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**The Flavor and Fragrance High Production Volume
Consortia**

The Aromatic Consortium

Final Revised Test Plan For Phenethyl alcohol

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Phenethyl alcohol

CAS No. 60-12-8

FFHPVC Aromatic Consortium Registration Number

**Submitted to the EPA under the HPV Challenge Program by:
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1 SUMMARY OF KEY HAZARD DATA FOR PHENETHYL ALCOHOL

Substance/Surrogate ¹	Endpoint	Value/Range ²	Reference
Physical Properties			
Phenethyl alcohol	Vapor pressure	0.097 mm Hg (25°C)	Vuilleumier, 1995
Phenethyl alcohol	Partition Coefficient	1.36	Sangster, 1989
Environmental Fate			
Phenethyl alcohol	Biodegradation ³	+ (OECD 301B)	Quest, 1994
Ecotoxicity			
Phenethyl alcohol	Fish	96-hr LC50=215 mg/L	BASF, 1998c
Phenethyl alcohol	Aquatic Invertebrates	48-hr EC50=287 mg/L	BASF, 1998a
Phenethyl alcohol	Aquatic Plant	72-hr EC50=490 mg/L	BASF, 1998b
Human Health			
Phenethyl alcohol	Repeat Dose ⁴ (route)	500 mg/kg (dermal, 90d)	Owston, 1981
Phenylacetic acid	Reproduction (route)	300 mg/kg (gavage, 28d)	Vollmuth, 1995
Phenethyl alcohol	Developmental (route)	266 mg/kg (diet, 13 wks) 143 mg/kg (dermal, 13 wks)	Bottomley, 1987 Palmer, 1986
Genotoxicity⁵			
Phenylacetic acid	<i>In vitro</i>	-	Heck, 1989; Norppa, 1983
Phenethyl alcohol			Wild, 1983
Phenethyl alcohol, 2-methyl			
Phenylacetaldehyde, 2-methyl	<i>In vivo</i>	-	Wild, 1983
Phenylacetic acid, isoeugenyl ester			

¹ Surrogate is a structurally related substance include a metabolic product or precursor of the named substance

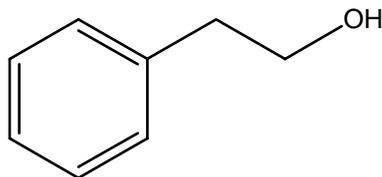
² Experimental value or values for a substance or group of substances in the chemical category

³ not biodegradable, (-); readily biodegradable, (+); ready and ultimately biodegradable, (++)

⁴ Value is the NOAEL or NOEL(route, duration)

⁵ (-), no significant genotoxic potential; (=/-), equivocal evidence; (+), positive evidence of genotoxicity

2 IDENTITY OF SUBSTANCE



Phenethyl alcohol

$C_8H_{10}O$

CAS No. 60-12-8

Synonyms:

Benzeneethanol

Ethanol, 2-phenyl-

(2-Hydroxyethyl)benzene

PEA

beta-Phenethyl alcohol

2-Phenylethanol

3 CATEGORY ANALYSIS

3.1 Introduction

In October of 1999, members of the United States flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical Right-to-Know Program. Members of these consortia are committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The Aromatic Consortium, as a member of the FFHPVC serves as an industry consortium to coordinate testing activities for aromatic substances under the Chemical Right-to-Know Program. Fourteen (14) companies are current members of the Aromatic Consortium. The Aromatic Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and, where needed, conducting additional testing. The test plan, category analysis and robust summaries presented below are the first phase of the Aromatic Consortium's commitment to the Chemical Right-to-Know Program.

