



## Review

# The FEMA GRAS assessment of phenethyl alcohol, aldehyde, acid, and related acetals and esters used as flavor ingredients

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## Abstract

This publication is the ninth in a series of safety evaluations performed by the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA). In 1993, the Panel initiated a comprehensive program to re-evaluate the safety of more than 1700 GRAS flavoring substances under conditions of intended use. Elements that are fundamental to the safety evaluation of flavor ingredients include exposure, structural analogy, metabolism, pharmacokinetics and toxicology. Flavor ingredients are evaluated individually and in the context of the available scientific information on the group of structurally related substances. Scientific data relevant to the safety evaluation of the use of phenethyl alcohol, aldehyde, acid, and related acetals and esters as flavoring ingredients is evaluated. The group of phenethylalcohol, aldehyde, acid, and related acetals and esters was reaffirmed as GRAS (GRASr) based, in part, on their self-limiting properties as flavoring substances in food, their rapid absorption, metabolic detoxication, and excretion in humans and other animals, their low level of flavor use, the wide margins of safety between the conservative estimates of intake and the no-observed-adverse effect levels determined from subchronic and chronic studies and the lack of significant genotoxic and mutagenic potential. This evidence of safety is supported by the fact that the intake of phenethyl alcohol, aldehyde, acid,

*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<sub>50</sub>, medianlethal dose; 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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and related acetals and esters as natural components of traditional foods is greater than their intake as intentionally added flavoring substances.

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## 1. Chemical identity

This review presents the key data relevant to the safety evaluation of 39 phenethyl alcohol derivatives and 4 phenoxyethyl alcohol derivatives. The group of flavoring ingredients includes the following: phenethyl alcohol (No. 1), 28 esters formed from phenethyl alcohol or phenylacetic acid (Nos. 2–13 and 22–36), six acetals of phenethyl alcohol or phenylacetaldehyde (Nos. 14, 15, 17–20), three related phenylacetaldehydes (Nos. 16,

37–39), and phenylacetic acid (No. 21). The four phenoxyacetic acid derivatives include phenoxyacetic acid (No. 40), an ester of phenoxyacetic acid (No. 41), an ester of phenoxyethyl alcohol (No. 42) and a homologue of phenoxyacetic acid (No. 43) (see Table 1).

The substances in this group are structurally related because they each have a 2-phenethyl or 2-phenoxyethyl-carbon skeleton containing a primary oxygenated functional group. Data indicates that the phenethyl

acetals and esters in this group are readily hydrolyzed to yield phenethyl alcohol, phenylacetaldehyde, or phenylacetic acid. Also, phenethyl alcohol and phenylacetaldehyde are readily oxidized to phenylacetic acid (Williams, 1959). Therefore, all of the substances in this group will eventually be hydrolyzed and oxidized to yield phenylacetic acid that will be excreted primarily in the urine in conjugated form. Similarly, the esters of phenoxyethyl alcohol or phenoxyacetic acid are hydrolyzed to the parent alcohol or acid. Phenoxyethyl alcohol is oxidized to phenoxyacetic acid that will be excreted primarily unchanged in the urine (Williams, 1959). Given their similar pharmacokinetic properties and their participation in a common metabolic pathway, the substances in this group are expected to exhibit a similar toxicologic profile (see Fig. 1).

## 2. Exposure

### 2.1. Flavor use and natural occurrence

Quantitative natural occurrence data and consumption ratios have been reported for 13 phenethyl alcohol derivatives in the group and demonstrate that their consumption occurs predominantly from traditional foods (i.e., consumption ratio >1). Consumption ratio relates the amount of a flavor ingredient that naturally occurs in traditional food to the amount added to processed foods as flavor ingredients [i.e., (annual consumption via food, kg)/(most recent reported volume as flavor ingredient, kg)] (Stofberg and Kirschman, 1985, 1987). Twenty flavoring ingredients in the group of phenethyl alcohol derivatives are natural components of foods. Phenethyl alcohol and its derivatives have been detected in beans, fruits, vegetables, cheeses, milk, oils, alcoholic and non-alcoholic beverages and a variety of other foods (Nijssen et al., 2003). Only one phenoxyacetic acid derivative is a natural component of food. Sodium 2-(4-methoxyphenoxy)propanoate (No. 43) has been detected in coffee (Rathbone et al., 1989). The consumption ratios for phenethyl alcohol, phenethyl acetate, phenethyl hexanoate, phenethyl octanoate, phenylacetaldehyde and phenylacetic acid are greater than or equal to 1 (Stofberg and Kirschman, 1985; Stofberg and Grundschober, 1987) (see Table 1).

The total annual volume of the 43 phenethyl alcohol derivatives is approximately 8334 kg in the USA (National Academy of Sciences, 1970, 1982, 1987; Lucas et al., 1999) (see Table 1). Greater than 50% of the total annual volume in the USA is accounted for by the use of phenethyl alcohol (No. 1), its corresponding acetate ester (No. 3), and phenylacetic acid (No. 21). Production volumes and intake values for each substance are reported in Table 1.

## 3. Hydrolysis, absorption, distribution, excretion and metabolism

### 3.1. Hydrolysis

Methods using artificial gastrointestinal juices have been used for studying the hydrolysis of esters (Longland et al., 1977). Prior to absorption, the esters and acetals in this group can be reasonably predicted to undergo *in vivo* hydrolysis (Williams, 1959) to yield phenethyl alcohol, phenoxyethyl alcohol, phenylacetaldehyde, phenylacetic acid, and phenoxyacetic acid. Phenethyl acetate (No. 3), methyl phenylacetate (No. 22), ethyl phenylacetate (No. 23), isopropyl phenylacetate (No. 25), isoamyl phenylacetate (No. 28) and citronellyl phenylacetate (No. 35) are rapidly hydrolyzed *in vitro* in simulated gastric juice and pancreatic juice (Longland et al., 1977) or in a buffered solution of pancreatin to corresponding phenethyl derivatives (Grundschober, 1977). Phenethyl acetate (No. 3), ethyl phenylacetate (No. 23), and isoamyl phenylacetate (No. 28) were not hydrolyzed by partially purified human plasma arylesterase (Augustinsson and Ekedahl, 1962).

The hydrolysis of aromatic acetals in simulated gastric juice and intestinal fluid supports the conclusion that the acetal functional group is hydrolyzed prior to absorption *in vivo*. *In vitro* incubation of 2-phenylpropanal dimethyl acetal (1 mM) with simulated gastric juice at 37 °C resulted in 97% hydrolysis in 1 h. Under the same experimental conditions, benzaldehyde propylene glycol acetal (1 mM) was 53% hydrolyzed in 5 h when compared to incubation of the acetal in 0.1 N HCl under reflux, which was 99% hydrolyzed (Morgareidge, 1962). Based on the *in vitro* and *in vivo* hydrolysis data collected for acetals, aliphatic acetals undergo hydrolysis in humans prior to absorption.

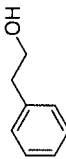



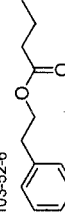

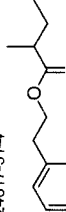
### 3.2. Absorption, distribution and excretion

When ingested as traditional foods, intentionally added ingredients of food, or as hydrolysis products resulting from either condition, phenethyl and phenoxyethyl alcohols, phenylacetaldehyde, and phenylacetic and phenoxyacetic acids are rapidly absorbed from the gastrointestinal tract. Once absorbed, the alcohols and aldehydes are rapidly oxidized to yield phenylacetic or phenoxyacetic acid derivatives that are subsequently excreted in the urine either free as in the case of phenoxyacetic acid or conjugated as in the case of phenylacetic acid (Williams, 1959; James et al., 1972; Caldwell, 1987; Sangster and Lindley, 1986; Hawkins and Mayo, 1986).

#### 3.2.1. Phenethyl alcohol (no. 1) and phenylacetic acid (no. 21)

Phenylacetic acid is a normal component of human urine (250–500 mg/24 h), forming mainly from the

Table 1  
Identity and exposure data for phenethyl alcohol, aldehyde, acid, and related acetals and esters used as flavor ingredients

Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume, kg <sup>a</sup>	Daily per capita intake ("eaters only") <sup>b</sup>		Annual volume in naturally occurring foods, kg <sup>c</sup>	Consumption ratio <sup>d</sup>
				µg/d	µg/kg bw/d		
1. Phenethyl alcohol	2858	60-12-8 	2517	332	6	695,796	276
2. Phenethyl formate	2864	104-62-1 	204	27	0.4	14	0.07
3. Phenethyl acetate	2857	103-45-7 	490	65	1	30069	61
4. Phenethyl propionate	2867	122-70-3 	24	3	0.05	216	9
5. Phenethyl butyrate	2861	103-52-6 	213	28	0.5	1	0.005
6. Phenethyl isobutyrate	2862	103-48-0 	435	57	1	+	NA
7. Phenethyl 2-methylbutyrate	3632	24817-51-4 	1	0.2	0.003	+	NA

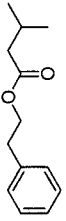
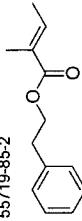
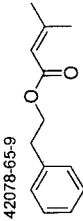

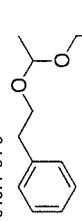
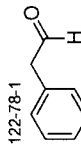
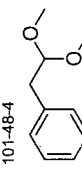
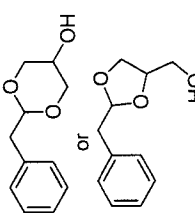
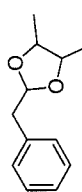
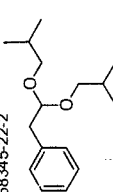
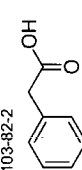
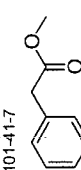
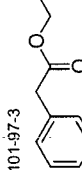
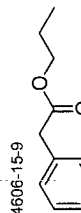
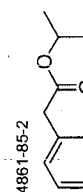
8. Phenethyl isovalerate	2871		204	27	0.4	432	2
9. Phenethyl hexanoate	3221		14	2	0.03	5732	409
10. Phenethyl octanoate	3222		1	0.1	0.002	3252	3252
11. Phenethyl tiglate	2870		9	1	0.02	–	NA
12. Phenethyl senecioate	2869		0.5	0.06	0.001	–	NA
13. Phenethyl phenylacetate	2866		590	78	1	–	NA
14. Acetaldehyde phenethyl propyl acetal	2004		45	6	0.1	–	NA
15. Acetaldehyde butyl phenethyl acetal	3125		0.3 <sup>e</sup>	0.05	0.0009	–	NA
16. Phenylacetaldehyde	2874		426	56	0.9	10,688	25
17. Phenylacetaldehyde dimethyl acetal	2876		344	45	0.8	+	NA

Table 1 (continued)

Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume, kg <sup>a</sup>	Daily per capita intake (“eaters only”) <sup>b</sup>		Annual volume in naturally occurring foods, kg <sup>c</sup>	Consumption ratio <sup>d</sup>
				µg/d	µg/kg bw/d		
18. Phenylacetaldehyde glyceryl acetal	2877	29895-73-6 	10	1	0.02	–	NA
19. Phenylacetaldehyde 2,3-butylene glycol acetal	2875	5468-06-4 	494	65	1	–	NA
20. Phenylacetaldehyde diisobutyl acetal	3384	68345-22-2 	3	0.4	0.006	–	NA
21. Phenylacetic acid	2878	103-82-2 	494	65	1	283	0.6
22. Methyl phenylacetate	2733	101-41-7 	172	23	0.4	55	0.3
23. Ethyl phenylacetate	2452	101-97-3 	132	17	0.3	41	0.3
24. Propyl phenylacetate	2955	4806-15-9 	2	0.3	0.005	–	NA
25. Isopropyl phenylacetate	2956	4861-85-2 	0.3 <sup>f</sup>	0.05	0.0009	–	NA

