidence of abnormalities related to administration of the test agent. The authors concluded that the no observable adverse effect level in the study was 10 mg/kg bw/d (Ford et al., 1983). This intake level is >100,000 times the estimated daily per capita intake ("eaters only")[1] of 0.04 μg/kg bw/d from use of *alpha*-isobutylphenethyl alcohol as a flavoring substance.

4.2.2.8. Methyl beta-naphthyl ketone (No. 38) and 1-(p-methoxyphenyl)-1-penten-3-one (No. 32). The two aromatic ketones were added to the diet of rat for 90 d using the same study protocol (Oser et al., 1965). Rats (15 M & 15 F) were provided food containing methyl *beta*-naphthyl ketone for a period of 90 d at dietary concentrations reported to result in daily intakes of 33.0 mg/kg bw/d and 36.9 mg/kg bw/d for males and females, respectively. Body weight and food consumption measurements, as well as clinical observations were performed regularly. Hematological and clinical chemistry evaluations were conducted at weeks 6 and 12. Liver and kidney weights were recorded at necropsy, and histological examinations were performed on prepared tissues from all major organs. Neither measurements of growth, hematology, and clinical chemistry, nor gross or histological observations at necropsy revealed any significant adverse effects. Dietary levels of 33.0 (males) and 36.9 (females) mg/kg bw/d that produced no adverse effects are >10,000 times the estimated daily per capita intake ("eaters only")[1] of 1 μg/kg bw/d from use as a flavoring substance.

No effects were observed when 1-(*p*-methoxyphenyl)-1-penten-3-one was added to the diet of rats at levels calculated to provide 12.7 and 15.2 mg/kg bw/d for males and females, respectively, for a period of 90 d. Dietary intakes of 12.7 (males) and 15.2 (females) mg/kg bw/d that produced no adverse effects are >1000 times the estimated daily per capita intake ("eaters only")[1] of 2 μg/kg bw/d, from use of 1-(*p*-methoxyphenyl)-1-penten-3-one as a flavoring substance.

4.2.2.9. Benzophenone (No. 35). Twenty to 32 Sprague-Dawley rats/dose/sex were maintained on diets containing benzophenone at levels calculated to provide a daily intake of 0, 20, 100 or 500 mg/kg bw/d for 28 d. Weekly measurement of body weight and food consumption revealed a significant decrease in final mean body weights for both sexes at the highest dose level. Hematological examinations, clinical chemistry determinations, and urine analysis conducted on all test and control rats on day 28 showed significantly decreased levels of red blood cells, hemoglobin, and hematocrit in mid- and high-intake level males and females. At necropsy, a significant increase in absolute and relative liver weights in mid- and high-intake level males and females and a significant increase in relative kidney weights in mid- and high-intake level males and all female treatment groups were reported. Low-intake level females had significantly increased relative liver weights. These changes were not observed at higher dietary levels. Histopathological examinations of the liver and kidney of all test and control animals and 18 additional organs from 5 males and 5 females randomly selected from the high-dose and control groups revealed significant increases of slight to moderate hepatocyte hypertrophy in mid- and high-dose males and females. No other treatment-related abnormalities were observed (Burdock et al., 1991).

In a second study, 16 male and 16 female Sprague-Dawley rats were administered 20 mg benzophenone/kg bw/d *via* the diet for a period of 90 d. Actual benzophenone intake was reported as 18.6 mg/kg bw/d for males and 21.6 mg/kg bw/d for females. General observations as well as body, liver, and kidney weight measurements

were performed according to the same protocol employed in the 28-d study (Burdock et al., 1991). Hematology evaluations, clinical chemistry determinations, and urine analyses conducted on day 90 revealed no apparent differences between test and control animals. Gross necropsies conducted for all test and control animals, and histopathological examinations performed on 20 organs (including liver and kidney) in 12 males and 12 females randomly selected from the treatment and control groups did not result in any findings attributable to administration of benzophenone (Burdock et al., 1991). The dietary intake levels of 18.6 (males) and 21.6 mg/kg bw/d (females) that produced no adverse effects are >10,000 times the estimated daily per capita intake ("eaters only")[1] of 0.2 μg/kg bw/d from use of benzophenone as a flavoring substance.

In a 14 week study performed by the NTP, benzophenone was provided to groups of F344/N rats (10/sex/dose) in the feed at concentrations of 0, 1250, 2500, 5000, 10,000, or 20,000 ppm. These concentrations in the feed are estimated by the study authors to provide a daily intake of 0, 75, 150, 300, 700, or 800 mg/kg bw for males and 0, 80, 160, 300, 700 or 1000 mg/kg bw for females (NTP, 2000). All animals were observed twice daily and clinical signs were recorded twice weekly. Body weights were measured weekly. Necropsies were performed on all animals, including those who died or were killed moribund during the study. All organs and tissues were evaluated for the presence of gross lesions. Histological examinations were performed on prepared tissues from all control and test animals that died during the study. Due to the potential photoionizing properties of benzophenone complete histopathology examination of the eyes was performed. Microsomal P450 content of the liver was also measured.

One female in the 20,000 ppm exposure group died on day 12. Due to the significant decrease in body weights for the remaining females in the 20,000 ppm group they were removed from the study at week 6. Body weights for males at 2500 ppm or greater and all female exposure groups were significantly reduced compared to controls. Clinical findings were noted as lethargy for the 20,000 ppm females and thinness in the 10,000 ppm males. Two males in the 20,000 ppm group had prolapsed penises. Males and females at the 20,000 ppm exposure level consumed significantly reduced amounts of food compared to controls and other exposure groups. Due to mortality and early removal from the study no 14 week clinical chemistry or hematology data were collected. Anemia was observed in males and females at 2500 ppm or greater as indicated by decreased hematocrit values, hemoglobin concentrations and erythrocyte levels. In male rats, increased reticulocyte counts were observed indicating an erythropoietic response. Platelet count decreases were seen in the 10,000 ppm male and 5000 and 10,000 ppm female exposure groups. Alanine aminotransferase activity levels were increased for the 10,000 ppm females and the 20,000 ppm males at 14 weeks and the 20,000 ppm females at 6 weeks. Sorbitol dehydrogenase was increased for the 10,000 ppm females. Minimal to marked increases in bile salts were reported at various time points in the study for all exposure groups. In contrast, minimal to mild decreases in alkaline phosphatase activity were reported at various time points for all exposure groups. Hyperproteinemia was persistent at week 14 for all exposed females and was accompanied by hyperalbuminemia. Hyperalbuminemia is an indicator of dehydration in animals. Creatinine concentration was decreased minimally at 5000 ppm and greater for males and females at all time points in a concentration dependent manner. Creatinine levels can be related to muscle mass in rats (Finco, 1989; Ragan, 1989), and rats in the higher exposure groups weighed less and had lower muscle mass than controls. Kidney and liver weights of all exposed male and female rats were significantly greater than controls with exception of the kidney weights from the 1250 ppm female group. The absolute heart and thymus weights of the 10,000 ppm males and the absolute thymus weights of females in the 5000 and 10,000 ppm exposure groups were significantly less than controls. Other organ weight differences were attributed to lower body weights of the exposed animals.

At necropsy, exposed males had significantly smaller seminal vesicles when compared to controls. There were no microscopic changes accompanying this gross finding. Increased kidney weights were accompanied by a broad range of microscopic findings. Papillary necrosis as indicated by acute coagulative necrosis of the distal tips of the renal papillae occurred in 20% of the 10,000 ppm and 60% ($p = 0.001$) of the 20,000 ppm exposure groups. In male rats exposed to 2500 ppm and higher and females at 10,000 and 20,000 ppm, well-demarcated, wedge-shaped areas of prominent dilatation were observed. These areas were based at the capsular surface and extended deep into the medulla. Within these areas tubules were dilated and generally empty but some contained fine granulated eosinophilic material. Increased incidences of severe focal tubule regeneration were observed in all exposure groups. Foci of tubule regeneration are a component of chronic nephropathy in the aging rat. Exposure-related increases in liver weights were attributed to hypertophy and/or cytoplasmic vacuolization of hepatocytes. All exposure groups of females showed increased centrilobular vacuolization of hepatocytes. Vacuolization was observed in all male exposure groups and indicated by a bubbly appearance in the cytosol. The 20,000 ppm male exposure group showed minimal hyperplasia of immature bile ducts in portal areas extending into adjacent sinusoids. Hypercellularity of the bone marrow in males and females and poorly developed seminiferous tubules in males were observed for the 20,000 ppm exposure groups. These findings were considered secondary to reduced body weight gain and inanition. No microscopic changes were observed for the eye or any substructures or tissues. Males and females exposed to 2500 and 5000 ppm and females exposed to 1250 ppm showed significant increases in liver microsomal CYP450.