3.2 Background Information

Phenethyl alcohol (PEA) or 2-phenylethanol is a simple aromatic primary alcohol. It is currently permitted by the U.S. Food and Drug Administration (FDA) for direct addition to food for human consumption as a flavoring substance and is considered by the Flavor and Extract Manufacturers' Association (FEMA) Expert Panel to be "generally recognized as safe" (GRAS) for its intended use as a flavoring substance [Hall, 1960]. In addition, a group of 42 phenethyl alcohol, phenylacetaldehyde, phenylacetic acid and related phenethyl esters and acetals have been approved for use as flavoring agents by both the FDA (CFR 172.515) and the World Health Organization's Joint Expert Committee on Food Additives [JECFA, 2002].

Phenethyl alcohol occurs naturally in more than 200 foods [Maarse *et al.*, 2000]. Quantitative natural occurrence data indicate that oral intake of phenethyl alcohol occurs predominantly from consumption of foods such as beer, wine, whiskey, olive oil, grapes, green and black tea, apple juice and coffee [Stofberg and Grundschober, 1987]. It has been estimated that approximately 700,000 kg of phenethyl alcohol is consumed annually as a natural component of foods.

Phenethyl alcohol is the main component of rose oil and is also found in neroli oil, ylang-ylang oil, carnation oil, and geranium oils. Therefore, phenethyl alcohol is used as a fragrance ingredient because of its rose-like odor in a wide variety of consumer products ranging from hydroalcoholic (typically in 70% ethanol) type products such as colognes and *eaux de toilette*, to cosmetics, soaps and detergents [Opdyke, 1975]. Such uses consumed approximately 1,000,000 pounds (lbs)/year in 1975 [Opdyke, 1975].

Phenethyl alcohol is also used as a flavor ingredient with an annual volume of use reported to be 2500 kg/year in the USA and 9900 kg/year in Europe [Lucas *et al.*, 1999; IOFI, 1995]. Therefore, greater than 99% of oral intake of phenethyl alcohol occurs from consumption of food containing naturally occurring phenethyl alcohol compared to the intake from its intentional use as a flavoring substance.

Phenethyl alcohol may be synthesized by a variety of methods including a Friedel-Crafts reaction of benzene and ethylene oxide, and by hydrogenation of styrene oxide [Bauer and Garbe, 1985].

3.3 Biochemistry of Phenethyl Alcohol and Derivatives

3.3.1 Introduction

Although phenethyl alcohol is the only substance being considered in this test plan and robust summaries, data on phenethyl esters are relevant to the hazard assessment in that phenethyl esters have been shown to readily hydrolyze to phenethyl alcohol *in vitro* and *in vivo* (see below). In addition, data is presented to demonstrate that phenethyl alcohol is rapidly converted *in vivo*, first to phenylacetaldehyde and then to phenylacetic acid.

Phenylacetic acid is then excreted mainly in conjugated form in man and other animals. These data support the conclusion that data on phenylacetaldehyde, phenylacetic acid and phenylacetate esters are relevant to the hazard assessment of phenethyl alcohol.

3.3.2 Hydrolysis

Prior to absorption, the esters of phenethyl alcohol and phenylacetic acid undergo *in vivo* hydrolysis [Williams, 1959] to yield phenethyl alcohol and phenylacetic acid, respectively. The half-life for hydrolysis of phenethyl acetate in simulated intestinal fluid (solution containing pancreatin at pH=7.5 and 37°C) was reported to be 29.7 minutes while the half-life in simulated gastric juice (solution containing pepsin at pH=1.2) was 300 minutes [Longland *et al.*, 1977]. Esters of phenylacetic acid are hydrolyzed at similar rates under these conditions. The half-lives for methyl phenylacetate and ethyl phenylacetate in gastrointestinal fluid were 96.7 and 100 minutes, respectively [Longland *et al.*, 1977]. In a similar *in vitro* hydrolysis experiment in simulated intestinal fluid [Grundschober, 1977], benzyl phenylacetate (36 ul/l), *o*-cresyl phenylacetate (9 mg/l), allyl phenyl acetate (50 ul/l), and isoamyl phenylacetate (less than 25 ul/l)) were 100% hydrolyzed after two hours at pH=7.5 and 37°C. Under the same conditions, citronellyl phenylacetate and isopropyl phenylacetate were 60 and 50% hydrolyzed after two hours. Based on the *in vitro* hydrolysis data collected for various phenethyl and phenylacetic acid esters undergo hydrolysis in humans prior to absorption.