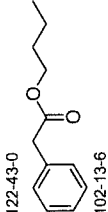
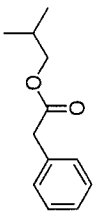
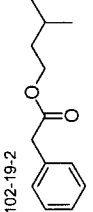
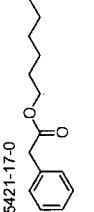
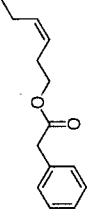
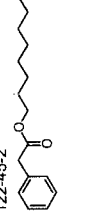
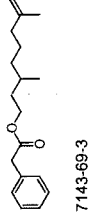
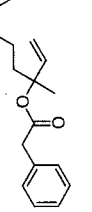
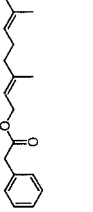
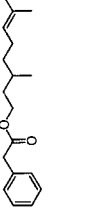
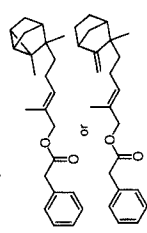
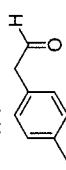
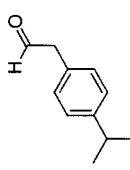
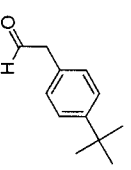
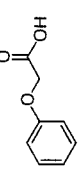
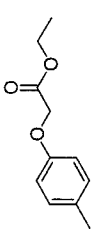
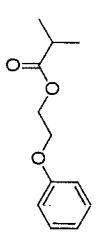
26. Butyl phenylacetate	2209	 122-43-0 102-13-6	25	3	0.06	+	NA
27. Isobutyl phenylacetate	2210	 168	168	22	0.4	+	NA
28. Isoamyl phenylacetate	2081	 102-19-2	222	29	0.5	+	NA
29. Hexyl phenylacetate	3457	 5421-17-0 42436-07-7	6 <sup>f</sup>	1	0.02	32	32
30. 3-Hexenyl phenylacetate	3633	 42436-07-7	0.4	0.05	0.0008	+	NA
31. Octyl phenylacetate	2812	 122-45-2	0.05	0.0006	0.0001	-	NA
32. Rhodinyl phenylacetate	2985	 10486-14-3 7143-69-3	7 <sup>f</sup>	1	0.02	-	NA
33. Linalyl phenylacetate	3501	 7143-69-3	0.1 <sup>f</sup>	0.02	0.0003	-	NA
34. Geranyl phenylacetate	2516	 102-22-7	14	2	0.03	-	NA
35. Citronellyl phenylacetate	2315	 139-70-8	14	2	0.03	-	NA

Table 1 (continued)

Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume, kg <sup>a</sup>	Daily per capita intake ("eaters only") <sup>b</sup>		Annual volume in naturally occurring foods, kg <sup>c</sup>	Consumption ratio <sup>d</sup>
				µg/d	µg/kg bw/d		
36. Santalyl phenylacetate	3008	1323-75-7 	5 <sup>E</sup>	0.9	0.01	—	NA
37. <i>p</i> -Toluacetaldehyde	3071	104-09-6 	23	3	0.05	—	NA
38. <i>p</i> -Isopropylphenylacetaldehyde	2954	4395-92-0 	0.05	0.006	0.0001	—	NA
39. Methyl <i>p</i> - <i>tert</i> -butylphenylacetate	2690	3549-23-3 	150	20	0.3	—	NA
40. Phenoxyacetic acid	2872	122-59-8 	0.9	0.1	0.002	—	NA
41. Ethyl ( <i>p</i> -tolylloxy)acetate	3157	67028-40-4 	0.5 <sup>c</sup>	0.09	0.001	—	NA
42. 2-Phenoxyethyl isobutyrate	2873	103-62-6 	830	109	2	—	NA



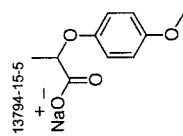
NA

+<sup>h</sup>

0.09

6

43



3773

43. Sodium 2-(4-methoxyphenoxy)propanoate

<sup>a</sup> From Lucas et al. (1999) or NAS (1970, 1982, 1987).

<sup>b</sup> Intake ( $\mu\text{g}/\text{person}/\text{day}$ ) calculated as follows:  $[(\text{annual volume, kg}) \times (1 \times 10^9 \mu\text{g}/\text{kg})] / [\text{population} \times \text{survey correction factor} \times 365 \text{ days}]$ , where population (10%, "eaters only") =  $26 \times 10^6$  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, 1982, 1987). Intake ( $\mu\text{g}/\text{kg bw}/\text{d}$ ) calculated as follows:  $[(\mu\text{g}/\text{person per day})/\text{body weight}]$ , where body weight = 60 kg. Slight variations may occur from rounding.

<sup>c</sup> Quantitative data for the United States reported by Stoffberg and Grundschober (1987).

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

<sup>e</sup> NAS (1987).

<sup>f</sup> NAS (1982).

<sup>g</sup> NAS (1970).

<sup>h</sup> Rathbone et al. (1988).

breakdown of phenylalanine by intestinal bacteria (Seakins, 1971) or via oxidative deamination of endogenous phenethylamine (Seakins, 1971; Richter, 1938). When administered orally, phenethylamine is rapidly metabolized to phenylacetylglutamine. Two human subjects, each fed a 300 mg dose of (*S*)-phenethylamine, excreted 60–62% of the administered dose as conjugated phenylacetic acid in the urine within 2–4.5 h (Seakins, 1971 and Richter, 1938). Also, greater than 80% of [<sup>14</sup>C]-(*S*)-phenethylamine fed to mice was rapidly excreted from urine as conjugated [<sup>14</sup>C]-phenylacetic acid (Block, 1953).

In humans, 26% of a 4000 mg oral dose of phenethyl alcohol (No. 1) is excreted in urine as phenylacetylglutamine within 24 h (Thierfelder and Schempp, 1917). In rabbits, 42% and 5% of a single 300 mg/kg bw oral dose of phenethyl alcohol is excreted in the urine as glycine and glucuronic acid conjugates, respectively, of phenylacetic acid within 24 h. The ether soluble acid extracted from the 24-h urine accounted for 61% of the dose (Bray et al., 1958). In an earlier study, 77% of 1300 mg/kg bw dose of phenethyl alcohol administered to rabbits via gavage was isolated from the 24-h urine as an ether soluble acid. No appreciable quantity (less than 0.5%) of free phenylacetic acid was recovered (Bray et al., 1946). In another study, it was reported that only 0.4–3.1% of an oral dose of phenylacetic acid was excreted unconjugated in the urine of rabbits (Tulane and Lewis, 1933).

Greater than 98% of a single oral dose of 80 mg of [carboxy-<sup>14</sup>C]-phenylacetic acid administered to each of three healthy human volunteers and three patients exhibiting phenylketonuria was excreted in the urine within 24 h as the glutamine conjugate (James et al., 1973). Greater than 98% of a 1 mg/kg oral dose of [carboxy-<sup>14</sup>C]-phenylacetic acid given to two male volunteers was excreted in the urine within 24 h (James et al., 1972). Based upon the results of studies using radiolabelled phenylacetic acid, it may be concluded that phenylacetic acid is rapidly absorbed and quantitatively excreted within 24 h.

### 3.2.2. Phenoxyethyl alcohol and phenoxyacetic acid (no. 40) derivatives

Once hydrolyzed, 2-phenoxyethanol is rapidly absorbed and oxidized to phenoxyacetic acid (No. 40), which is then excreted almost exclusively in the urine in unconjugated form (see Fig. 2).

The fate of 2-phenoxyethanol in rats and humans has been investigated (Howes, 1988). Greater than 90% of an oral dose of 15.6, 27.4 or 160.7 mg/kg bw of [2-<sup>14</sup>C]-phenoxyethanol given to male Colworth rats by gavage is excreted in the urine within 24 h. In a female rat, approximately 90% of a 27.4 mg/kg dose was also excreted in the urine within 24 h. Approximately 2% and 1.3% of the ingested dose was recovered from expired air of female and male rats, respectively. The rate

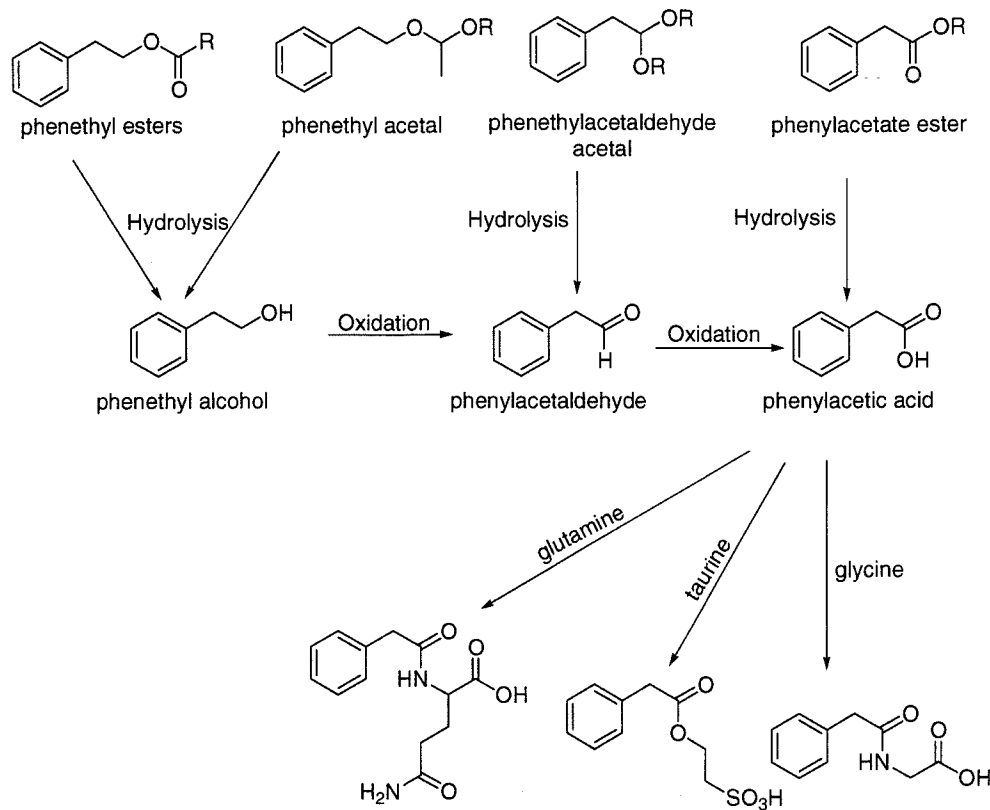


Fig. 1. Metabolism of phenethyl alcohol and related substances in animals.

of intestinal absorption was rapid with 60–70% of the excreted <sup>14</sup>C detected at 3 h and greater than 95% of the total 4-day urinary <sup>14</sup>C detected within the first 24 h. Trace amounts of radioactivity were detected in the feces. Four days after dosing only trace amounts of radioactivity remained in the carcasses, primarily in the liver (<0.2% of dose), fat and muscle. At 4 days the <sup>14</sup>C level in the blood was low (0.001%) (Howes, 1988).

A human volunteer ingested a dose of 10.63 mg of unlabelled 2-phenoxyethanol. Urine samples were pooled daily for 4 days. Quantitative urinary elimination (12.08 mg, 104% of 2-phenoxyethanol dose) of phenoxyacetic acid, mainly in the unconjugated form, was reported within 24 h. Minor amounts (0.34 mg) of phenoxyacetic acid were detected at day two, but there was no detectable amount at days three and four (Howes, 1988).

Phenoxyacetic acid was fed to male rabbits at a level of 100–200 mg/kg bw. When glycine was also adminis-

tered, it was given in amounts corresponding to three equivalents of the acid feeding level. In this test, 44–72% of phenoxyacetic acid was recovered unchanged in the urine within 6 h and 82–105% in 24 h. There was no evidence of conjugation with either glucuronic acid or glycine even when the diet was supplemented with glycine. After a rabbit received an oral dose of 500 mg of the glycine conjugate of phenoxyacetic acid, 30% of the dose was excreted unconjugated in the urine after 18 h (Levey and Lewis, 1947). In another study, 55% of an oral dose of an unspecified amount of phenoxyacetic acid was obtained in the urine of dogs (Thierfelder and Schempp, 1917), and 61% in the urine of humans (Thierfelder and Schempp, 1917). No evidence for glycine or glucuronic acid conjugation was observed.