All exposure groups showed increased levels of pentoxyresorufin dealkylase activity. The 1250 ppm (75 mg/kg bw for males, 80 mg/kg bw for females) exposure is roughly 375,000 times the estimated daily per capita human intake (“eaters only”) of 0.2 μg/kg bw/d from use of benzophenone as a flavoring substance.

4.2.2.10. Benzoin (No. 37). Groups of F344 rats (5 M & 5 F) were administered 0, 100, 316, 1000, 3160, or 10,000 mg benzoin/kg bw/d in the diet for a period of 14 d. Experimental parameters were evaluated according to the same protocol employed in the corresponding mouse study (NTP, 1980). Three females receiving the highest intake level died before the end of the study. A dose-related decrease in weight gain was observed in male test animals (statistical significance not specified). None of the females receiving benzoin in the diet gained significant weight, and those at the highest intake level lost weight. Necropsies revealed a solid, silvery-white substance in the stomachs of rats in the two highest dietary intake groups (NTP, 1980).

In a subsequent 90-d study, F344 rats (10 M & 10 F) were administered 0, 500, 1500, 5000, 15,000, or 50,000 ppm benzoin in the diet for a period of 90 d. Dietary concentrations were calculated (FDA, 1993) to provide corresponding average daily intake levels of 0, 50, 150, 500, 1500, and 5000 mg/kg bw, respectively. Experimental parameters were evaluated according to the same protocol employed in the corresponding mouse study (NTP, 1980). Body weight gains in males and females receiving the three highest intake levels were reduced by more than 10% with respect to controls (statistical significance not specified). At necropsy, greenish cortices were observed in the kidneys of the following animals: 4 males and 2 females receiving 5000 mg/kg bw/d, one male receiving 500 mg/kg bw/d, and one female receiving 50 mg/kg bw/d. Liver discoloration was observed in one to four females at each intake level. Interstitial nephritis was observed in all treatment groups and severity was dose-related. Scattered vacuolated hepatocytes were observed in the livers of females at the two highest dietary intakes (NTP, 1980).

In a second 90-d study conducted at lower dietary intake levels to determine a concentration that would not cause interstitial nephritis, F344 rats (10/sex/group) were administered 0, 30, 60, 125, 250, or 500 ppm benzoin in the diet. Dietary concentrations were calculated (FDA, 1993) to provide corresponding average daily intake levels of 0, 3, 6, 12.5, 25, and 50 mg/kg bw, respectively. Experimental parameters were evaluated according to the same protocol employed in the corresponding mouse study (NTP, 1980). No deaths occurred. Interstitial nephritis was observed only in the kidneys of males at the 2 highest intake levels. Evidence of nephritis in all other test groups was comparable to controls (NTP, 1980). The highest dose level showing no adverse renal effects is >40,000 times the estimated daily *per capita* intake (“eaters only”) of 0.3 μg/kg bw/d from use of benzoin as a flavoring substance.

4.3. Long term studies of toxicity and carcinogenicity

4.3.1. Benzophenone (No. 35)

4.3.1.1. Rats. Groups of F344/N rats (50 M & 50 F) were maintained on diets containing 0, 312, 625, or 1250 ppm of benzophenone for 105 weeks (NTP, 2006). These dietary levels were calculated to provide a daily intake of 0, 15, 30, or 65 mg/kg bw, respectively. All animals were observed twice daily and clinical signs were recorded monthly. Body weights were measured weekly for the first 12 weeks, and monthly thereafter. Necropsies were performed on all animals, including those who died or were killed moribund during the study. All organs and tissues were evaluated for the presence of gross lesions, which were histopathologically examined. Histological examinations were performed on prepared tissues from all control and test animals that died during the study. Organs of the treated rats that were targeted for potential neoplastic and nonneoplastic effects were histopathologically examined.

Although mortality rates for male rats approached 100% (96%) in the 1250 ppm group, mortality was also significant for the control group (56%), the 312 ppm group (46%), and the 625 ppm group (38%). A majority of the control animals that died during the study did so by week 93. No significant differences in mortality rates were reported between controls and any of the three test groups of female rats. Mean body weights of males in the control and test groups peaked between weeks 62 (1250 ppm) to 86 (control) and then steadily decreased until the end of the study. The kidney was noted to be the principal target organ for toxic and neoplastic responses in male rats. Chronic nephropathy and mineralization of the medulla were reported in essentially all (90–100%) control and test animals. The severity of chronic nephropathy increased with dose. A dose-dependent increase in the incidences of renal tubule hyperplasia (1/50, 5/50, 20/50, and 23/50) and pelvic transitional epithelial hyperplasia (1/50, 11/50, 29/50, and 34/50) were reported. Upon microscopic evaluation of standard sectioning of the kidney, a non-statistically significant increase was reported in the incidence of renal tubule adenomas (1/50, 1/50, 2/50, and 4/50). When microscopic evaluations were extended to step sectioning, the incidences (2/50, 2/50, 4/50, and 8/50) of renal tubular neoplasms increased to become statistically significant at the two highest dietary levels.

The NTP concluded that: “*Under the conditions of these 2-year studies, there was some evidence of carcinogenic activity of benzophenone in male F344/N rats based on increased incidences of renal tubular cell adenoma.*”

The results of this 2-year bioassay for benzophenone in the male F344/N rat mimic those of other NTP bioassays for other substances. The classic profile of results involve poor survival, mean body weight changes, chronic nephropathy, and associated renal toxicity that are specific to this strain and sex of rat. Analysis by NTP researchers (Haseman et al., 1998) have shown the survival rates of feeding study and control F344 male rats have decreased

significantly over the last decade (66 and <50%, respectively). One of the major causes of death is severe chronic nephropathy that has been increasing in incidence in more recent control groups (Eustis et al., 1994). This species- and sex-specific phenomenon, in all probability, reflects the sensitivity of the male rat kidney to chronic progressive nephropathy, focal tubular and pelvic transitional urothelial hyperplasia, and specific tumorigenic responses. The interaction of test substances with spontaneous, age-related renal disease in laboratory rats has recently been reviewed (Hard, 1998; Lock and Hard, 2004). Based on a comprehensive review of renal tumors of all types reported in NTP bioassays, it seems that the interaction of chemical agents and spontaneous chronic progressive nephropathy occurs at two levels; one, to exacerbate the rate of chronic progressive nephropathy, and two, to stimulate tubule hyperplasia into foci of atypical hyperplasia eventually leading to adenomas. The induction of tumors via this pathway normally produces a minimal response in male rats leading to a low incidence of tumors of relatively small size and low grade that develop late in life.

In the benzophenone study, poor survival, especially in control and high dose animals, severely reduced the sensitivity of the study for detecting the presence of a carcinogenic response in chemically-exposed groups of male rats. Excessive mortality in the control that occurred primarily during the last quarter of the study limited the ability to detect the renal effects resulting from chronic nephropathy. Mean body weights of both control and test males peaked long before study termination (week 86 for control males to week 62 for high dose males) suggesting that systemic changes related to chronic nephropathy occurred and the overall health of the animals was adversely affected. These weight changes are similar to those observed in numerous other bioassays for other substances (Hard, 1998). Nevertheless, the severity of the chronic nephropathy was significantly greater with increasing dose as seen by increased renal tubule hyperplasia, increased hyperplasia of the transitional epithelium of the pelvis, increased renal tubule adenoma in both single section evaluation and step section evaluation (see Table 8 in NTP report chronic nephropathy in male rat) (NTP, 2006).