3.3.3 Absorption and Excretion

In vivo data in animals support the conclusion that phenethyl alcohol is rapidly absorbed and rapidly oxidized to phenylacetic acid. When ingested as traditional foods or intentionally added ingredients of food, or as hydrolysis products resulting from either condition, phenethyl alcohol is rapidly oxidized first to phenylacetaldehyde and then to phenylacetic acid. Phenylacetic acid is subsequently conjugated primarily with glutamine in humans or glycine in rodents and then excreted in the urine [Williams, 1959; El Marsy *et al.*, 1956; James *et al.*, 1972; Caldwell, 1987; Sangster and Lindley, 1986; Hawkins and Mayo, 1986].

The glutamine conjugate of phenylacetic acid is the only metabolite identified in the 24-hour urine of humans given a 4,000 mg oral dose of phenethyl alcohol [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 hours. The ether soluble acid extracted from the 24 hour urine accounted for greater than 61% of the dose [Bray *et al.*, 1958]. In an earlier study, 77% of 1300 mg/kg bw dose of phenethyl alcohol orally (gavage) administered to rabbits was isolated from the 24-hour urine as an ether soluble acid. No appreciable quantity (less than 0.5%) of free phenylacetic acid was recovered [Bray *et al.*, 1946].

The fate of administered phenylacetic acid supports the conclusion that this acid is readily conjugated and excreted mainly from the urine. The greater than 90% of an oral dose of phenylacetic acid given to rabbits is excreted in conjugated form. Only 0.4 – 3.1% was excreted unconjugated [Tulane and Lewis, 1933]. Greater than 98% of a 1 mg/kg oral dose of [carboxy-¹⁴C]-phenylacetic acid given to two male humans was excreted in the urine within 24 hours [James, *et al.*, 1972]. Greater than 98% of a single oral dose of 80 mg of [carboxy-¹⁴C]-phenylacetic acid administered to each of 3 control humans and 3 patients exhibiting phenylketouria was excreted in the urine within 24 hours [James *et al.*, 1973]. Based upon the results of studies using radio labeled phenylacetic acid, it may be concluded that phenylacetic acid is rapidly absorbed and quantitatively excreted within 24 hours.

Other acute studies in humans have investigated the effects of phenylacetic acid on various biochemical parameters. Although blood sugar levels were reduced in guinea pigs (13-60%) fed 380-750 mg/kg bw of phenylacetic acid, no effect on glucose levels was observed over a 2-6 hour period when a human was fed a 3,000 mg dose [Stewart, 1962]. Phenylacetic acid fed in a single 20 mg/kg dose to each of five male humans resulted in a two to four fold increase in urinary indoleacetic acid levels [Tashian, 1960]. Presumably, the phenylacetic acid deactivates tryptophan decarboxylase resulting in increased conversion of tryptophan to indoleacetic acid. Incubation of high concentrations of phenylacetic acid with human plasma caused prolonged clotting and increased thrombin activity [Nour-Eldin, 1968]. One-hour incubation of human cerebral spinal fluid with the

phenylacetic acid produced up to 91% inhibition of galactosyl transferase activity utilized in cerebral glycoprotein synthesis [Ko *et al.*, 1973]. No changes in total nitrogen in urine as well as the ammonia were noted after rabbits were fed 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].

3.3.4 Metabolism

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. For example, the glutamine conjugate (*i.e.*, phenylacetylglutamide) is the principal urinary metabolite in man while the glycine conjugate (phenylaceturic acid) predominates in rats and rabbits [James *et al.*, 1972].