A series of studies performed with 2-(4-methoxyphenoxy)propanoate (No. 43) indicate that this phenoxyacetic acid derivative is also rapidly absorbed and excreted unchanged in the urine. Groups of three male and three female rats were dosed by oral gavage with [<sup>14</sup>C]-sodium

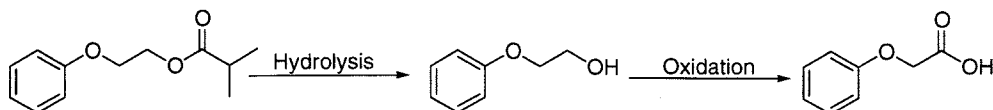


Fig. 2. Metabolism of 2-phenoxyethanol and related substances in animals.

2-(4-methoxyphenoxy)propanoate at 50 mg/kg and 500 mg/kg (5  $\mu\text{Ci}$ ). Urine was collected at 6, 12, 24, 48, 72, 96, and 120 h. Regardless of dose, the majority of radioactivity (87.6–97.6%) was eliminated primarily in the urine in the first 24 h, with a majority (group mean activity 59–76% of dose) of elimination occurring within the first 6 h. Only 0.8–4.6% was detected in the feces. The authors noted that urine contamination of feces may be responsible for detected radioactivity. At 120 h, urine radioactivity levels in females were approximately twice those of males, suggesting that the rate of elimination in males is faster than in females (Brown et al., 1986).

In the same study, 30 male and 27 female rats were dosed by oral gavage with [ $^{14}\text{C}$ ]-sodium 2-(4-methoxyphenoxy)propanoate at 50 mg/kg (5  $\mu\text{Ci}/\text{ml}$ ) 18 days after mating. Groups of three male and three female animals were sacrificed 0.5, 1, 3, 5, 7, 12, 24, 48, 72 and 96 h after dosing. After sacrifice, blood was sampled and tissues and organs were removed. Sodium 2-(4-methoxyphenoxy)propanoate was rapidly absorbed and eliminated by both male and female rats. Only 12.5% (range 6.3–22.9%) and 2.6% remained in the stomach of males and females after 0.5 h indicating that absorption in females was slightly faster than in males. Total radioactivity was greatest in the liver (2.5% for males and 3.1% for females) and kidney (2.1% for males and 2.5% for females) with the concentration of radioactivity (dpm/g) being 3.5 times higher in the kidney than in the liver. Loss of radioactivity is rapid from the tissue and organs with elimination being essentially complete with 24 h.

In pregnant female CD rats dosed by oral gavage with [ $^{14}\text{C}$ ]-sodium 2-(4-methoxyphenoxy)propanoate at 50 mg/kg (5  $\mu\text{Ci}/\text{ml}$ ), examination of fetuses and amniotic fluid revealed that radioactivity crosses the placenta, although within 3 h its elimination from the fetus and placenta is complete. Elimination of radioactivity from amniotic fluid is complete in 12 h. Radioactivity is detected within the uterine muscle for up to 72 h, although by 7 h, the level is less than 0.05% of the dose (Brown et al., 1986). The authors note that the radioactivity retained within the uterine muscle is within the level of impurities reported for administered acid (Brown et al., 1986). The authors suggested that the acid may bind to uterine muscle protein. In a subsequent *in vitro* test using bovine serum albumin, sodium 2-(4-methoxyphenoxy)propanoate binds to protein with equilibrium (83–85% in bound fraction) being reached within 2 h (Brown et al., 1986). The authors concluded that although acid binds to plasma protein, it is still rapidly eliminated from the blood (Brown et al., 1986).

Plasma measurements confirm that [ $^{14}\text{C}$ ]-sodium 2-(4-methoxyphenoxy)propanoate is rapidly absorbed and eliminated from male and pregnant female rats. The highest plasma level was at the first sampling time point (0.5 h). At 12 h, plasma radioactivity is only

0.3% of the level achieved at 0.5 h. Repeated daily dosing results in 90% of the steady state plasma level after ten days. However, the steady state is still <1% of the plasma concentration at 0.5 h. Thus, repeat dosing has little effect on the plasma concentration time profile (Brown et al., 1986).

In a subsequent pharmacokinetic study, three male and three female rats each received a single intravenous dose of [ $^{14}\text{C}$ ]-sodium 2-(4-methoxyphenoxy)propanoate at a level of 50 mg/kg (10  $\mu\text{Ci}$ ). Blood samples were collected at 5, 15, 30, and 60 min, 2, 4, 6, 12, and 24 h post-dosing until concentrations of radioactivity were near the limit of detection (Hawkins and Mayo, 1986). The highest plasma concentration achieved at 5 min declined rapidly over the next hours. Mean concentrations of radioactivity declined from 194 and 213  $\mu\text{g}$  2-(4-methoxyphenoxy)propanoic acid/ml plasma at 5 min to 11.2 and 15.5  $\mu\text{g}/\text{ml}$  plasma at 1 h in male and female rats, respectively. Mean elimination half-lives of radioactivity of 13 and 14 min were estimated during this time for male and female rats, respectively (Hawkins and Mayo, 1986). Concentrations of radioactivity then declined to 0.25 and 0.32  $\mu\text{g}/\text{ml}$  plasma in male and female rats, respectively, at 6 h. No radioactivity was detected in male rats after 6 h or in female rats after 24 h. The mean area under the curve (AUC) in male rats of 77  $\mu\text{ghml}^{-1}$  compared to that of 102  $\mu\text{ghml}^{-1}$  in the female indicated slower elimination of radioactivity in females. The authors conclude that sodium 2-(4-methoxyphenoxy)propanoate was rapidly eliminated from the vascular system of the male and female rat (Hawkins and Mayo, 1986).

In a subsequent investigation of the effect of repeated exposure on metabolism and excretion, a group of five male and five female rats were fed 5000 ppm of sodium 2-(4-methoxyphenoxy)propanoate (ca. 250 mg/kg bw per day (FDA, 1993)) for 17 weeks. The study was conducted in three phases. Two unlabelled doses of 300 mg/kg bw and one uniformly [ $^{14}\text{C}$ ]-ring labelled dose of 310 mg/kg bw (5  $\mu\text{Ci}$ ) in males and 318 mg/kg bw (5  $\mu\text{Ci}$ ) in females of the sodium salt were given on separate occasions with two weeks between each dose. In the study with the labelled dose, 99.37% and 103.8% of the radioactivity was excreted in the urine of males and females, respectively, with less than 1% of the dose being detected in the feces. The 0–6 h urine samples accounted for 83% of the radiolabel and 6–12 h samples accounted for 17% of the radiolabel. No radioactivity was detected in the whole blood, plasma or washed red blood cells seven days after administration. Greater percentage of urinary metabolites were recovered in the unlabelled studies than were orally administered indicating that the test material was not completely eliminated prior to the oral administration. However, in both studies, 90% of the recovered material was excreted in the first 6 h post-dosing and the majority of the remainder in the 6–12 h urine

sample. The author concludes that prolonged exposure to sodium 2-(4-methoxyphenoxy)propanoate does not affect the metabolism and disposition of this compound in the rat (Hill, 1986).

The disposition of sodium 2-(4-methoxyphenoxy)propanoate has also been investigated in three healthy male volunteers. Volunteer A received a 114.7 mg capsule, volunteer B received a 112.9 mg capsule and volunteer C received a 114.3 mg capsule of the test compound. Urine analysis demonstrates that approximately 75% of the dose was eliminated within 72 h, of which 85% was recovered within 24 h (Sangster and Lindley, 1986). In another study, five healthy male volunteers were given an oral dose of 100 mg of [2-<sup>14</sup>C]-sodium 2-(4-methoxyphenoxy)propanoate (50  $\mu$ Ci) and approximately 97% of the dose was recovered within five days of administration, with approximately 93% recovered in the first 24 h (Caldwell, 1987). According to the author, the data demonstrate that sodium 2-(4-methoxyphenoxy)propanoate is rapidly absorbed and rapidly eliminated from the body via the urine.

Based on the extensive data available for the absorption, distribution and elimination of phenethyl and phenoxyethyl alcohols and phenylacetic and phenoxyacetic acids, it is concluded that following hydrolysis, the parent alcohols are predominantly converted to the corresponding carboxylic acids that are then rapidly excreted in either the free or conjugated form in the urine. The pharmacokinetic profiles for the rat and human with phenoxyethyl alcohol derivatives indicate that the rat is an acceptable model for human risk assessment.

### 3.3. Metabolism

#### 3.3.1. Phenethyl alcohol derivatives

Phenethyl alcohol is successively oxidized to phenylacetaldehyde and phenylacetic acid in vivo. Phenylacetic acid undergoes species-specific conjugation with a variety of amino acids, amines, or glucuronic acid followed by excretion almost exclusively in the urine (James et al., 1972) (see Fig. 1).

Phenethyl alcohol is readily oxidized to phenylacetaldehyde by an assortment of NAD<sup>+</sup>-dependent alcohol and aldehyde dehydrogenases (Bosron and Li, 1980). The highest activity of mammalian alcohol dehydrogenases (ALDH) occurs in the liver where they exhibit broad substrate specificity for the oxidation of primary aliphatic and aromatic alcohols. Human liver ALDH shows decreased  $K_m$ <sup>1</sup> with increasing lipophilicity.

<sup>1</sup> The Michaelis–Menten constant,  $K_m$ , is the concentration of the specific substrate at which a given enzyme yields one-half its maximum velocity. Michaelis–Menten equation:  $v_0 = V_{max}[S]/K_m + [S]$ .

Table 2

Human mitochondrial aldehyde dehydrogenase (ALDH-2) and cytosolic isoenzyme (ALDH-1)<sup>a</sup>

	ALDH1	ALDH2
$K_m$ (nM)	5500 $\pm$ 1200	29 $\pm$ 4
$V_{maxrel}$ (%) (% in relation to $V_{max}$ of acetaldehyde)	380 $\pm$ 40	153 $\pm$ 20
$k_{cat}$ <sup>b</sup>	3000 $\pm$ 340	1800 $\pm$ 200

<sup>a</sup> Klyosov (1996).

<sup>b</sup>  $k_{cat}$  values are based on molecular weights for the tetrameric enzymes of 230,000 (ALDH-1) and 240,000 (ALDH-2). Specific activities of purified ALDH-1 and -2 from five different batches were 3.4  $\pm$  0.6 and 4.9  $\pm$  0.8  $\mu$ mol min<sup>-1</sup> (mg of protein<sup>-1</sup>), respectively, at pH 9.5. This corresponds to  $k_{cat}$  values of 782  $\pm$  138 min<sup>-1</sup> for ALDH-1 and 1176  $\pm$  192 min<sup>-1</sup> for ALDH-2.

However,  $V_{max}$ <sup>2</sup> remains essentially constant suggesting that the rate-limiting step does not involve the binding or release of the alcohol or aldehyde intermediate (Pietruszko et al., 1973).

Once formed, phenylacetaldehyde is oxidized by inducible aldehyde dehydrogenases from rat liver cytosol. In the rat, these isoenzymes can be induced by phenobarbital (Simpson et al., 1985). The  $K_m$  and  $V_{max}$  values of human mitochondrial aldehyde dehydrogenase (ALDH-2) and cytosolic isoenzyme (ALDH-1) for oxidation of phenylacetaldehyde (Table 2) indicate rapid conversion to phenylacetic acid (Klyosov, 1996).