In addition, sex-specificity for renal pathology can, in part, be understood in terms of gender-specific differences for renal clearance of the major urinary metabolites. The majority of ingested benzophenone metabolizes via reduction to yield the glucuronic acid conjugate of diphenylmethanol (benzhydrol) (Nakagawa et al., 2000). As for other aliphatic and aromatic ketones (e.g., acetophenone) a steady state develops between the ketone and corresponding alcohol *in vivo*. Conjugation of the alcohol form and subsequent renal clearance of the conjugate shifts the steady state, decreasing the *in vivo* concentration of the ketone. The fact that female rats experience higher plasma concentrations of benzophenone than male rats suggests that renal clearance is slower for females (NTP, 2006). Conversely, renal tubular concentrations of the conjugate of diphenylmethanol are expected to be higher in males. The observed gender difference is supported by other biochemical evidence.

Also relevant is the difference between male and female rats in the transport of organic anions such as glucuronic acid conjugates in the proximal tubules. Although substances can pass into the cell from the lumen, they can also accumulate in tubule cells from the interstitial compartment by a variety of transporters. Uptake of substances from the peritubular plasma across the basolateral membranes is mediated by an organic anion transporter (OAT) comprised of approximately 550 amino acids (Tojo et al., 1999; Kojima et al., 2002). Although several isoforms have been identified, OAT1 has been detected exclusively in the S1, S2, and S3 sections of the proximal tubules. Messenger OAT1 RNA expression is significantly higher in male Sprague-Dawley rats compared to female rats (Buist et al., 2002). Therefore, the male rat is expected to experience greater glucuronide loads in the proximal tubules. This may be of little consequence when the organic moiety is relatively polar. However, when the conjugate is bound to a hydrophobic moiety (such as diphenylmethanol or *alpha*-methylbenzyl alcohol), increased renal tubule toxicity is anticipated. The fact that the pattern of renal pathology for the 2-year bioassay for *alpha*-methylbenzyl alcohol mirrors that of benzophenone in male and female rats supports the conclusion that dose-dependent loading in the proximal tubule is a part of the basis for understanding the origin of the difference in renal pathology between the male and female F344/N rat, in addition to the normally present sex differences in urinary protein and aging chronic nephropathy.

The severity scores of the aging nephropathy were 1.3, 2.4, 3.3 and 3.8 for the 0, 312, 625 and 1250 ppm groups of males, respectively. Aging chronic nephropathy (Hard and Khan, 2004; Hard and Seely, 2005; Haseman et al., 2003; Seely et al., 2002) is considerably more severe in males compared to female rats in most strains, including the F344. Although the reason for this difference is not completely understood, the much higher urinary concentration of protein, primarily because of $alpha_{2\upsilon}$ globulin, in male rats is considered a major contributing factor.

Several modes of action for renal carcinogenesis in rats and mice have recently been summarized (Lock and Hard, 2004). One of these modes is a marked increase in the severity of the aging chronic nephropathy associated with an increase of tumors, mostly adenomas. One of the characteristics of aging chronic nephropathy is increased renal tubular degeneration and regenerative hyperplasia. Under normal circumstances, the degree of tubular proliferation is insufficient to generate atypical hyperplastic foci or adenomas. However, if the chronic nephropathy increases in severity, as in the 2-year rat study, there is considerably more tubular degeneration, but more importantly, an increase in tubular proliferation, hyperplastic foci, atypical hyperplastic foci and an increase in the incidence of adenomas. The findings of the 2-year rat study support a conclusion that

increased severity of aging chronic nephropathy is an explanation for the renal tubular effects produced by benzophenone, with a correlating dose–response change in tubular proliferation response, a predominant (or exclusive) effect in males compared to females, the lack of other changes (i.e., tubular necrosis, *alpha*$_{2u}$ globulin, increased apoptosis) that could explain the effect, and the lack of such effects in the mouse. Thus, in the 2-year rat study with benzophenone, the increasing severity of the aging chronic nephropathy can be concluded to be largely responsible for the renal tubular proliferation in the male rat. This mode of action is not relevant to human renal carcinogenesis, and thus, these rat tumors indicate no renal carcinogenic risk in humans.

The dose-related increased incidences of renal pelvis transitional (urothelial) cell hyperplasia seen in this study can also be explained by the increasing severity of aging chronic nephropathy with dose. One of the manifestations of aging chronic nephropathy is marked deposits of calcium salts (especially calcium oxalate) in the medulla, cortico-medullary junction, and at the fornices of the renal pelves. The amount of mineralization increases, both with incidence and severity, with increasing severity of the aging chronic nephropathy. The calcium deposition at the renal fornices extends along the renal pelvis and is associated with transitional cell proliferation (hyperplasia). Under exceptional circumstances, the degree of transitional cell proliferation can lead to a papilloma or carcinoma of the kidney pelvis.

The increasing incidences of transitional cell hyperplasia seen in this study were attributed to the increasing severity of the aging chronic nephropathy by the NTP itself. Supporting this conclusion is the lack of a hyperplastic effect in the urinary bladder. If the chemical itself was directly producing the transitional cell proliferation effect, rather than indirectly by increasing the severity of the aging chronic nephropathy, the effect would be expected to be greater in the bladder than in the kidney pelvis in the absence of urinary tract obstruction (Hanai et al., 2002). In addition, the effect was not seen in female rats and was not present in mice of either sex.

Thus, the kidney pelvis transitional cell hyperplasia seen in rats administered benzophenone is due to increased severity of the aging chronic nephropathy and is not indicative of a risk to humans.

Excessive mortality, late-stage reduction in body weights, the presence of chronic nephropathy in all control and test groups, and the dose-dependent exacerbation of renal tubule hyperplasia by the test agent or more likely, the principal metabolite, may eventually lead to an increase in the incidence of renal tubule adenomas in male rats. Since the carcinogenic and hyperplastic effects are secondary to renal toxicity and specific to the male F344/N rat, the results have no relevance to the safety of benzophenone in humans. In the event this study was to be used in a human health assessment, however, it should be noted that there was no difference in the incidence of tumors in control and the lowest dose group of male rats. This dietary level of 15 mg/kg bw/d is 750,000 times the estimated daily *per capita* intake of benzophenone as a flavoring agent in the US (0.00002 mg/kg bw/d or 0.02 μg/kg bw/d).

4.3.1.2. Mice. Neoplastic and nonneoplastic lesions associated with the dietary administration of 0, 312, 625, or 1250 ppm of benzophenone to mice developed principally in the liver in males (see data summary below). The dietary concentrations were estimated to provide a daily intake of 40, 80, or 160 mg/kg bw for males and 35, 75, or 150 mg/kg bw for females. Although there was a slight decrease in survival in females on the 1250 ppm diet, there was no significant difference in survival between the control group and any of the three treatment groups. Mean body weights of females in the 35, 70, and 150 mg/kg bw per day test groups were less than controls after weeks 86, 52, and 37, respectively (see Table 3).

There was an increase in the incidence of hepatocellular adenomas in males (control, 11/50; 40 mg/kg, 15/50; 80 mg/kg, 23/50; 160 mg/kg, 23/52) that was statistically significant at the two highest dietary levels. The incidence of hepatocellular carcinomas was greater in control males than in any group of test group males (control, 8/50;

Table 3
Data summary of the incidences of hepatocellular neoplasms associated with the dietary administration of benzophenone to mice for two years (NTP, 2006)

	Control	40 mg/kg	80 mg/kg	160 mg/kg
Male mice				
Hepatocellular adenoma	11/50 (22%)	15/50 (30%)	23/50 (46%)	23/50 (46%)
Hepatocellular carcinoma	8/50 (16%)	5/50 (10%)	6/50 (12%)	6/50 (12%)
Hepatoblastoma	0/50 (0%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Combined rates[a]	18/50 (36%)	20/50 (40%)	25/50 (50%)	29/50 (58%)
		35 mg/kg	75 mg/kg	150 mg/kg
Female mice				
Hepatocellular adenoma	5/50 (10%)	4/50 (8%)	10/50 (20%)	8/50 (16%)
Hepatocellular carcinoma	0/50 (0%)	1/50 (2.5%)	0/50 (0%)	1/50 (2.5%)
Combined rates[b]	5/50 (10%)	5/50 (10%)	10/50 (20%)	9/50 (18%)

[a] Historical incidence for 2-year dietary (NTP-2000 diet) control groups (mean ± standard deviation): 145/460(32.4% ± 9.1%); range 20–47%.
[b] Historical incidence: 53/457 (11.8% ± 3.1%); range 8–16%.