Phenethyl alcohol is readily oxidized to phenylacetaldehyde by an assortment of NAD⁺-dependent alcohol and aldehyde dehydrogenases [Bosron and Li, 1980]. Highest activities of mammalian alcohol dehydrogenase (ALDH) occur 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 ⁶ with increasing lipophilicity. However, V_{max} ⁷ 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. These isoenzymes were 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 A1) indicate rapid conversion to phenylacetic acid [Klyosov, 1996].

⁶ The Michaelis-Menten constant is the concentration of the specific substrate at which a given enzyme yields one-half its maximum velocity. Michaelis-Menten equation: $v_0 = \frac{V_{max}[S]}{K_m + [S]}$
where v_0 =initial rate at substrate concentration [S]

Table A1. Human mitochondrial aldehyde dehydrogenase (ALDH-2) and cytosolic isoenzyme (ALDH-1)⁸

	ALDH1	ALDH2
K_m (nM)	5500 + 1200	29 + 4
V_{m rel}(%)	380 + 40	153 + 20
% in relation to V_m of acetaldehyde		
K_{cat}⁹	3000 + 340	1800 + 200

Phenylacetaldehyde, 3- and 4-chlorophenylacetaldehyde are effectively oxidized to the corresponding phenylacetic acid derivatives when incubated with rat hepatic microsomal dehydrogenase containing NAD⁺ as a coenzyme. The rates of oxidation for the 3- and 4-chloro derivatives are markedly slow than that for 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 hours [Kay and Raper, 1922].

3.3.5 Conjugation of Phenylacetic Acid

Although phenylacetic acid (No.21) 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

⁷ V_{max} is the maximum rate or velocity of an enzymatic reaction which means that the enzyme is saturated with its substrate

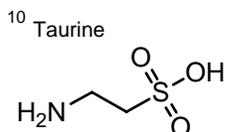
⁸ (Klyosov, 1996)

⁹ 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 5 different batches were 3.4±0.6 and 4.9±0.8 μmol min⁻¹ (mg of protein⁻¹), respectively, at pH 9.5. This corresponds to k_{cat} values of 782±138 min⁻¹ for ALDH-1 and 1176 ±192 min⁻¹ for ALDH-2.

metabolic options available to phenylacetic acid are conjugation 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-¹⁴C]phenylacetic acid is excreted within 24 hours as glutamine and taurine¹⁰ 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 to 10,000 mg doses of the acid over a 97day 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 conjugate phenylacetic acid with glutamine and to a lesser extent, taurine. However, significant quantities of acid (1-44%) are excreted free. 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 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 (No. 21) 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.



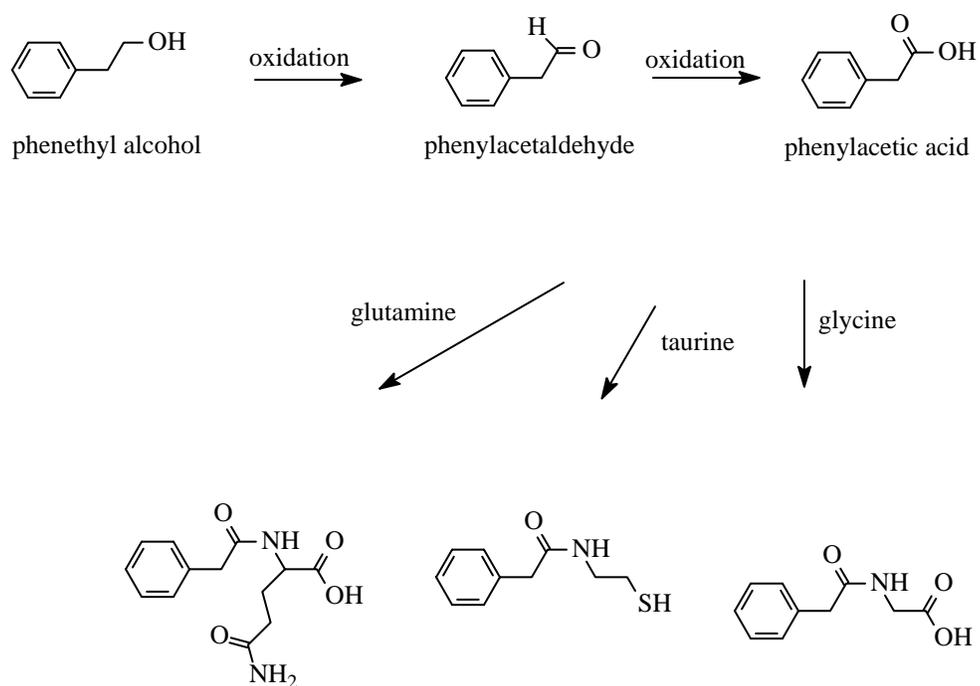
The capacity for glutamine conjugation has been studied in 3 volunteers and 3 patients exhibiting phenylketouria [James *et al.*, 1973]. Each subject was given a single 80 mg dose of [carboxy-¹⁴C]-phenylacetic acid. The average excretion of phenylacetylglutamine (measured as mmoles/g creatinine) by phenylketourics was approximately 5 times that of the control subjects indicating that the glutamine conjugation mechanism is able to cope with large amounts of phenylacetic acid produced by phenylketourics. 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 [¹⁴C]-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 hours [Koss and Lamprecht, 1968]. Significant levels of free phenylacetic acid have been observed at high dose levels [Teuchy *et al.*, 1971, James *et al.*, 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.