Phenylacetaldehyde, 3- and 4-chlorophenylacetaldehyde are effectively oxidized to the corresponding phenylacetic acid derivatives when incubated with rat hepatic microsomal dehydrogenase containing NAD<sup>+</sup> as a coenzyme. The rates of oxidation for the 3- and 4-chloro derivatives are markedly slower than that of the parent phenylacetaldehyde (Martini and Murray, 1996). In dogs, 32% of a 1900 mg/kg bw dose of phenylacetaldehyde (No. 16) given to dogs is rapidly oxidized and excreted as the glycine conjugate within 48 h (Kay and Raper, 1922).

#### 3.3.2. Phenylacetic acid

Although phenylacetic acid has been extensively studied, investigations conducted prior to 1950 on human metabolism (Shiple and Sherwin, 1922; Wagreich et al., 1940; Power and Sherwin, 1927; Ambrose et al., 1933) failed to account for the endogenous level of 250–500 mg/kg per day of phenylacetic acid conjugated with glutamine (Stein et al., 1954) present in human urine and did not adequately characterize the array of urinary conjugates that formed from phenylacetic acid. More recent work demonstrates that conjugation is both dose-dependent and species-specific. The major metabolic options available to phenyl acetic acid are conjuga-

<sup>2</sup>  $V_{max}$  is the maximum rate or velocity of an enzymatic reaction which is indicative of all of the enzyme active site(s) is complexed with substrate.

tion with glucuronic acid, glycine, taurine, or glutamine, and elimination as the free acid.

In two adult male humans an average of 91% and 7% of a 1 mg/kg bw oral dose of [carboxy-<sup>14</sup>C]phenylacetic acid is excreted within 24 h as glutamine and taurine<sup>3</sup> conjugates, respectively. Unlike most other animals, only a trace of the glycine conjugate has been detected in humans (James et al., 1972). The distribution and type of conjugation is relatively unaffected by continued ingestion of phenylacetic acid. After being fed thirty-four 1000–10,000 mg doses of the acid over a 97-day period, one human excreted greater than 90% of the administered dose as the phenylacetylglutamine conjugate (Ambrose et al., 1933). Similar to humans, Old and New World monkeys form phenylacetic acid conjugates with glutamine and to a lesser extent, taurine. However, significant quantities of acid (1–44%) are excreted unconjugated. In carnivores (e.g., dog, cat, ferret), glycine conjugation predominates with no detectible amounts of glutamine conjugation. Likewise in rodents and lagomorphs (rabbits), phenylacetic acid is excreted primarily as the glycine conjugate. Unconjugated phenylacetic acid and minute amounts of taurine conjugates are also excreted. In rats, greater than approximately 94% of an 80 mg/kg dose of phenylacetic acid given by intraperitoneal injection is excreted as the glycine conjugate (James et al., 1972).

Clearly, the nature of the amino acid used for conjugation is a function of species. Sources and amounts of available amino acids will alter the conjugating ability of different species. In humans, endogenous sources of glutamine include those from waste urea nitrogen. Ingestion of 5000 mg per day of phenylacetic acid for three consecutive days resulted in a 25–78% decrease in urine urea nitrogen levels (Shiple and Sherwin, 1922). Glutamine may be supplied by blood plasma glutathione (reduced tripeptide, Glu-Cys-Gly). An 18–23% reduction in the plasma tripeptide level was observed within a few hours of human ingestion of a 4000 mg dose of phenylacetic acid.

The capacity for glutamine conjugation has been studied in three volunteers and three patients exhibiting phenylketonuria (James et al., 1973). Each subject was given a single 80 mg dose of [carboxy-<sup>14</sup>C]-phenylacetic acid. The average excretion of phenylacetylglutamine (measured as mmoles/g creatinine) by phenylketonurics was approximately five times that of the control subjects indicating that the glutamine conjugation mechanism is able to cope with large amounts of phenylacetic acid produced by phenylketonurics. The mechanism for conjugation of glutamine with phenylacetic acid probably involves formation of a phenylacetic acid coenzyme-A

(CoA) intermediate (Moldave and Meister, 1957). One hour perfusion of human kidney or incubation of human liver homogenate with [<sup>14</sup>C]-glutamine and phenylacetyl-CoA results in the formation of the radioactive conjugate in the respective yields of 5% and 13%.

In rodents (e.g., rats) endogenous levels of unconjugated phenylacetic acid may occur at dose levels at which glycine conjugation is capacity-limited, presumably by the supply of endogenous glycine (Gregus et al., 1993). Only small amounts of the glycine conjugate enter the bile. Less than 10% of a 100 mg/kg bw oral dose of phenylacetic acid was collected from the bile of rats over 4 h (Koss and Lamprecht, 1968). Significant levels of free phenylacetic acid have been observed at high dose levels (Teuchy et al., 1971, 1972). Prolonged elevated levels of free phenylacetic acid may be associated with toxicologic effects similar to those observed with high dose levels of other carboxylic acids where conjugation is glycine-limited. Conversely, in humans high levels of glutamine available for conjugation allow metabolic pathways to cope with high levels of endogenously formed phenylacetic acid.

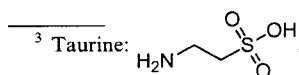
### 3.3.3. Phenoxyethyl derivatives

In rats, greater than 90% of a 15.6, 27.4 or 160.7 mg/kg bw dose in males and 27.4 mg/kg bw in females of [2-<sup>14</sup>C]-2-phenoxyethanol is excreted in the urine as phenoxyacetic acid within 24 h (Howes, 1988). Essentially, an entire oral dose of 10.63 mg of unlabelled 2-phenoxyethanol could be accounted for in the urine of one healthy male human volunteer as 2-phenoxyacetic acid. The majority of the acid was excreted unconjugated (Howes, 1988).

The urine of male rats collected for 6 h after an oral dose of 500 mg/kg (5 $\mu$ C<sub>i</sub>) of [<sup>14</sup>C]-sodium 2-(4-methoxyphenoxy)propanoate revealed two excretion products, identified as 2-(4-methoxyphenoxy)propanoic acid (90%) and the *O*-demethylated metabolite  $\pm$ 2-(4-hydroxyphenoxy)propionic acid (7–10%) (Brown et al., 1986).

Rats (5/sex/group) were fed 5000 ppm per day, which corresponds to 250 mg/kg bw per day, [<sup>14</sup>C]-sodium 2-(4-methoxyphenoxy)propanoate for 17 weeks. The majority (92–96%) of the dose was excreted as the unchanged acid, 2-(4-methoxyphenoxy)propanoic acid, while 6–7% of the dose in male rats and 8–9% of the dose in female rats was excreted as the *O*-demethylated metabolite  $\pm$  2-(4-hydroxyphenoxy)propionic acid (Hill, 1986).

Each of three human males received a single dose of 113–114 mg 2-(4-methoxyphenoxy)propanoic acid. Urinary metabolites included 2-(4-methoxyphenoxy)propanoic acid (65%) and 2-(4-hydroxyphenoxy)propanoic acid (10%) (Sangster and Lindley, 1986). In another study, approximately 97% an oral dose of 100 mg of [2-<sup>14</sup>C]-sodium 2-(4-methoxyphenoxy)propanoate (50  $\mu$ C<sub>i</sub>) given to five healthy male volunteers is excreted in the urine as 2-(4-methoxyphenoxy)propanoic acid of



administration. Greater than 93% was recovered from the urine in the first 24 h (Caldwell, 1987). The data show that sodium 2-(4-methoxyphenoxy)propanoate is rapidly absorbed and rapidly eliminated in the urine mainly as the unchanged acid. Minor amounts undergo *O*-demethylation to yield the corresponding *O*-demethylation metabolite 2-(4-hydroxyphenoxy)propanoic acid.

In summary, phenethyl and phenoxyethyl alcohol derivatives are rapidly oxidized to the corresponding carboxylic acids. Subsequently in humans, phenylacetic acid is conjugated primarily with glutamine and excreted in the urine while phenoxyacetic acid is excreted unconjugated almost exclusively in the urine.

### 3.3.4. Biochemical studies

Other studies in rodents and humans have investigated the effects of phenylacetic acid on various biochemical parameters, mostly associated with phenylketonuria. Although blood sugar levels were reduced in guinea pigs (12–60%) fed 380–750 mg/kg bw of phenylacetic acid, no effect on glucose levels was observed over a 2–6 h period when a volunteer was fed a 3000 mg dose (ca. 50 mg/kg bw (FDA, 1993)) (Stewart, 1962). Phenylacetic acid administered orally to fasted guinea pigs in doses of 375, 500 or 750 mg/kg bw resulted in a reduction in blood sugar of 12%, 44% and 60% of the initial values, respectively, within 2–4 h (Stewart, 1962). Rabbits were as sensitive as guinea pigs to the hypoglycemic action of phenylacetic acid (Stewart, 1962). Orally administered phenylacetic acid (62.5 mg/kg bw) also produced a drastic reduction in blood sugar in cats, which was sustained for at least 5 h (Stewart, 1962).

Phenylacetic acid fed in a single 20 mg/kg bw dose to each of five male human volunteers resulted in a two- to four-fold increase in urinary indole-3-acetic acid levels (Tashian, 1960). Presumably, the phenylacetic acid deactivates tryptophan decarboxylase resulting in increased conversion of tryptophan to indole-3-acetic acid. Incubation of a suspension (0.1 ml or 0.1 g/100 ml) of phenylacetic acid with human plasma caused prolonged clotting and increased thrombin activity (Nour-Eldin, 1968). No changes in total nitrogen and ammonia content were noted in the urine after rabbits were fed a diet containing neutralized (with sodium carbonate) phenylacetic acid (0.5 g first day followed by 1.0 g the next day). An increase in amino acid and an insignificant decrease in urea output were noted in urine (Hijikata, 1922).

In a study of the effect of various phenyl-substituted acids on glycosyl transferase activity in cerebral spinal fluid (CSF), 1-h incubation of human CSF with phenylacetic acid (2–10 mM) produced up to 91% inhibition of galactosyl transferase activity utilized in cerebral glycoprotein synthesis. The authors hypothesized that inhibi-

tion of these enzymes may play a key role in pathogenesis of phenylketonuria (Ko et al., 1973).

In a study of the bacteriocidal activity of phenethyl alcohol, incubation of 0.25% (2500 µg/ml) of phenethyl alcohol with *Escherichia coli* strains DG75 and H500T resulted in reversible inhibition of DNA synthesis and cell division as measured by labelled thymidine uptake and cell counts, respectively. Cells remained viable and RNA and protein synthesis capable. Increased sedimentation during treatment was interpreted as due to either increased formation of nucleoid bodies containing the replicating mechanism of the cell or increased cell lysis due to PEA's detergent action (Brunner and Treick, 1982). The authors concluded that PEA-treated cells could be used to evaluate the formation and composition of nucleoid bodies during bacteriocidal activity.

Numerous studies have evaluated the effect of phenethyl alcohol and a range of pharmaceuticals on excision repair of UV-induced DNA damage in non-standard *Escherichia coli* strains. Solutions (0.2% and 0.4%) of phenethyl alcohol were incubated with UV-damaged *E. coli* strains H/r 30 and B (wild-type). Phenethyl alcohol inhibited excision repair of membrane associated DNA as measured by a decrease liquid holding recovery (LHR in cells has been related to repair of UV-damaged DNA in *E. coli*) and a decrease in the removal of thymine dimers. The authors hypothesized that excision repair may required that DNA is bound to the cell membrane. The test article may inhibit that binding (Tachibana and Yonei, 1985). Similar results were obtained in an earlier study. It was shown that 0.05–0.5% solutions of phenethyl alcohol result in the detachment of DNA from the DNA-membrane complex in *E. coli* B cells. The effect is concentration dependent. Similar results were obtained with *Escherichia coli* NG30 (RecA mutant) (Tachibana et al., 1982). In a related study, phenethyl alcohol inhibited the removal of thymine dimers from DNA that is thought to be the incision step of excision repair in *E. coli* (Tomiyama et al., 1986). Phenethyl alcohol and a wide range of pharmaceuticals have been shown to inhibit excision repair of UV-damaged DNA in *E. coli* (Yonei, 1980; Todo and Yonei, 1983; Tachibana and Yonei, 1985).