40 mg/kg, 5/50; 80 mg/kg, 6/50; 160 mg/kg, 6/52), while the incidence of hepatoblastomas in test groups was <6% and not statistically different from controls. The incidence of combined hepatocellular adenomas, carcinomas, and blastomas in males maintained on the 625 and 1250 ppm diets was increased ($P < 0.01$) compared to the control group (control, 18/51; 40 mg/kg, 20/50; 80 mg/kg, 25/50; 160 mg/kg, 29/52) (see Table 1).

Non-neoplastic effects in the liver of males included an increase in clear cell foci, eosinophilic foci and mixed cell foci and a significant increase in centrilobular hepatocellular hypertrophy, multinucleated cells, and hepatic necrosis in all treatment groups and cystic degeneration in the two highest dietary groups. Chronic active inflammation was observed but the effect was also prevalent in control animals.

Female mice exhibited a low incidence of nonneoplastic and neoplastic lesions compared to male mice. There was no significant difference in the incidence of combined hepatocellular adenomas and carcinomas (control, 5/50; 35 mg/kg, 5/50; 75 mg/kg, 10/50; 150 mg/kg, 9/52). The historical incidence of adenomas and combined adenomas and carcinomas for mice on the NTP-2000 diet were 9.6% and 11.8%, respectively. A much lower incidence of non-neoplastic effects were observed in females compared to males. The incidence of clear cell foci, eosinophilic foci, and mixed cell foci was slightly increased over controls. Although hepatocyte hypertrophy was reported in test groups there was no significant difference in multinucleated cells, necrosis, inflammation, or cystic degeneration between test and control groups. These data support the conclusion that the male mouse liver exhibits increased susceptibility to neoplastic changes compared to the female mouse.

Also, a statistically significant ($P = 0.03$, 5/50) increase of histiocytic sarcoma was reported in female mice maintained on the 625 ppm diet. The incidence was lower (3/50; not statistically significant) in the 1250 ppm group and was absent in the low 312 ppm and control groups. The authors noted that the historical incidence of histiocytic sarcoma in 2-yr feed (NTP-2007 diet) controls was 2/459 ($0.3\% \pm 0.8\%$, range 0–2%) and was 18/1258 ($1.5\% \pm 2.2\%$, range 0–8%) for all routes of administration. Thus, the incidences were nearly within the range of historical controls, and were not dosage dependent in this study (NTP, 2006).

Under the conditions of these 2-year gavage studies, the NTP report concluded: "*There was some evidence of carcinogenic activity of benzophenone in male B6C3F$_1$ mice based on increased incidences of hepatocellular neoplasms, primarily adenoma. There was some evidence of carcinogenic activity in female B6C3F$_1$ mice based on increased incidences of histiocytic sarcoma.*"

The primary neoplastic effects observed in the treated mice in the 2-year NTP gavage study were associated with the liver. The high incidence of hepatocellular adenomas and carcinomas in both control and treated groups of male and female mice is indicative of the sensitivity of the B6C3F$_1$ mouse liver to neoplastic changes. The high incidences of hepatocellular adenoma in treated and control male mice were not significantly different. The incidences of nonneoplastic lesions and adenomas in the male control group were higher than any group of treated females. Although the incidence of hepatocellular adenomas and combined hepatocellular adenomas and carcinomas in all groups of treated male mice was greater than in the control group, there was no difference in the incidence of malignant neoplasms (hepatocellular carcinomas and hepatoblastomas). The incidence of hepatocellular neoplasms (adenomas and combined adenomas and carcinomas) in treated females was not significantly different from the control group, and the overall incidence of neoplastic and nonneoplastic lesions was significantly less than in males.

The profile of neoplastic responses is consistent with the historically high levels of background hepatocellular neoplasms in male and female B6C3F$_1$ mice (Maronpot et al., 1987). The historical spontaneous incidence of liver neoplasms in control male and female B6C3F$_1$ mice has revealed background incidences of combined hepatocellular adenoma and carcinomas of 32.4% for males with a range of 20–47% and 11.8 % for females with a range of 8–16% (NTP, 2006). Similar incidences were reported in the benzophenone study. Based on the above discussion, observations of hepatic neoplasms in the NTP mouse bioassay are not relevant to the safety of benzophenone in humans at low levels of intake 0.2 mg/kg bw per day from use as a flavor ingredient. This conclusion is based on: the high incidence of spontaneous hepatocellular neoplasms (adenomas and carcinomas) in the male B6C3F$_1$ mouse, the absence of consistent dose–response data, the benign nature of the lesion (adenomas), the lack of hepatocellular neoplastic effects in the parallel rat study (discussed above), and the relatively high dose levels administered compared to the intake of 0.2 mg benzophenone/kg bw per day from intended use as a flavoring ingredient.

The comparison of the incidence of histiocytic sarcomas in female mice {5/50 (10%) at 625 ppm and 3/50 (6%) at 1250 ppm} to the low historical incidence {2/459 ($0.3\% \pm 0.8\%$, range 0–2%)} of these neoplasms in recent NTP-2000 feed studies was the basis for the conclusion of "*some evidence of carcinogenic activity in female B6C3F$_1$ mice*" (NTP, 2006). Although the NTP report did not consider the appearance of histiocytic sarcoma to be sufficient evidence of compound-related carcinogenesis, low non-statistically significant increases (i.e., 1/50 at 30 mg/kg bw and 2/50 at 65 mg/kg bw) in histiocytic sarcoma were observed in female rats at the two highest dietary levels.

The female mouse historical database upon which the above comparison was based is relatively small (7 dietary studies containing a total of 359 [not 459, as reported in the original study] female mice; incidence of 2/359 or 0.6%). When the database is expanded to include control animals maintained on the NTP-2000 diet and administered the test article by all routes of administration, the

incidence of histiocytic sarcomas increases (1.5% ± 2.2%, range 0–8%) and incidence of tumors in the benzophenone study (10% and 6%) are nearly within the historical range.

In order to evaluate the relevance of these tumors to the potential cancer risk for humans consuming benzophenone as a flavoring agent, it is important to note that histiocytic sarcomas were only found at dose levels inducing severe toxicity. Increased incidences of hepatic and renal lesions were recorded in both species, and increased incidences of splenic extramedullary hematopoiesis was reported in female mice. For example, the majority of female mice (4 of 5) that exhibited histiocytic sarcomas also showed hepatocellular adenomas. In the female rat mid- and high-dose groups, there were marked decreases in the incidences of thyroid C-cell hyperplasia and mammary gland fibroadenomas which may be indicative of high doses of benzophenone interfering with hormones such as calcitonin and estradiol (Boorman et al., 1996).

Clearly, low incidences of histiocytic sarcomas occurred in only one sex, and only then at dose levels inducing severe toxicity and possibly affecting hormonal balance, rendering it highly likely that the occurrence of these tumors is a high-dose phenomenon that becomes manifest in severely affected female rats and mice. The estimated daily *per capita* intake of benzophenone as a flavoring agent in the US (0.00002 mg/kg bw/d) is more than 750,000 times lower than the lowest dietary level examined (15 mg/kg bw/d) at which no histiocytic sarcomas were found. Therefore, the occurrence of these neoplasms in the present study is considered a high-dose phenomenon without any relevance for assessing the potential cancer risk of the use of benzophenone as a food flavor.