3.3.6 Summary

Based on the above data it is concluded that phenethyl esters and phenylacetate esters are readily hydrolyzed to phenethyl alcohol and phenylacetic acid, respectively. Once absorbed phenethyl alcohol is successively oxidized to phenylacetaldehyde and phenylacetic acid. Phenylacetic acid is then efficiently excreted mainly in the urine as the glutamine conjugate in man or the glycine conjugate in other animals. Therefore, the use of hazard data for precursor esters or the predominant *in vivo* metabolite phenylacetic acid is relevant to the hazard assessment of phenethyl alcohol.

FIGURE 1. METABOLISM OF PHENETHYL ALCOHOL



4 TEST PLAN

4.1 Chemical and Physical Properties

4.1.1 Melting Point

The measured melting point of phenethyl alcohol has been reported to be $-27\text{ }^{\circ}\text{C}$ [CRC, 1986; Merck, 1996]. Based on the input data of $-27\text{ }^{\circ}\text{C}$, the calculated melting point of phenethyl alcohol is reported to be $-6.0\text{ }^{\circ}\text{C}$ (adapted Joback method) [MPBPVP EPI Suite, 2000a].

4.1.2 Boiling Point

The measured boiling point of phenethyl alcohol has been reported to be $218\text{ }^{\circ}\text{C}$ [CRC, 1986] and $219 - 221\text{ }^{\circ}\text{C}$ at 750 mm Hg [Merck, 1996]. Based on input values of $218.2\text{ }^{\circ}\text{C}$ for boiling point and $-27\text{ }^{\circ}\text{C}$ for melting point, the calculated boiling point is $224.8\text{ }^{\circ}\text{C}$ (adapted Stein and Brown Method) [MPBPVP EPI Suite, 2000a].

4.1.3 Vapor Pressure

Two measured values for vapor pressure of phenethyl alcohol are in good agreement. The vapor pressure has been reported to be 0.0868 mm Hg at $25\text{ }^{\circ}\text{C}$ [MPBPVP EPI Suite, 2000b] and 0.0707 mm Hg at $30\text{ }^{\circ}\text{C}$ [Vuilleumier, 1995]. Based on input values of $218.2\text{ }^{\circ}\text{C}$ for boiling point and $-27\text{ }^{\circ}\text{C}$ for melting point, the calculated vapor pressure is 0.0222 mm Hg at $25\text{ }^{\circ}\text{C}$ [MPBPVP EPI Suite, 2000a].