Phenylacetaldehyde (No. 16) typically causes an initial large magnitude, short duration fall in blood pressure followed by a smaller magnitude, longer duration rise in pressure (Wingard et al., 1955; Romano et al., 1954). Phenylacetaldehyde inhibited both sodium/potassium ( $\text{Na}^+/\text{K}^+$ ) activated ATPase and  $\text{Mg}^{2+}$ -ATPase. In addition, this compound also inhibited  $\text{K}^+$  ion-dependent *p*-nitrophenylphosphatase activity. The  $\text{Na}^+/\text{K}^+$ -ATPase was much more sensitive than  $\text{Mg}^{2+}$ -ATPase of  $\text{K}^+$ -activated phosphatase to inhibition by aldehydes. The inhibition of  $\text{Na}^+ + \text{K}^+$ -ATPase by aldehyde was

reversible and was non-competitive with ATP or  $K^+$  as the variable substrate or activator, respectively. Addition of cysteine or mercaptoethanol protected the enzymes from inhibition by aldehydes. All aldehydes, including acetaldehyde, were more potent inhibitors of  $Na^+ + K^+$ -ATPase activity than was ethanol (Erwin et al., 1975). Glutathione peroxidase is inactivated by phenylacetaldehyde in vitro (Tabatabaie and Floyd, 1996).

#### 4. Toxicological studies

##### 4.1. Acute toxicity

Oral  $LD_{50}$  values have been reported for 24 of the 39 phenethyl alcohol derivatives and are summarized in Table 3. In rats,  $LD_{50}$  values are in the range from 1500 mg/kg bw for phenethyl alcohol to greater than 15,390 mg/kg bw phenethyl phenylacetate, demonstrating that the oral acute toxicity is very low (Jenner et al., 1964, 1971; Keating, 1972; Moreno, 1972a,b, 1973a,b,c,d,e, 1974a,b, 1976a,b,c, 1977a,b,c,d, 1979, 1980, 1982; Levenstein, 1973; Russell, 1973; Levenstein, 1974; Zaitsev and Rakhmanina, 1974; Beroza et al., 1975; Mallory et al., 1982, 1987).

In mice,  $LD_{50}$  values are in the range from 2250 mg/kg for phenylacetic acid to 15,488 mg/kg for linalyl phenylacetate (Colaiani, 1967; Zaitsev and Rakhmanina, 1974; Levenstein, 1975).

Oral  $LD_{50}$  values have also been reported in guinea pigs. These values are in the range from 2250 mg/kg for phenylacetic acid to 3890 mg/kg for phenethylacetaldehyde (Zaitsev and Rakhmanina, 1974).

Oral  $LD_{50}$  values have been reported for three of the four phenoxyethyl alcohol derivatives and are also summarized in Table 3. In rats,  $LD_{50}$  values are in the range from 1500 for phenoxyacetic acid to greater than 5000 mg/kg bw for phenoxyacetic acid, 2-phenoxyethyl isobutyrate, and sodium 2-(4-methoxyphenoxy)propanoate. These data demonstrate that the oral acute toxicity of phenoxyacetic acid and related substances is very low in the rat (Moreno, 1973a,b,c,d,e, 1976a,b,c; Piccirillo et al., 1983; Cummins, 1985; Burdock and Ford, 1990).

##### 4.2. Short-term studies of toxicity

The results of short-term, long-term and carcinogenicity studies are summarized in Table 3 and described below. These studies demonstrate that phenethyl and phenoxyethyl alcohol derivatives exhibit a low toxic potential.

###### 4.2.1. Phenethyl senecioate (no. 12) and isopropylphenylacetaldehyde (no. 38)

A control and a test group, each consisting of 16 male and female Charles River CD rats, were housed in pairs

of the same sex and given ad libitum access to water and food. The concentration of the test material in the diet was adjusted during the study to maintain constant daily levels of dietary intake of 1.52 mg/kg bw for males or 1.84 mg/kg bw for females of phenethyl senecioate or 16.98 mg/kg bw for males or 18.77 mg/kg bw for females of isopropylphenylacetaldehyde for 90 days. During weeks 7 and 13, hematological examination and clinical chemistry (blood urea) parameters were measured. After 90 days, all animals were killed, subjected to a detailed necropsy examination and liver and kidney weights were measured. A wide range of tissues and organs from each animal were preserved and histopathological examinations were performed on major organs and tissues (Posternak et al., 1969).

There was no significant difference in body weight gain between test and control animals. Kidneys of male rats fed phenethyl senecioate were slightly enlarged, however, these slight increases in the relative weight became even less marked when test values were compared with values for laboratory wide control groups. The authors concluded that the changes in kidney weight were not toxicologically significant. In the study with isopropylphenylacetaldehyde, no effects were observed in either male or female rats (Posternak et al., 1969). The NOAEL's of 16.98 and 18.77 mg isopropylphenylacetaldehyde/kg bw per day for males and females, respectively, are at least 18,000,000 times the estimated daily per capita intake<sup>4</sup> ("eaters only") of 0.001  $\mu$ g/kg bw from use of isopropylphenylacetaldehyde as a flavoring ingredient in the USA (see Table 1).

Groups of Osbourne–Mendel rats (10/sex/group) were maintained on diets containing 0, 20, 1000, 2500, or 10,000 ppm of phenethyl senecioate. These dietary levels are calculated to provide an average daily intake of 0, 1.50, 125, or 500 mg of phenethyl senecioate/kg bw for 4 months (FDA, 1993). The following tissues were taken from 11 control animals and 12 animals in the 10,000 ppm group: brain, pituitary, eye, salivary gland, thyroid, lung, heart, liver, spleen, pancreas, kidney, adrenal, stomach, duodenum, ileum, colon, urinary bladder, ovary and uterus or testes and prostate, bone marrow and skeletal muscle. The kidneys, liver and adrenals with occasionally other tissues were taken from the remaining animals. Slight degenerative changes were seen in some kidneys; however, this is not unexpected in

<sup>4</sup> Intake ( $\mu$ g/person/day) calculated as follows:  $[(\text{annual volume, kg}) \times (1 \times 10^9 \mu\text{g/kg})] / [\text{population} \times \text{survey correction factor} \times 365 \text{ days}]$ , where population (10%, "eaters only") =  $26 \times 10^6$  for the USA; where correction factor = 0.8 for the Lucas et al. (1999) and 0.6 National Academy of Sciences, 1970, 1982, 1987 and anticipated volumes represents the assumption that only 80% and 60% of the flavor volume, respectively, was reported in the surveys (National Academy of Sciences, 1970, 1982, 1987; Lucas et al., 1999). Intake ( $\mu$ g/kg bw/d) calculated as follows:  $[(\mu\text{g/p/d}) / \text{body weight}]$ , where body weight = 60 kg. Slight variations may occur from rounding off.

Table 3  
Acute, short- and long-term toxicity studies for phenylethyl alcohol, aldehyde, acid, and related acetals and esters used as flavor ingredients

	Flavoring ingredient	Oral acute studies		Reference	Short-term and long-term studies		NOAEL (mg/kg bw)	Reference
		Oral LD <sub>50</sub> mg/kg bw (species)	Species, sex <sup>a</sup>		Time (days)/route			
1	Phenethyl alcohol	800 (Mouse)		Fassett (1963)	Rat; M, F	392/Drinking water	120 <sup>b,c</sup>	Johannsen and Purchase (1969)
1	Phenethyl alcohol	400 (Guinea pig)		Fassett (1963)	Rat; M	120/Gavage	50.8 <sup>b</sup>	Zaitsev and Rakhmanina (1974)
1	Phenethyl alcohol	2190 µl (Rat)		Hofmann (1974)				
1	Phenethyl alcohol	1800 (Rat)		Rumyantsev et al. (1987)				
1	Phenethyl alcohol	1500 (Rat)		Moreno (1982)				
1	Phenethyl alcohol	1790 (Rat)		Jenner et al. (1964)				
1	Phenethyl alcohol	2540 (Rat)		Zaitsev and Rakhmanina (1974)				
1	Phenethyl alcohol	2540 (Mouse)		Zaitsev and Rakhmanina (1974)				
1	Phenethyl alcohol	2540 (Guinea pig)		Zaitsev and Rakhmanina (1974)				
1	Phenethyl alcohol	1693 (Rat)		Mallory et al. (1982)				
2	Phenethyl formate	3.22 ml/kg (Rat)		Levenstein (1973)				
3	Phenethyl acetate	>5000 (Rat)		Moreno (1973a)	Rat; M	120/Gavage	73.4 <sup>b</sup>	Zaitsev and Rakhmanina (1974)
3	Phenethyl acetate	5200 (Rat)		Rumyantsev et al. (1987)				
3	Phenethyl acetate	3670 (Rat)		Zaitsev and Rakhmanina (1974)				
3	Phenethyl acetate	3670 (Mouse)		Zaitsev and Rakhmanina (1974)				
3	Phenethyl acetate	3670 (Guinea pig)		Zaitsev and Rakhmanina (1974)				
4	Phenethyl propionate	3980 (Rat)		Beroza et al. (1975)				
4	Phenethyl propionate	4000 (Rat)		Moreno (1973b)				
5	Phenethyl butyrate	4.6 ml/kg		Levenstein (1974)				
6	Phenethyl isobutyrate	>5000		Shelanski and Moldovan (1971)				
7	Phenethyl 2-methylbutyrate	>5000		Moreno (1982)				
11	Phenethyl tiglate	>5000		Levenstein (1974)				
12	Phenethyl senecioate				Rat; M, F	90/Diet	1.52 (M) <sup>b</sup> 1.84 (F) <sup>b</sup>	Posternak et al. (1969)
13	Phenethyl phenylacetate	3190		Zaitsev and Rakhmanina (1974)	Rat; M, F	119/Oral	1000 <sup>b</sup>	Hagan et al. (1967)
13	Phenethyl phenylacetate	15390		Jenner et al. (1964)				
13	Phenethyl phenylacetate	3190		Zaitsev and Rakhmanina (1974)				
13	Phenethyl phenylacetate	3190		Zaitsev and Rakhmanina (1974)				
14	Acetaldehyde phenethyl propyl acetal	>5000		Moreno (1978)				
16	Phenylacetaldehyde	1550		Moreno (1977a)				





Osbourne-Mendel rats of this age. In older rats all gradations of chronic interstitial nephritis from slight to marked is a common finding. The author concludes that the gross and histopathological examination of a wide variety of tissues failed to reveal any alterations that could be related to administration of the test material (Taylor, 1970). The NOAEL of 500 mg/kg bw per day is at least  $5 \times 10^8$  times the estimated daily per capita intake<sup>4</sup> (“eaters only”) of 0.001  $\mu\text{g}/\text{kg}$  bw from use of phenethyl senecioate as a flavoring ingredient in the USA (see Table 1).

#### 4.2.2. Phenethyl phenylacetate (no. 13)

Groups of 10 male and 10 female Osbourne-Mendel rats were provided phenethyl phenylacetate in the diet at concentrations of 0, 1000, 2500 or 10,000 ppm which corresponds to an average daily intakes of 0, 50, 250, or 500 mg/kg bw per for 17 weeks (FDA, 1993). Measurement of body weight and food intake recorded weekly showed no significant difference between test and control animals at any intake level. At termination, hematological examinations revealed no effects due to administration of the test substance. At necropsy, no differences were reported in major organ weights between test and control animals. Gross examination of tissue of all animals was unremarkable and histopathological examination of six-eight animals, equally represented by gender, for the high-dose group and the control group revealed no treatment-related lesions (Hagan et al., 1967). The highest dose level, 500 mg/kg bw per day of phenethyl phenylacetate that produced no effects in rats is at least 500,000 times the estimated daily per capita intake<sup>4</sup> (“eaters only”) of 1  $\mu\text{g}/\text{kg}$  bw from use of phenethyl phenylacetate as a flavoring ingredient in the USA (see Table 1).