4.3.2. α-Methylbenzyl alcohol (No. 1)

4.3.2.1. Rats. Groups of F344/N rats (50 M & 50 F) were administered 0, 375, or 750 mg *alpha*-methylbenzyl alcohol/kg bw/d by corn oil gavage 5 d per week for a period of 103 weeks. Evaluations and necropsies were performed according to the same protocol used in the corresponding 103-week mouse study (NTP, 1990).

Deaths were reported in the following groups prior to the end of the study: high-dose males (49/50) and females (39/50), low-dose males (42/50) and females (24/50), control males (15/50) and females (16/50). It should be noted that 8 high-dose males, 14 high-dose females, 9 low-dose males, 4 low-dose females, one control male, and one control female were killed accidentally. Most deaths occurred within the second year of the study. The NTP suggested that delayed mortality was likely attributable to cumulative and progressive renal toxicity which may have compromised excretion and was caused by high dose levels and an increase in circulating *alpha*-methylbenzyl alcohol. The NTP also stated that an increase in agent concentration in the blood could result in CNS depression and loss of vital functions.

Mean body weights of surviving high-dose male rats were 12–32% lower than controls at the end of the study. Mean body weights of surviving low-dose male rats were 10–20% less than controls. Surviving high-dose females weighed 12–19% less than controls at the end of the study. Surviving low-dose females had final body weights comparable to controls. Exacerbation of age-related renal nephropathy was reported in more than half of the dosed and control females, and nearly all dosed and control males. However, this age-related renal disease was judged to be more severe in dosed male rats than in vehicle controls. Renal tubular cell adenomas occurred with significantly greater frequency in high-dose males (10%) than in control rats (0%). Significantly increased incidence of parathyroid hyperplasia, heart calcification, glandular stomach calcification, and fibrous bone osteodystrophy occurred in both male dose groups. These effects were likely a secondary response to a mineral imbalance caused by impairment of renal function (secondary hyperthyroidism). Centrilobular necrosis of the liver was observed at significantly increased incidences in high and low-dose males when compared to controls. Inflammation of the forestomach was also observed at increased frequency in dosed males. Significantly increased incidence of lung congestion was prevalent among low- and high-dose females. Pulmonary hemorrhage and foreign matter was significantly more prevalent in high-dose males and females than in control rats. Inflammation in the nasal cavity and salivary gland were observed at significantly increased incidences in dosed males. These symptoms are commonly associated with gavage accidents.

The NTP concluded that: "*Under the conditions of these 2-year gavage studies, there was some evidence of carcinogenic activity of α-methylbenzyl alcohol for male F344/N rats, as shown by increased incidences of renal tubular cell adenomas and adenomas or adenocarcinomas* (*combined*), *and no evidence for carcinogenic activity for female F344/N rats administered 375 or 750 mg/kg.*"

The NTP remarked that the "renal toxicity was characterized by severe nephropathy and related secondary lesions in the dosed rats, and excessive mortality occurred during the last quarter of the studies. Poor survival reduced the sensitivity of the studies for detecting the presence of a carcinogenic response in both chemically exposed groups of male rats and the high dose group of female rats" (NTP, 1990).

The interpretation of the renal pathology observed specifically in male rats given *alpha*-methylbenzyl alcohol is, in large part, presented in the discussion of the NTP study on benzophenone. In addition, excessive reduction in body weights and prevalence of gavage-related deaths are significant inadequacies in the 2-year NTP study of *alpha*-methylbenzyl alcohol in rats. Administration of the substance in the diet rather than via the gavage route, and the employment of doses equivalent to some realistic multiple of expected human consumption would have increased survival and allowed for more interpretable results. The low dose level of 375 mg/kg bw/d is 350,000 times the estimated daily *per capita* intake of *alpha*-methylbenzyl

alcohol as a flavoring ingredient (1 μg/kg bw/d). The excessive doses used in the NTP study probably accounted for the high mortality and development of severe nephropathy and secondary lesions in the kidney (Smith and Ford, 1993).

4.3.2.2. Mice. Groups of 50 male and 50 female B6C3F$_1$ mice were administered 0, 375, or 750 mg *alpha*-methylbenzyl alcohol/kg bw/d by corn oil gavage 5 d per week for a period of 103 weeks (NTP, 1990). All animals were observed twice daily and clinical signs were recorded monthly. Body weights were measured weekly for the first 12 weeks, and monthly thereafter. Necropsies were performed on all animals, including those that died or were killed moribund during the study. All organs and tissues were evaluated for the presence of gross lesions, which were histopathologically examined. Histological examinations were performed on prepared tissues from all control and high-dose animals, as well as low-dose animals dying during the first 21 months of the study. For all dosed mice, organs targeted for potential neoplastic and nonneoplastic effects were histopathologically examined.

Survival of treated mice was comparable to controls. Mean body weights of high-dose females were 8–16% lower than controls from week 72 to study termination. Significant lung congestion was observed in high-dose males (control, 0/50; low-dose, 0/50, high-dose, 7/50) and females (0/50, 0/50, 7/50). Pulmonary hemorrhage and foreign matter was observed in 6/50 high-dose males and 1/49 control males. These effects are commonly associated with the gavage technique. No other negative effects were observed. The NTP concluded "*There was no evidence of carcinogenic activity of α-methylbenzyl alcohol for male and female B6C3F$_1$ mice administered 375 or 750 mg/kg for 2 years*" (NTP, 1990).

4.3.3. Benzoin (No. 37)

4.3.3.1. Rats. Groups of 50 male or 50 female F344 rats were administered 0, 125, or 250 ppm benzoin or 0, 250, or 500 ppm benzoin, respectively, in the diet for a period of 104 weeks. Dietary concentrations were calculated (FDA, 1993) to provide corresponding average daily intake levels of 0, 12.5, and 25 mg/kg bw for males and 0, 25, and 50 mg/kg bw for females. Experimental parameters were evaluated according to the same protocol employed in the corresponding mouse study (NTP, 1980).

Survival was significantly reduced in low-intake level males (50%) with respect to controls (72%). Survival in other dosed groups was comparable to controls. No agent-related clinical signs were observed. Mean body weights of dosed rats were normal throughout the study. A dose-related increase in lymphomas and leukemias in male rats was not significant ($p > 0.05$). A dose-related increase in the incidence of hyperplasia of the adrenal medulla was observed in male rats (control, 8%; low-intake level, 16%; high-intake level, 38%). These foci were described by the authors as "very small collections of medullary cells with basophilic cytoplasm and nuclei smaller than those of normal pheochromocytes." A dose-dependent increase in frequency of chronic nephritis was observed in both sexes (males: 33/49, 41/49, 45/50; females: 7/50, 19/49, 29/50). The effect was not significant in male rats, given the high incidence of chronic nephritis in the control group. The chronic inflammation observed in the kidney was qualitatively similar to that commonly observed in aging rats. Given that the degenerative, proliferative, and inflammatory lesions occurred with comparable frequency in control and treated rats, NTP concluded that, "*under the conditions of this bioassay, benzoin was not carcinogenic for male or female F344 rats*" (NTP, 1980).

4.3.3.2. Mice. Groups of 50 male and 50 female B6C3F$_1$ mice were administered 0, 2500, or 5000 ppm benzoin in the diet for a period of 104 weeks (NTP, 1980). Dietary concentrations were calculated (FDA, 1993) to provide corresponding average daily intake levels of 0, 375, and 750 mg/kg bw, respectively. Animals were observed twice daily while clinical signs and presence of palpable masses were recorded weekly. Body weights were measured every 2 weeks for the first 12 weeks and monthly thereafter. Necropsies were conducted on all test and control animals at study termination. Gross and microscopic examinations were performed on the major organs and tissues of all animals, including those that died during the study. Gross lesions were also microscopically examined. It should be noted that some evaluations were limited by autolysis and cannibalization.

Survival of dosed mice was comparable to controls. No compound-related clinical signs were observed. After week 44, body weights of low- and high-dose females were reduced by about 10% with respect to controls. Survival rates for male or female treatment groups did not differ significantly from control animals. Neoplastic and nonneoplastic lesions were observed in treated mice at frequencies comparable to those historically observed in aged B6C3F$_1$ mice. The NTP concluded "*There was no evidence of carcinogenic activity of benzoin for male and female B6C3F$_1$ mice administered 375 or 750 mg/kg for 2 years*" (NTP, 1980). The dose level of 750 mg benzoin/kg bw/d that produced no adverse effects in male mice is >1,000,000 times the estimated daily per capita intake ("eaters only")[1] of 0.3 μg benzoin/kg bw/d from use as a flavoring substance.

4.4. Genotoxicity studies

Predominantly negative results are reported in *in vitro* genotoxicity assays for aromatic ketones, secondary alcohols, and related esters. The most studied substance, benzoin, is also the one exhibiting the most positive results in *in vitro* assays. Benzoin exhibits mild antibacterial activity and is well known as a topical antiseptic. Chemically, benzoin is an *alpha*-hydroxyketone that may enolize and, to some extent, complex with metal ions. The results reported for benzoin are inconsistent in that, a positive

Table 4
In vitro genotoxicity studies on benzyl derivatives

#	Flavoring ingredient	Test system	Test object	Maximum concentration of substance	Result	References
1	*alpha*-Methylbenzyl alcohol	Ames	*S. typh.* TA98, TA100, TA1535, TA1537	Up to 6666 μg/plate[a,b]	Negative	NTP (1990)
1	*alpha* -Methylbenzyl alcohol	Ames	*S. typh.* TA98, TA 100, TA1535, A1537	33–6666 μg/plate[a,b]	Negative	Zeiger et al. (1987)
1	*alpha* -Methylbenzyl alcohol	SCE	CHO cells	33–1000 μg/ml[a,b]	Negative	NTP (1990)
1	*alpha* -Methylbenzyl alcohol	Chr. Abs	CHO cells	1000–2500 μg/ml[a] 1000–4000 μg/ml[b]	Negative Positive	NTP (1990)
8	Acetophenone	Ames	*S. typh.* TA98, TA100, TA1535, TA1537	360 μg/plate[a,b]	Negative	Florin et al. (1980)
8	Acetophenone	Ames	*S. typh.* TA97, TA102	10–1000 μg/plate[a,b]	Negative	Fujita and Sasaki (1987)
8	Acetophenone	Ames	*S. typh.* TA98, TA100, TA2637	50–1000 μg/plate[a,b]	Negative	Nohmi et al. (1985)
8	Acetophenone	Chr. Abs.	CHO cells	800–1200 μg/ml[a] 600–1000 μg/ml[b]	Negative Positive	Sofuni et al. (1985)
12	Acetanisole	Mutation	*E. coli* WP2, WP2*uvr*A-	1–1000 μg/ml[a,b]	Negative	McMahon et al. (1979)
12	Acetanisole	Mutation	*S. typh.* G45, TA1535, TA100, C3076, TA1537, D3052, TA1538, TA98	1–1000 μg/ml[a,b]	Negative	McMahon et al. (1979)
19	4-(*p*-Methoxyphenyl)-2-butanone	Ames	*S. typh.* TA98, TA100, TA1535, TA1537, TA1538	Up to 3600 μg/plate[1,2]	Negative	Wild et al. (1983)
20	4-Phenyl-3-buten-2-ol	Ames	*S. typh.* TA98, TA100, TA1535, TA1537, TA1538	Up to 3600 μg/plate[1,2]	Negative	Wild et al. (1983)
21	4-Phenyl-3-buten-2-one	Ames	*S. typh.* TA98, TA100, TA1535, TA1537	10–3000 μg/plate[b] (TA100 only)	Positive	Prival et al. (1982)
21	4-Phenyl-3-buten-2-one	Ames	*S. typh.* TA98, TA100, TA1535, TA1537	10–3000 μg/plate[a] (TA100) 10–3000 μg/plate[a,b] (TA98, TA1535, TA1537)	Negative	Prival et al. (1982)
25	Propiophenone	Mutation	*S. typh.* TA98, TA100, TA1535, TA1537, TA1538, G46, C3076, D3052	0.1–1000 μg/ml[a,b]	Negative	McMahon et al. (1979)
25	Propiophenone	Mutation	*E. coli* WP2, WP2*uvr*A-	0.1–1000 μg/ml[a,b]	Negative	McMahon et al. (1979)
26	*alpha* -Propylphenethyl alcohol	Ames	*S. typh.* TA98, TA100, TA1535, TA1537, TA1538	Up to 3600 μg/plate[a,b]	Negative	Wild et al. (1983)
30	Ethyl benzoylacetate	Ames	*S. typh.* TA98, TA100, TA1535, TA1537, TA1538	Up to 3600 μg/plate[a,b]	Negative	Wild et al. (1983)
32	1-(*p*-Methoxyphenyl)-1-penten-3-one	Ames	*S. typh.* TA98, TA100, TA1535, TA1537, TA1538	Up to 3600 μg/plate[a,b]	Negative	Wild et al. (1983)
34	1-Phenyl-1,2-propanedione	Ames	*S. typh.* TA100	Up to 148 μg/plate[a]	Negative	Dorado et al. (1992)
35	Benzophenone	Ames	*S. typh.* TA97, TA98, TA100, TA1535, TA1537	3–1000 μg/plate[a,b]	Negative	Mortelmans et al. (1986)
37	Benzoin	Ames	*S. typh.* TA97, TA98, TA100, TA102	32–1000 μg/plate[a,b]	Negative	Baker and Bonin (1985)
37	Benzoin	Ames	*S. typh.* TA97, TA98, TA100, TA102	20–5000 μg/plate[a,b]	Negative	Matsushima et al. (1985)
37	Benzoin	Ames	*S. typh.* TA98, TA100, TA1535, TA1537, TA1538	50–5000 μg/plate[a,b]	Negative	Rexroat and Probst (1985)
37	Benzoin	Ames	*S. typh.* TA97, TA98, TA100, TA1535	33–1000 μg/plate[a] 33–1000 μg/plate[b]	Positive Negative	Zeiger and Haworth (1985)
37	Benzoin	MLA	Mouse	300–900 μg/ml[a,b]	Negative	Lee and Webber (1985)
37	Benzoin	MLA	Mouse	15.6–250 μg/ml[a] 5–20 μg/ml[b]	Negative Positive	Myhr et al. (1985)
37	Benzoin	MLA	Mouse	1–1000 μg/ml[a] 0.1–10 μg/ml[b]	Negative Positive	Oberly et al. (1985)
37	Benzoin	MLA	Mouse	125–1000 μg/ml[a,b]	Positive	Styles et al. (1985)

37	Benzoin	SCE	CHO cells	5–2000 μg/ml[a,b]	Negative	Gulati et al. (1989)
37	Benzoin	SCE	CHO cells	125–500 μg/ml[a,b]	Negative	Lane et al. (1985)
				1000 μg/ml[a]	Negative	
				1000 μg/ml[b]	Positive	
37	Benzoin	SCE	Human lymphocytes	1–100 μg/ml[a,b]	Negative	Obe et al. (1985)
37	Benzoin	Chr. Abs.	CHO cells	50–2000 μg/ml[a,b]	Negative	Gulati et al. (1989)
37	Benzoin	Chr. Abs.	Hamster liver fibroblasts	20–200 μg/ml[a]	Negative	Danford (1985)
37	Benzoin	Chr. Abs.	Hamster lung fibroblasts	10 μg/ml[a,b]	Negative	Ishidate and Sofuni (1985)
				20–40 μg/ml[a]	Positive	
37	Benzoin	Chr. Abs.	Hamster liver fibroblasts	20–200 μg/ml[a]	Negative	Parry (1985)
37	Benzoin	UDS	Rat hepatocytes	212 μg/ml	Positive	Glauert et al. (1985)
37	Benzoin	UDS	Rat hepatocytes	0.106–212 μg/ml	Negative	Probst and Hill (1985)
37	Benzoin	Micronucleus	CHO cells	0.2–212 μg/ml[a,b]	Negative	Douglas et al. (1985)
37	Benzoin	Micronucleus	Hamster lung cells	5–81 μg/ml[a]	Negative	Li et al. (1993)
				5–20 μg/ml[b]	Negative	
				40–81 μg/ml[b]	Positive	
38	Methyl *beta*-naphthyl ketone	Ames	*S. typh.* TA98, TA100, TA1535, TA1537, TA1538	Up to 3600 μg/plate[a,b]	Negative	Wild et al. (1983)