#### 4.2.3. Ethyl (*p*-tolylxy)acetate (no. 41)

Charles River CD rats, (16/sex/group) were housed in pairs of the same sex and given ad libitum access to water and food and the concentration of ethyl (*p*-tolylxy)acetate in the diet was maintained at levels of dietary intake of 15.98 for females and 14.61 mg/kg bw for males for 90 days. Weekly measurements of body weight, food intake, and efficiency of food utilization revealed no difference between test and control groups. Hematological and blood-urea examinations performed on 50 percent of the animals at week seven and on all the animals at week 13 revealed normal values. At necropsy, there was no difference in liver and kidney weights between test and control groups. A wide range of tissues and organs from each animal were preserved and histopathological examinations. Based on gross and histopathological examination, there was no evidence of alterations that could be associated with administration of the test substance (Posternak et al., 1969). The NOAEL's of 15.98 mg/kg bw per day for females and

14.61 mg/kg bw per day for males are at least 1,400,000 times the estimated daily per capita intake<sup>4</sup> (“eaters only”) of 0.001  $\mu\text{g}/\text{kg}$  bw from use of ethyl (*p*-tolylxy)acetate as a flavoring ingredient in the USA (see Table 1).

#### 4.2.4. Sodium 2-(4-methoxyphenoxy)propanoate (no. 43)

A 14-day study was conducted with sodium 2-(4-methoxyphenoxy)propanoate administered in the feed of rats of the Sprague-Dawley derived, CD strain. Groups of 10 male and 10 female rats were maintained on diets containing 0, 4000, 12,000, 25,000, or 50,000 ppm. These dietary levels are calculated to provide an estimated daily intake of 0, 200, 600, 1250 or 2500 mg/kg bw, respectively, of sodium 2-(4-methoxyphenoxy)propanoate (FDA, 1993). Reduced body weight gain was associated with reduced food consumption at 1250 or 2500 mg/kg bw dietary levels. Water consumption was increased in both sexes of the two highest exposure groups. Elevated plasma alkaline phosphatase (ALP) and asparatate aminotransferase (AST) levels were reported for half of the animals in the 2500 mg/kg bw per day group in both sexes. The increases were within normal range for females given 1250 or 2500 mg/kg bw per day. Male and female rats at 2500 mg/kg bw per day showed lower blood reticulocyte counts, but there were no other hematological changes associated with the treatment. There was no evidence of pathology associated with administration of the test substance. The author concluded that the data from this study show that a dietary intake levels in the range of 1000–1250 mg/kg bw would be suitable for a subsequent 90-day study (Hill and Wood, 1983).

In the 90-day study, groups of 20 male and 20 female CD rats received sodium 2-(4-methoxyphenoxy)propanoate in the diet at levels of 0, 5000, 10,000, or 20,000 ppm, which corresponds to estimated daily intake levels of 0, 250, 500, or 1000 mg/kg bw, respectively (FDA, 1993). Body weight gain was reduced in males (17%) and females (20%) in the 1000 mg/kg bw per day intake level group. Males in the 500 mg/kg bw per day group also showed a 7% reduction in bodyweight gain. The authors did not consider the figures to be statistically significant. Hematological examination and blood chemical determinations revealed normal values. There were no treatment related changes in urine cellularity, volume, specific gravity, pH; protein; bilirubin; ketone, blood, glucose and sodium concentration. There was however a reduction in urine potassium levels in males at the 1000 mg/kg bw per day dietary level during weeks 6 and 13, and in females fed 500 and 1000 mg/kg bw per day during week 13 of the treatment period. There were no microscopic pathology changes related to administration of the test substance (Hill and Wood, 1986). The NOAEL of 250 mg/kg bw per day is greater than 2,000,000 times the daily per capita intake<sup>4</sup> (“eaters

only”) of 0.09 µg/kg bw from use of sodium 2-(4-methoxyphenoxy)propanoate as a flavoring ingredient in the USA (see Table 1).

#### 4.3. Long-term studies of toxicity and carcinogenicity

##### 4.3.1. Phenethyl alcohol (no. 1)

Groups of male and female Wistar albino rats (20/sex/group) were given a mixture of compounds dissolved in tap water as their only drinking source for 56 weeks. This mixture included 6000 mg/kg bw ethyl alcohol (6%), 4 mg/kg bw ethyl acetate (0.004%), 120 mg/kg bw isoamyl alcohol (0.12%), 120 mg/kg bw phenethyl alcohol (0.12%), 200 mg/kg bw isobutyl alcohol (0.2%), and 200 mg/kg bw acetic acid (0.2%). A control group of 20 rats/sex was maintained on tap water only. All groups of rats were fed a stock ration ad libitum and their weights were recorded weekly. The activity of alcohol dehydrogenase (ADH), glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and the protein content were determined at two-four week intervals in the livers of rats. At study termination, liver, kidney, heart, spleen, and lung were examined histologically. There was a slight non-statistically significant decrease in the mean body weight of both groups at 28–29 weeks compared to 53–56 weeks. There was no difference in absolute or relative liver weight between the test and control groups. There was a slight increase in GOT activity between 28 and 56 weeks in both test groups. No significant abnormalities were observed in any of the organs examined. Six animals contracted pneumonia and were discarded. Pneumonia was common in the rats at termination, equally distributed in all groups including the control group. The authors concluded that the mixture of chemicals tested did not produce any effects in the parameters tested (Johannsen and Purchase, 1969).

#### 4.4. Genotoxicity studies

Mutagenicity/genotoxicity testing has been performed on seven representative phenethyl alcohol derivatives and three phenoxyethyl alcohol derivatives. The results of these tests are summarized in Table 4 and described below.

##### 4.4.1. In vitro

Short-term genotoxicity assays indicate that phenethyl alcohol and related compounds have no or low mutagenic/genotoxic potential. Phenethyl alcohol, phenylacetaldehyde, phenylacetic acid, ethyl phenylacetic acid, isobutyl phenyl acetate, isoamyl phenylacetate and *p*-tolylacetaldehyde have been tested for mutagenicity by the Ames assay using different strains of *Salmonella typhimurium* (e.g., TA98, TA100, TA1535,

TA1537, TA1538) in the presence or absence of S-9 fraction. All the compounds tested using the Ames test at concentrations up to 5000 µg/ml or 50 mg/plate were found to be negative (Florin et al., 1980; Kato et al., 1989; Heck et al., 1989; Ishidate et al., 1984; Fujita et al., 1994; Oda et al., 1979; Rapson et al., 1980).

There was no evidence of mutagenicity in the standard Ames assay when various strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) were incubated with up to 3600 µg ethyl (*p*-tolyl)oxyacetate/plate (Wild et al., 1983), 3600 µg 2-phenoxyethyl isobutyrate/plate (Wild et al., 1983) or up to 5000 µg sodium 2-(4-methoxyphenoxy)propanoate/plate (Varley and Trenchard-Morgan, 1985), with and without metabolic activation.

The results of spore rec assay using *Bacillus subtilis* H17 and M45 for ethyl phenylacetate and isoamyl phenylacetate are not conclusive. In one study, 21 µl/disk of ethyl phenylacetate was incubated with *B. subtilis* H17 and M45 in a spore-rec assay. Based on the difference in the zone of inhibition (0.8 mm) between the two strains, ethyl phenylacetate was concluded to be not mutagenic (Oda et al., 1979). In a second study at a lower concentration, 20 µl/disk of ethyl phenylacetate was incubated with *B. subtilis* H17 and M45 in the same spore-rec assay. The difference in the zone of inhibition (>8 mm) between the two strains was reported as positive evidence of mutagenicity (Yoo, 1986). Contradictory data are also reported for isoamyl phenylacetate. When 20 µl/disk of isoamyl phenylacetate was incubated with *B. subtilis* H17 and M4, a weak (2–5 mm zone difference) positive response was reported in one study (Oda et al., 1979) and a negative response in the other (Yoo, 1986).

Phenylacetaldehyde and *p*-tolylacetaldehyde were studied in an *E. coli* system at an unspecified concentration (Kato et al., 1989 and Ohshima et al., 1989). Phenylacetaldehyde was tested in *E. coli* strain WP2uvrA/pKM101 by the preincubation assay (Kato et al., 1989). *p*-Tolylacetaldehyde was studied in *E. coli* strain PQ37 (Ohshima et al., 1989). There was no evidence of mutagenicity in either assay. In another *E. coli* assay, 200–1600 µg/plate of ethyl phenylacetate showed no evidence of mutagenicity when incubated with *E. coli* WP2uvrA (Yoo, 1986). The contradictory results reported in the two studies (Yoo, 1986; Oda et al., 1979), the negative result in the WP2 uvrA strain, and the fact that phenethyl alcohol exhibits bacteriocidal activity in *E. coli* (Brunner and Treick, 1982) support the conclusion that the results of spore rec assay using *B. subtilis* H17 and M45 should not be included in the overall assessment of genotoxic potential of these substances.

No increase in a sister chromatid exchange (SCE) was observed when human whole-blood lymphocyte cultures were exposed to 2-phenethyl alcohol for 72 h (Norppa and Vainio, 1983). Also, no increase in unscheduled DNA synthesis (UDS) was noted when rat hepatocytes

Table 4  
In vitro and in vivo genotoxicity studies on phenethyl alcohol, aldehyde, acid and related acetals and esters used as flavoring ingredients

#	Substance name	Test system in vitro	Test object	Maximum concentration of substance	Result	Reference
<i>In vitro</i>						
1	Phenethyl alcohol	Ames test	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative <sup>a</sup>	Florin et al. (1980)
1	Phenethyl alcohol	Sister chromatid exchange assay		Not specified	Negative	Norppa and Vainio (1983)
16	Phenylacetaldehyde	Ames test	<i>S. typhimurium</i> TA98, TA100, TA104	Not specified	Negative <sup>a</sup>	Kato et al. (1989)
16	Phenylacetaldehyde	Bacterial mutation	<i>Escherichia coli</i> WP2uvrA/pKM101	Not specified	Negative <sup>a</sup>	Kato et al. (1989)
21	Phenylacetic acid	Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	1000 µg	Negative <sup>a</sup>	Heck et al. (1989)
21	Phenylacetic acid	Unscheduled DNA synthesis	Rat hepatocytes	500 µg	Negative	Heck et al. (1989)
21	Phenylacetic acid	Mouse Lymphoma Forward Mutation Assay	Mouse lymphoma L5178Y tk ± cells	1500 µg	Negative <sup>a</sup>	Heck et al. (1989)
23	Ethyl phenylacetate	Ames test	<i>S. typhimurium</i> TA92, TA94, TA98, TA100, TA1535, and TA1537	5 mg	Negative <sup>a</sup>	Ishidate et al. (1984)
23	Ethyl phenylacetate	Bacterial mutation assay	<i>E. coli</i> WP2uvrA(trp-)	200–1600 µg/plate	Negative <sup>b</sup> below	Yoo (1986)
23	Ethyl phenylacetate	Rec assay	<i>Bacillus subtilis</i> H17(rec+)	21 µg/disk	Negative <sup>b</sup>	Oda et al. (1979)
23	Ethyl phenylacetate	Rec assay	and M45(rec-)			
23	Ethyl phenylacetate	Rec assay	<i>B. subtilis</i> H17(rec+) and M45(rec-)	20 µL/disk	Positive <sup>b</sup>	Ishidate et al. (1984)
23	Ethyl phenylacetate	Chromosomal aberration	Chinese hamster fibroblast cells	1 mg/mL	Negative <sup>a</sup>	Fujita et al. (1994)
27	Isobutyl phenylacetate	Ames test	<i>S. typhimurium</i> TA97 and TA104	0.1 mg/plate	Negative <sup>a</sup>	Oda et al. (1979)
28	Isoamyl phenylacetate	Ames test	<i>S. typhimurium</i> TA98 and TA100	10 µg/plate	Negative <sup>a,b</sup>	Oda et al. (1979)
28	Isoamyl phenylacetate	Ames test	<i>S. typhimurium</i> TA98 and TA100	50 µg/plate	Lethal <sup>a,b</sup>	Oda et al. (1979)
28	Isoamyl phenylacetate	Rec assay	<i>B. subtilis</i> H17(rec+) and M45(rec-)	20 µg/disk	Positive <sup>b</sup>	Oda et al. (1979)
28	Isoamyl phenylacetate	Rec assay	<i>B. subtilis</i> H17(rec+) and M45(rec-)	20 µL/disk	Negative <sup>b</sup>	Yoo (1986)
37	<i>p</i> -Tolylacetaldehyde	Ames test	<i>S. typhimurium</i> TA100	1000 µg/plate	Negative	Rapson et al. (1980)
37	<i>p</i> -Tolylacetaldehyde	Bacterial mutation	<i>E. coli</i> PQ37	Not specified	Negative	Ohshima et al. (1989)
41	Ethyl ( <i>p</i> -tolylloxy)acetate	Ames test	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	3600 µg/plate	Negative <sup>a</sup>	Wild et al. (1983)
42	2-Phenoxyethyl isobutyrate	Ames test	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	3600 µg/plate	Negative <sup>a</sup>	Wild et al. (1983)
43	Sodium 2-(4-methoxyphenoxy)propanoate	Ames test	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537	5000 g/plate	Negative <sup>a</sup>	Varley and Trenchard-Morgan (1985)
<i>In vivo</i>						
42	2-Phenoxyethyl isobutyrate	Micronucleus induction assay	Mouse bone marrow cells	1875 mg/kg bw	Negative <sup>c</sup>	Wild et al. (1983)
43	Sodium 2-(4-methoxyphenoxy)propanoate	Micronucleus induction assay	Mouse bone marrow cells	2000 mg/kg bw	Negative	Aisquith and Pickering (1985)
	Isoeugenol phenylacetaldehyde	Micronucleus induction assay	Mouse bone marrow cells	2820 mg/kg bw	Negative <sup>c</sup>	Wild et al. (1983)
	Isoeugenol phenylacetaldehyde	Base test	<i>Drosophila melanogaster</i>	25 mM	Negative	Wild et al. (1983)

<sup>a</sup> With and without metabolic activation.

<sup>b</sup> Foreign language article, data available from English abstract and/or tables.

<sup>c</sup> Administered via intraperitoneal route.

were incubated with phenylacetic acid (Heck et al., 1989). Incubation of 1000 µg/ml of ethyl phenylacetate with Chinese hamster fibroblast cells for 48 h produced 3% cells with chromosomal aberrations. Based on the study guideline that assays showing greater than 10% aberrations were considered positive in the chromosomal aberration assay, ethyl phenylacetate was not mutagenic in this assay (Ishidate et al., 1984).

#### 4.4.2. *In vivo*

Results of *in vivo* genotoxicity assays were negative for a phenylacetate ester and 2-phenoxyethyl isobutyrate, and for sodium 2-(4-methoxyphenoxy)propanoate. There was no significant increase in micronucleated polychromatic erythrocytes in mice following ip injections of 625–1875 mg 2-phenoxyethyl isobutyrate/kg bw (Wild et al., 1983). In another micronucleus test, sodium 2-(4-methoxyphenoxy)propanoate was given by gavage in doses of 500–2000 mg/kg bw to mice. A higher number of micronucleated polychromatic erythrocytes were reported only at the 500 mg/kg bw dose in males at 24 h. The authors did not consider this to be of any biological significance since (1) the positive result was due to one male animal, (2) the controls had somewhat lower numbers of micronuclei than usually seen in males at 24 h, and (3) there was a dose-related decrease in PCE/NCE (polychromatic erythrocytes/normochromatic erythrocytes) ratios for females sacrificed at 48 h and both males and females sacrificed at 72 h for the 1000 and 2000 mg/kg bw dose groups (Aisquith and Pickering, 1985).

A structurally related phenyl acetate ester, isoeugenol phenylacetate, was also the subject of a mouse micronucleus assay. Groups of NMRI male and female mice were injected ip with dose levels of 1128–2820 mg/kg bw of isoeugenol phenylacetate. After 30 h they were sacrificed and the mean number of micronucleated polychromatic erythrocytes per 1000 normochromatic erythrocytes was measured. There was no evidence of an increase in micronucleated polychromatic erythrocytes at any dose level (Wild et al., 1983). Also, there was no evidence of an increase in sex-linked lethal mutations when *Drosophila melanogaster* were fed a solution of 25 mM isoeugenol phenylacetate for 3 days (Wild et al., 1983).

#### 4.4.3. *Conclusion*

Overall, the results from the *in vitro/in vivo* studies indicate that this group of compounds and related flavoring substances are neither mutagenic nor genotoxic. Two of the three positive *in vitro* tests reported for this group of flavoring ingredients were directly contradictory. The uniformly negative *in vivo* genotoxicity assays confirm the results of the *in vitro* assays that phenethyl alcohol and phenoxyethyl alcohol and their structurally related aldehydes, acids, esters, and acetals

show no genotoxic potential. This is supported by metabolic data that indicated that the members of this group are rapidly converted to the corresponding acid that is readily excreted by humans in free or conjugated form.

#### 4.5. *Other relevant studies*

##### 4.5.1. *Phenethyl alcohol (no. 1) and phenylacetic acid (no. 21)*

Screening studies performed by one group of investigators during the 1980s reported that low dose levels of phenethyl alcohol and phenylacetic acid produce teratogenic effects resembling Fetal Alcohol Syndrome (Mankes et al., 1983, 1984, 1985). These results are contradicted by the results of another study in which phenethyl alcohol given to pregnant rats at high doses at critical periods of embryogenesis does not cause any visible anomalies in embryonal development (Burdock et al., 1987). More recent comprehensive studies conducted with high dose levels of phenethyl alcohol given either by oral (Bottomley et al., 1987) or dermal (Palmer et al., 1986) routes of exposure have demonstrated that this group of substances exhibits a very low order of reproductive and developmental toxicity.

In the original studies (Mankes et al., 1983, 1984, 1985), pregnant Long Evans rats were given oral doses of 4.3, 43 or 432 mg/kg of phenethyl alcohol by gavage during days 6–15 of gestation. The average birth weight and pup size of all treated groups were significantly lower than those of the control group, but the change was not dose related. In fact, birth weights were greater in the mid-dose group than in controls. Mean litter size was greater in the high dose group (13) than in either the two lower doses (9) or controls (12). Also, there was no embryoletality in the high dose group while embryoletality was 18% at 43 mg/kg and 10% at 4.3 mg/kg. The authors reported a clear dose related increase in the percentage of malformations in live offspring (100% at the 432 mg/kg level, 93% at 43 mg/kg and 50% at 4.3 mg/kg). Malformations were mainly in ocular malformation, neural tube defects, hydronephrosis and limb defects (Mankes et al., 1983). In abstracts of subsequent studies reported by the same authors (Mankes et al., 1984, 1985), dose levels of phenethyl alcohol equivalent to 0.02% and 24% of the oral LD<sub>50</sub> were administered to pregnant Long Evans rats. Intrauterine growth retardation (birth weight reductions) and embryoletality were reported at all dose levels. These observations are inconsistent with those of the original study.

Microencapsulated phenethyl alcohol was administered in the diet to nulliparous Sprague–Dawley rats at levels of 1000, 3000 and 10,000 ppm during the period of major organogenesis (days 6–15 post coitum). Achieved intake of phenethyl alcohol was calculated for dams during the treatment period, values were

adjusted to take account of the assayed content of test material in the microcapsules used and indicated that the actual intake was about 83, 266, and 799 mg/kg per day for groups designated 1000, 3000 and 10,000 ppm, respectively. Effects in the dams were limited to transient suppression of maternal food consumption, resulting in slight weight loss during the first two days of treatment at the high dose level only. Effects in the offspring were minimal with malformations limited to a total of five pups (three in control and two in the mid-dose). The number and type of visceral anomalies were comparable between control and treated groups. Of fetuses exhibiting skeletal anomalies, an increased incidence of incomplete calcification was noted only in the 799 mg/kg group. This was considered to be a possible consequence of the earlier impairment of maternal weight gain. There were no obvious differences between control and treated groups for skeletal variants, number of live young, embryoletality, implants, litter weight, mean fetal weight or sex ratio (Bottomley et al., 1987). The NOAEL of 266 mg phenethyl alcohol/kg bw per day for teratogenic effects is at least 40,000 times the estimated the daily per capita intake<sup>4</sup> (“eaters only”) of 6 µg/kg bw from use of phenethyl alcohol as a flavoring ingredient in the USA (see Table 1).

The effect of phenethyl alcohol on pregnancy of rats was studied (Palmer et al., 1986). Phenethyl alcohol was applied topically at concentrations of 0, 0.14, 0.43 or 1.40 ml/kg during Day 6–15 of pregnancy. The dermal levels of exposure are approximately equal to 0, 140, 430, and 1400 mg/kg, respectively, and were chosen so that the intermediate dose was roughly equivalent to the highest dosage used in a previous oral study (Mankes et al., 1983). The highest dose was designed to extend the range in case of differential absorption by the dermal route. An occlusive bandage was used to minimize oral ingestion of the test material and this was put on immediately after dosing and not removed until the following day. The animals were killed on day 20 of pregnancy and in utero development assessed by determination of litter values and examination of the fetuses for soft tissue and skeletal changes. At 1.40 ml/kg per day, there was clear evidence of both maternal toxicity including lethality, suppression of mean food intake and growth rate and embryo-fetal toxicity indicated by resorption, embryo-fetal wastage, reduction in mean litter size, depression of fetal weight, a wide range of soft tissue and skeletal changes, incomplete ossification. For the latter, the pattern of response and the comprehensive nature of the morphological changes were considered by the authors, to be beyond those that would occur merely as a secondary consequence of the maternal response. In this study, 0.43 ml/kg per day was considered close to the threshold of maternal toxicity but while there was no evidence of an adverse effect on litter values, there was a dose-dependent increase in some of the morphological

changes recorded in fetuses. A dermal concentration of 0.14 ml/kg per day did not elicit any adverse effects in the litter values. Based on the overt effects on fetal development at the higher dermal concentrations, the slight differences in morphological changes between the 0.14 ml/kg concentration and controls (cervical rib(s) thoracic vertebral irregularities), the authors concluded that the 0.14 ml/kg dermal concentration level is a threshold for developmental toxicity in the rat.

#### 4.5.2. Phenoxyethyl alcohol

Phenoxyethyl alcohol was continuously administered to male and female CD-1 mice in the feed at levels of 0.25, 1.25 and 2.5% for 18 weeks. These dietary levels correspond to an estimated daily intake of 375, 1875 and 3700 mg/kg bw of phenoxyethyl alcohol, respectively (FDA, 1993). There was no effect on the number of pairs able to reproduce at least one litter or the sex of pups born alive in this  $F_0$  continuous breeding study. Reductions in litter size and fewer live pups/litter were reported for the highest dose level when compared to the control group and the two lower doses. There was also a dose-related decrease in live pup weight for the middle and high dose pairs. Live male pup weight ( $F_1$ ) was also decreased for the low dose pairs ( $F_0$ ), which correspond to a decrease in food consumption through week 18 reported for the low dose  $F_0$  pairs. Necropsy of control and high dose  $F_0$  mice revealed decreased body weights (6% less) and increased absolute liver weights (14% higher) for treated high dose males compared to control animals. Absolute liver weight was also increased in high dose female mice. No effects on other organ weights were reported and sperm indices revealed no effects of test compound.