*Not reported which method was used i.e. plate incorporation method or preincubation method.
[a] Without metabolic activation.
[b] With metabolic activation.

report of genotoxicity in one assay (Zeiger and Haworth, 1985) is negative in the same assay under the same conditions in other reports (Baker and Bonin, 1985; Matsushima et al., 1985; Rexroat and Probst, 1985). The absence of any consistent evidence of *in vivo* genotoxicity or carcinogenicity of benzoin in rats and mice supports the conclusion that benzoin exhibits a low potential for genotoxicity. Given the extensive data available for benzoin, it will be discussed separately.

4.4.1. In vitro

In vitro mutagenicity/genotoxicity testing has been performed with 14 aromatic substituted secondary alcohols, ketones, and related esters used as flavor ingredients (see Table 4). Negative results were reported in the standard Ames assay when various strains of *Salmonella typhimurium* (TA 97, TA 98, TA 100, TA 102, TA 1535, TA 1537, TA 1538, and TA 2637) were incubated with 33–6666 μg *alpha*-methylbenzyl alcohol/plate (NTP, 1990; Zeiger et al., 1987), 10–1000 μg acetophenone/plate (Florin et al., 1980; Fujita and Sasaki, 1987; Nohmi et al., 1985), 3600 μg methyl *beta*-napthyl ketone/plate (Wild et al., 1983), 3600 μg 4-(*p*-methoxyphenyl)-2-butanone/plate (Wild et al., 1983), 3600 μg 4-phenyl-3-buten-2-ol/plate (Wild et al., 1983), 3600 μg *alpha*-propyl phenethyl-alcohol/plate (Wild et al., 1983), 3600 μg 1(*p*-methoxyphenyl)-1-penten-3-one (Wild et al., 1983), 3–1000 μg benzophenone/plate (Mortelmans et al., 1986), up to 148 μg 1-phenyl-1,2-propanedione/plate (Dorado et al., 1992), and 3600 μg ethyl benzoylacetate/plate (Wild et al., 1983) (see Table 4). Although 4-phenyl-3-buten-2-one was negative in three strains of *S. typhimurium* (TA 98, TA 1535, and TA 1537) (Prival et al., 1982), concentrations of 10–3000 μg/plate gave positive results in TA100 with S-9 metabolic activation, but negative results without it. Mutagenicity assays performed using a modified Ames technique gave negative results in *S. typhimurium* and *E. coli* at concentrations of 1–1000 μg acetanisole/ml and 0.1–1000 μg propiophenone/ml with and without metabolic activation (McMahon et al., 1979).

Genotoxicity was not evident when *in vitro* sister chromatid exchange (SCE) assay was performed in Chinese hamster ovary (CHO) cells at concentrations of 33–1000 μg *alpha*-methylbenzyl alcohol/ml (NTP, 1990). Chromosomal aberrations were absent when CHO cells were incubated with 1000–2500 μg *alpha*-methylbenzyl alcohol/ml (NTP, 1990) or 800–1200 μg acetophenone/ml (Sofuni et al., 1985) without metabolic activation. Positive results were reported in the same studies when CHO cells were incubated with 1000–4000 μg *alpha*-methylbenzyl alcohol/ml (NTP, 1990) or 600–1000 μg acetophenone/ml (Sofuni et al., 1985) with metabolic activation.

4.4.1.1. Benzoin. In four Ames assays using the same strains of *Salmonella typhimurium* at similar concentrations (Baker and Bonin, 1985; Matsushima et al., 1985; Rexroat and Probst, 1985; Zeiger and Haworth, 1985), only one

reported an increase in the frequency of reverse mutations (Zeiger and Haworth, 1985). In a preincubation modification of the Ames assay, an increase in the number of reverse mutants was reported when *S. typhimurium* was incubated with 33–1000 μg benzoin/plate without metabolic activation. Addition of S9 abolished all mutagenic effects (Zeiger and Haworth, 1985). These results are inconsistent with the negative results reported in the 3 other Ames tests with doses of 20–5000 μg benzoin/plate with and without metabolic activation (Baker and Bonin, 1985; Matsushima et al., 1985; Rexroat and Probst, 1985).

Results in the *in vitro* mouse lymphoma forward mutation assay (MLA) with benzoin were negative at concentrations of 1–1000 μg/ml without metabolic activation (Lee and Webber, 1985; Myhr et al., 1985; Oberly et al., 1985) and 300–900 μg/ml with metabolic activation (Lee and Webber, 1985). Positive results in mouse lymphoma assays performed with S9 included one at concentrations of 0.1–10 μg/ml (Oberly et al., 1985), and another at 5–200 μg benzoin/ml (Myhr et al., 1985). Positive results were also reported in one study at 125–1000 μg benzoin/ml with and without S9 (Styles et al., 1985). When interpreting the results of *in vitro* mammalian cell assays performed before 1986, and particularly the MLA assay, it is important to remember that the investigators usually did not properly control pH and ionic strength of the test media. Mammalian cells *in situ* rely on complex regulatory mechanisms to maintain homeostatic conditions and those in culture are not equipped to respond to environmental changes; therefore, it is important that culture media used to support *in vitro* mammalian cell assays be maintained at a pH of approximately 6.8–7.5. Reduced pH of test media or changes in culture osmolality due to air-oxidizable test agents (*e.g.* benzoin) may cause false positive results in mammalian cell assays, especially those that employ S9 metabolic activation systems. Studies have shown that increased acidity facilitates the breakdown of S9 components into mutagenic agents (Brusick, 1986).

Genotoxicity was not evident when *in vitro* sister chromatid exchange (SCE) assay was performed in Chinese hamster ovary (CHO) cells at concentrations of 5–2000 μg benzoin/ml (Gulati et al., 1989; Lane et al., 1985) with and without S9 activation. In one study, an increase in sister chromatids was reported at a concentration of 1000 μg benzoin/ml with the addition of S9 (Lane et al., 1985). These results are inconsistent with those reported by Gulati et al. (1989). No evidence for SCE was found in human lymphocytes at concentrations of 1–100 μg benzoin/ml (Obe et al., 1985).

In the chromosomal aberration assay, negative results were reported in CHO cells incubated with 50–2000 μg/ml with or without metabolic activation (Gulati et al., 1989). Negative results were also reported when hamster liver fibroblasts were incubated with 200 μg/ml without metabolic activation (Danford, 1985; Parry, 1985). Incubation of hamster lung fibroblasts with 10 μg/ml with and without S9 activation also failed to produce chromosomal aberrations (Ishidate and Sofuni, 1985). Significant increases in chromosomal aberrations were reported in hamster lung fibroblasts at 20–40 μg/ml with and without metabolic activation (Ishidate and Sofuni, 1985).

An assay to detect unscheduled DNA synthesis (UDS) in rat hepatocytes revealed negative results at concentrations of 0.106–212 μg/ml (Probst and Hill, 1985). A different UDS study reported positive results in rat hepatocytes at 212 μg/ml (Glauert et al., 1985). A micronucleus assay performed on CHO cells at concentrations up to 212 μg/ml gave negative results with and without metabolic activation (Douglas et al., 1985). In a micronucleus assay using hamster lung cells, negative results were found at concentrations of 5–81 μg/ml without metabolic activation and 5–20 μg/ml with metabolic activation. Positive results were reported at 40–81 μg/ml with metabolic activation (Li et al., 1993).

4.4.1.2. Summary of in vitro genotoxicity. Generally aromatic secondary alcohols and ketones showed no genotoxic potential in *in vitro* test systems with the exception of benzoin, which showed equivocal genotoxic potential. It can be concluded that equivocal results obtained in some assays (MLA) are most probably due to the experimental conditions in these *in vitro* test systems. As is shown below, benzoin shows no significant evidence of genotoxicity *in vivo* and no evidence of carcinogenicity in long-term studies in mice and rats (NTP, 1980).

4.4.2. In vivo

Results of *in vivo* genotoxicity assays were predominantly negative (see Table 5). There was no increase in micronucleated polychromatic erythrocytes (PCEs) in mice following i.p. injections of 876 mg methyl beta-naphthyl ketone/kg bw (Wild et al., 1983), 1426 mg 4-(*p*-methoxyphenyl)-2-butanone/kg bw (Wild et al., 1983), or 200–500 mg benzophenone/kg bw (NTP, 2006). Benzophenone in the feed of B6C3F1 mice at 1250 to 20,000 ppm for 14 weeks showed no increase in the frequency of micronucleated normochromatic erythrocytes in the peripheral blood of males or females (NTP, 2006).

4.4.2.1. Benzoin. There was no increase in micronucleated polychromatic erythrocytes (PCEs) in mice following i.p. injections of 1750 mg benzoin/kg bw (Shelby et al., 1993). Results were negative when the same assay was performed following a single oral dose of 5000 mg/kg bw (Sheldon, 1989). The only reported positive *in vivo* increase in micronucleated polychromatic erythrocytes was in a micronucleus assay in which i.p. injections of 250–2000 mg benzoin/kg bw (Ishidate and Odagiri, 1989) were used. These results are inconsistent with those reported at similar concentrations in two other studies (Shelby et al., 1993; Wild et al., 1983).

The ability of benzoin to induce sister chromatid exchange (SCE) was also studied *in vivo* (McFee and Lowe, 1989). Results were negative in mouse bone marrow cells

Table 5
In vivo genotoxicity studies on benzyl derivatives

#	Substance name	Test system	Test object	Maximum concentration of substance	Result	Reference
19	4-(*p*-Methoxyphenyl)-2-butanone	Micronucleus	Mouse bone marrow cells	Up to 1426 mg/kg bw	Negative	Wild et al. (1983)
35	Benzophenone	Micronucleus	Mouse bone marrow cells	100–500 mg/kg bw	Negative	NTP (2004)
35	Benzophenone	Micronucleus	Mouse peripheral blood cells	40–160 mg/kg bw (M); 35–150 mg/kg bw (F) (1250–20,000 ppm)	Negative	NTP (2004)
37	Benzoin	Micronucleus	Mouse bone marrow cells	250–2000 mg/kg bw	Positive	Ishidate and Odagiri (1989)
37	Benzoin	Micronucleus	Mouse bone marrow cells	438–1750 mg/kg bw	Negative	Shelby et al. (1993)
37	Benzoin	Micronucleus	Mouse bone marrow cells	Up to 5000 mg/kg bw	Negative	Sheldon (1989)
37	Benzoin	UDS DNA Strand Breaks	Rat hepatocytes	750 mg/kg bw	Negative	Bermudez et al. (1989)
37	Benzoin	UDS	SHE cells	750 mg/kg bw	Negative	Working (1989)
37	Benzoin	SCE	Mouse bone marrow cells	Up to 3000 mg/kg bw	Negative	McFee and Lowe (1989)
37	Benzoin	Chr. Abs.	Mouse bone marrow cells	Up to 1500 mg/kg bw	Negative	McFee and Lowe (1989)
38	Methyl *beta*-napthyl ketone	Micronucleus	Mouse bone marrow cells	Up to 876 mg/kg bw	Negative	Wild et al. (1983)

following i.p. injections of 3000 mg/kg bw. The same authors reported negative results in a chromosomal aberration assay where mice were given i.p. injections of 1500 mg benzoin/kg bw.

A test for unscheduled DNA synthesis (UDS) revealed negative results in SHE cells following administration of 750 mg/kg bw to rats by gavage (Working, 1989). In another assay, 750 mg/kg bw was administered to male rats by corn oil gavage, while the control group received corn oil alone. Hepatocytes were isolated 12, 24, or 48 h after dosing. Benzoin administration did not induce UDS in rat hepatocytes. However, an 8–9-fold increase over controls in the fraction of cells in the S-phase was increased 24 h after treatment (Bermudez et al., 1989).

There is no consistent evidence of genotoxicity for benzoin in the series of *in vivo* assays. The lack of *in vivo* evidence of genotoxicity for this substance is consistent with the lack of any carcinogenic potential in the 2-year bioassay in mice and rats.

5. Other relevant studies

In a developmental toxicology study, groups of 10 female Sprague-Dawley rats were administered 0, 75, 225 or 750 mg/kg bw per day of acetophenone via gavage for a minimum of 14 d through day three of lactation. There were no parental deaths. Mating and fertility indices and mean gestation length were unaffected by acetophenone administration up to 750 mg/kg bw per day. The live birth index, pup survival during lactation and pup body weights were decreased for the 750 mg/kg bw per day group offspring. The authors of this abstract concluded that the NOAEL for reproductive effects was 225 mg/kg bw per day (Kapp et al., 2003).

In a developmental toxicity study, 25 female CD rats were administered 0, 100, 200 or 300 mg/kg bw per day of benzophenone via gavage on gestational days 6 through 19 (NTP, 2002). Dams were monitored for clinical signs, food and water intake and body weight. At necropsy on gestational day 20 maternal clinical condition; body, liver, paired kidney and gravid uterine weights; pregnancy status and number of corpus lutea were recorded. In the gravid uterus the numbers of resorbed, dead or live fetuses were recorded. All live fetuses were weighed, sex recorded and examined for external morphological anomalies. Approximately half of the fetuses were examined for visceral anomalies including internal head structures, and the remainder of the fetuses were examined for skeletal irregularities. Confirmed pregnancy rates were 88–100% per group. No treatment related maternal deaths occurred. Clinical signs observed were lethargy, piloerection, weight loss and rooting in the bedding after dosing. Maternal body weight gain was reduced for all doses from gestational days 6–9 and at the high dose from days 9–12. Increased body weight gain was observed for the mid-dose group on gestational days 9–12 and 19–20 and for the high dose group on gestational days 18–20. Gestational weight gain corrected for gravid uterine weight was reduced at all doses. Maternal liver and kidney weights were significantly increased at all doses. A significant decrease in feed intake was noted for all high-dose groups throughout the study. Relative water intake throughout the study was comparable between test animals and controls. Average fetal weight per litter decreased relative to increasing dose of benzophenone. There was no

effect on prenatal viability or overall incidences of fetal malformations. The incidence of unossified sternebrae was increased at all doses and the incidence of extra rib on Lumbar I was increased at the mid- and high-doses. Overall, the authors concluded that maternal toxicity and the LOAEL were 100 mg/kg bw per day but that a NOAEL or LOAEL for developmental toxicity were not achieved by this study (NTP, 2002).

The choleretic activity of 1-phenyl-1-propanol has been investigated as a possible treatment for decreased hepatic function. In male Wistar rats (8), single 25 or 50 mg/kg bw doses of 1-phenyl-1-propanol administered by gavage increased choleretic activity (*i.e.*, biliary flow and bilirubin excretion) by 50% and 100%, respectively, after 1.5 h. Normal activity returned within 6 h (Riva et al., 1966).

Approximately 30 min after intraduodenal administration of a 100 mg dose of 1-phenyl-1-propanol, healthy human subjects (50) exhibited a 78% increase in bile excretions and an 89% increase in bilirubin excretion compared to pre-treatment values (Brugel et al., 1956).

6. Recognition of GRASr status

The group of aromatic substituted secondary alcohols, ketones and related esters 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 secondary alcohols, ketones and related esters was reaffirmed as GRAS (GRASr) based, in part, on 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.

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