In the second part of this study, phenoxyethyl alcohol was administered after weaning to the last  $F_1$  litter from all dose levels at the same dose levels provided to the  $F_0$ . There was a dose-related decrease in body weight from birth to post-natal day 74 at the 1.25% and 2.5% dose levels in the  $F_1$  generation. Increased mortality was reported for the middle and high dose levels from weaning until post-natal day 74, with marked effects in the high dose group (6/56 pups surviving until mating on post natal day 74). Consequently the high dose group was not included in the rest of the study. Mating of the  $F_1$  generation revealed no treatment related effects on  $F_2$  litter size or sex ratio. Live pup weight ( $F_2$  generation) was decreased at the 1.25% dose level by 7%. Necropsy of the control and 1.25%  $F_2$  mice revealed decreased body weight (13% less), decreased absolute testis weight (16% less), decreased relative seminal vesicles weight (14% less) in the male mice and decreased body weight (7%) in the female mice compared to control animals. No effects were reported on epididymal sperm concentration, motility or morphology

(National Toxicology Program, 1984). The decrease in body weight reported for the live male pups ( $F_1$ ) born to the low dose pairs ( $F_0$ ) was barely statistically significant and its biological relevance is questionable given it was observed in the male live pups only (National Toxicology Program, 1984). Given these results, it may be concluded that the NOAEL under conditions of this study for reproductive effects is 0.25% phenoxyethyl alcohol which corresponds to a dose level of 375 mg/kg bw per day.

#### 4.5.3. Phenoxyacetic acid (no. 40)

Phenoxyacetic acid was administered by gavage to pregnant mice on one of gestation days 8–15 at a dose of 800–900 mg/kg bw or on three consecutive days (7–9, 10–12, or 13–15) at a dose of 250–300 mg/kg bw. On gestation day 18, treated females were sacrificed. Uteri were exposed and examined to determine the number of live, dead, and resorbed fetuses. Live fetuses were examined for gross external malformation and weighed. Fetuses from each litter were further examined for visceral abnormalities and skeletal malformations. No statistically significant differences between control and treated animals were reported (Hood et al., 1979).

#### 4.5.4. Sodium 2-(4-methoxyphenoxy)propanoate (no. 43)

An investigation of the toxicity of sodium 2-(4-methoxyphenoxy)propanoate in the pregnant rat was conducted to determine suitable dose levels for a second study. Groups of five time-mated Charles River female rats were dosed once daily by gavage with suspensions of the test substance from day 6 to 15 of pregnancy. The dose levels were 0, 100, 500, 1000, and 5000 mg/kg per day. Surviving animals were sacrificed on day 20 of pregnancy and gross necropsy was performed. There were no observed adverse effects at 100 and 500 mg/kg per day doses. At 1000 and 5000 mg/kg per day dose levels, there were no treatment related maternal clinical signs of toxicity, mortality or abnormal necropsy findings. Embryonic and fetal development was not affected by treatment with the test substance. Maternal bodyweight gain and food consumption were adversely affected at 1000 mg/kg per day. Food consumption was significantly lower ( $P < 0.01$ , Student's *t*-test) than the controls throughout the dosing period. Body weight gain at this level was lower than the controls from the onset of dosing. These differences were considered to be treatment related even though statistical significance was not achieved (probably because of the small group size). However, there was no mortality or adverse necropsy findings. There was a trend towards a slightly lower fetal bodyweight (not statistically significant), which may be related to the reduced maternal food consumption. Two animals in the 5000 mg/kg per day group were found dead on day eight of pregnancy, having re-

ceived two doses of sodium 2-(4-methoxyphenoxy)propanoate. The remaining three animals were reported to be in poor condition on day seven, having received one dose, and were sacrificed. The author also reported brown stains on the fur and piloerection in most animals. Additionally, there were lesions of the stomach mucosa, which included inflammation, hemorrhage or ulceration in four animals. From this data, the author concluded that 1000 mg/kg per day is a suitable highest dose level for the main teratology study. They realize that although slight toxicity was observed at this level, there were no maternal deaths (Ridgway, 1985).

The following study was designed to investigate the effects of sodium 2-(4-methoxyphenoxy)propanoate on the embryonic and fetal development of the rat when administered during the period of organogenesis. Groups of 24 time-mated female rats of the CD strain were dosed once daily by gavage with suspensions of sodium 2-(4-methoxyphenoxy)propanoate from days 6–15 inclusive of pregnancy. The dose levels were 0, 100, 300, and 1000 mg/kg per day. The animals were sacrificed on day 20 of pregnancy and necropsy performed. The fetuses were subjected to detailed external, visceral and skeletal examinations. There were no maternal deaths caused by 2-(4-methoxyphenoxy)propanoate. Treatment related clinical signs were reported at the 1000 mg/kg per day dose level only. The test substance caused a transient slight retardation in maternal body weight gain at all levels at the start of the dosing period. The statistically significant difference of  $P < 0.05$  and  $P < 0.001$  occurred on days 6–7 for the 100 and 300 mg/kg per day dose and days 6–7 and 6–9 for the 1000 mg/kg per day dose, respectively. Maternal food consumption was not affected. There were no abnormal maternal necropsy findings and no evidence of teratogenicity or embryo/fetotoxicity. Implantation, post-implantation loss, fetal weight, sex ratio and incidence of fetal abnormalities were not affected by treatment with the test substance. The author concluded that sodium, 2-(4-methoxyphenoxy)propanoate administered during the period of organogenesis showed no evidence of teratogenicity or embryo/fetotoxicity (Ridgway, 1986).

#### 4.5.5. Phenethyl alcohol (no. 1) and phenethyl acetate (no. 3)

Male rats were given 50.8 mg/kg bw of phenethyl alcohol or 73 mg/kg bw phenethyl acetate daily via gavage for four months (Zaitsev and Rakhmanina, 1974). At day 40 and 140 cholinesterase activity, serum enzymes such as aldolases, amylase, sorbitol dehydrogenase, aspartate (AST) and alanine aminotransferase (ALT), content of thiol groups, and total serum protein in the blood serum were monitored. The treatment with the phenethyl alcohol resulted in an increase in cholinesterase and ALT activities and increased content of thiol group in the blood at day 40. Serum protein content

decreased by 7.15 g/100 ml after 40 days of treatment with phenethyl alcohol and phenethyl acetate. The treatment with phenethyl acetate also resulted in an increase in cholinesterase activity. No significant changes were reported at day 140.

#### 4.5.6. Phenylacetic acid (no. 21)

A rapid screening protocol was used to evaluate the potential immunotoxicity of a large number of flavoring ingredients, including phenylacetic acid. This protocol incorporated key elements of the National Toxicology Program's (NTP) tier testing strategy, including body weight, lymphoid organ weight and cellularity, as well as functional tests of both humoral, antibody plaque-forming cell (PFC), response to sheep erythrocytes and cell-mediated, *Listeria monocytogenes* bacteria challenge, immunity. The test compounds were administered orally to groups of 10–20, 6–8 week old female CD-1 or B6C3F<sub>1</sub> mice. Decreases in body weight, spleen and/or thymus weights or a decrease in spleen cellularity may be indicative of depressed immune competence. Quantitation of the number of antibody-producing plasma cells, the end result of antigen driven B-cell differentiation, following immunization with a T-cell dependent antigen such as SRBC (Sheep Red Blood Cells) provides information about the functional integrity of, and communication among, several cell populations important in antibody-mediated immunity. These cells include T-cells, B-cells, and macrophages. The *Listeria* model system was selected because both the pathogenesis of this infection and the host's immune response are similar in mice and humans. The model system is extremely useful to assess immunosuppression since both immunocompetent T-cells and macrophages are required to control infection and supply protective immunity. Phenylacetic acid at doses as high as 100 mg/kg per day produced no effect on spleen weight, thymus weight, spleen cellularity, anti-SRBC PFC response and *Listeria* mortality (Vollmuth et al., 1989).

Phenylacetic acid was shown to be devoid of any immunomodulatory effects (Gaworski et al., 1994). The immunotoxicity tier testing strategy was used to evaluate the effects of 35 commonly used food-flavoring ingredients on humoral and cell-mediated immune responses. Female CD-1 or B6C3F<sub>1</sub> mice were administered 250, 500 or 1000 mg phenylacetic acid/kg bw intragastrically on a daily basis for five days. *Listeria monocytogenes* bacterial challenge was conducted to assess cell-mediated immunity. Humoral immunity was measured by the antibody PFC response to sheep erythrocytes. Body weights, lymphoid organ weights and spleen cellularity were also measured. Cyclophosphamide served as an immunosuppressive positive control agent. The results indicated that the phenylacetic acid did not modulate the cell-mediated or humoral response at any dose level tested.

#### 4.5.7. Sodium 2-(4-methoxyphenoxy)propanoate (no. 43)

The effects of sodium 2-(4-methoxyphenoxy)propanoate and ibuprofen on prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) levels and leucocyte counts in subcutaneously implanted carrageenan-soaked sponges in the rat were studied. Oral administration of sodium 2-(4-methoxyphenoxy)propanoate at 3, 10, 30, and 100 mg/kg bw in male and female rats did not alter leucocyte migration into subcutaneously implanted carrageenan-soaked sponges. In general, the test substance had no significant or dose-related effect on the PGE<sub>2</sub> content from the sponges. However, there was a marked increase in the PGE<sub>2</sub> levels in male rats treated with sodium 2-(4-methoxyphenoxy)propanoate at 30 mg/kg bw and a significant reduction in PGE<sub>2</sub> levels in females treated with the test substance at 100 mg/kg bw. The reference standard ibuprofen at 100 mg/kg bw did not alter leucocyte counts but significantly reduced the PGE<sub>2</sub> content of the sponges in both male and female rats. The authors conclude that this reduction is consistent with the anti-inflammatory action of ibuprofen and that sodium 2-(4-methoxyphenoxy)propanoate could have a slight inhibitory effect on prostaglandin biosynthesis (Algate et al., 1986a,b).

The next study by the same authors looked at the effects of orally administered sodium 2-(4-methoxyphenoxy)propanoate and ibuprofen on the carrageenan-induced edema in rats. They found that sodium 2-(4-methoxyphenoxy)propanoate produced a statistically significant inhibition of the carrageenan-induced edema at the 10, 30, and 100 mg/kg dose levels. This anti-inflammatory effect was apparent at the 3-h (30 and 100 mg/kg) and 6-h (10, 30 and 100 mg/kg) observation times. Ibuprofen caused a statistically significant inhibition in the edema at all three intervals with peak activity recorded at the 6-h observation time. The authors concluded that sodium 2-(4-methoxyphenoxy)propanoate and the reference standard ibuprofen have activities which suppress the inflammatory response induced by the irritant carrageenan; however, the activity demonstrated by 2-(4-methoxyphenoxy)propanoate was reported to be of minor consequence (Algate et al., 1986a).

The effects of sodium 2-(4-methoxyphenoxy)propanoate (lactisole) on glucose tolerance and insulin secretion in healthy human subjects were investigated. Eight healthy male volunteers received on two separate occasions, at least seven days apart, either a glucose solution (75 g glucose) or a glucose solution containing 80 mg of the test substance. Blood samples were collected in 30-min intervals for 3 h, and then hourly, until 5 h after the glucose load. Glucose, insulin, C-peptide and glucagon levels were assayed. The data indicate that lactisole had no effect on glucose metabolism, insulin, C-peptide or glucagon secretion under the conditions of the study (Marks, 1988).



## 5. Recognition of GRASr status

The group of phenethylalcohol, aldehyde, acid, and related acetals and 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 phenethylalcohol, aldehyde, acid, and related acetals and esters was reaffirmed as GRAS (GRASr) based, in part, on their self-limiting properties as flavoring substances in food; their rapid absorption, metabolic detoxication, and excretion in humans and other animals; their low level of flavor use; the wide margins of safety between the conservative estimates of intake and the no-observed-adverse effect levels determined from subchronic and chronic studies and the lack of significant genotoxic and mutagenic potential. This evidence of safety is supported by the fact that the intake of phenethyl alcohol, aldehyde, acid, and related acetals and esters as natural components of traditional foods is greater than their intake as intentionally added flavoring substances.

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