4.1.4 n-Octanol/Water Partition Coefficients

The reported log Kow of phenethyl alcohol is 1.36 [Sangster, 1989; KOWWIN EPI Suite, 2000b]. Log Kow was also calculated resulting in a value of 1.57 [KOWWIN EPI Suite, 2000a]. The agreement between measured and calculated values confirms the experimental value of log Kow for phenethyl alcohol of 1.36.

4.1.5 Water Solubility

The measured water solubility for phenethyl alcohol is 22,200 mg/L [WSKOWWIN EPI Suite, 2000b] and 20,340 mg/L [Merck, 1996]. Based on an experimental melting point of -27°C and a log Kow of 1.36, the calculated water solubility is reported to be 32,720 mg/L at 25°C [WSKOWIN EPI Suite, 2000a].

4.1.6 New Testing Required

None.

4.2 Environmental Fate and Pathways

4.2.1 Photodegradation

The calculated photodegradation half-life for phenethyl alcohol is 12.6 hours [AOPWIN EPI Suite, 2000]. The calculations are based on measured rate constants for radical reactions of OH, O₃ and NO₃ with organic substrates [AOPWIN EPI Suite, 2000]. The short half-life is consistent with the presence of reactive benzylic hydrogen and alcoholic OH function in phenethyl alcohol. Therefore, the half-life can be considered reliable.

4.2.2 Stability in Water

Phenethyl alcohol will not hydrolyze in water. As a primary alcohol, phenethyl alcohol exhibits hydrogen bonding with water. The molecule is expected to be relatively stable in water in the absence of oxidative microorganisms. The HENRYWIN model predicts that the volatilization half-lives of phenethyl alcohol are 2529 hours in river water and 27,680 days in lake water [Mackay D. *et al.*, 1996a, 1996b].

4.2.3 Biodegradation

Phenethyl alcohol has been subjected to a CO₂ production test according to OECD Guideline 301B [Quest International Ltd., 1994]. The total biodegradation was 106.3% after 28 days with 10% degradation in approximately 1 day. Phenethyl alcohol can be considered to be readily and ultimately biodegradable.

The calculated value of 103.0% linear biodegradation probability is in agreement with experimental values [BIOWIN EPI Suite, 2000].

4.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model [Mackay, 1996a; 1996b] through the EPA EPI Suite 2000 program. The input parameters used were molecular weight, measured melting point (-27 °C), boiling point (218.2 °C), vapor pressure (0.089 mm Hg

at 25 °C), water solubility (20,340 mg/L) and log Kow (1.36). The model predicts that phenethyl alcohol will be distributed primarily to the water (46%) and soil (51.6%) with half lives of 360 hours in each compartment. The predicted persistence time is 312 hours which is consistent with the fact that phenethyl alcohol is readily biodegradable within 28 days.

In these environmental compartments, released phenethyl alcohol exhibits a potential to be oxidized to the corresponding carboxylic acid. Because of its use in food and cosmetics, soaps and detergents, the majority of phenethyl alcohol will enter the environment primarily *via* a sewage treatment plant and will be rapidly and extensively biodegraded.

4.2.5 New Testing Required

None. Phenethyl alcohol has been shown to be readily and ultimately biodegradable. While fugacity calculations estimate that the bulk will end up in soil and water, this does not take into account the principal uses of phenethyl alcohol, which would result in exposure *via* a sewage treatment plant allowing for rapid and extensive biodegradation.

4.3 Ecotoxicity

4.3.1 Acute Toxicity to Fish

Phenethyl alcohol has been subjected to a 96-hour static acute toxicity test according to the German guideline 38-414 with Golden Orfe (*Leuciscus idus*). An LC50 of 215 mg/L, a NOEL of 100 mg/L and an LC100 of 464 mg/L were reported [BASF AG, 1988c]. The experimental value [ECOSAR EPI Suite, 2000] of LC50 of 230 mg/L is conservative since it approximates experimental LC0 value.

4.3.2 Acute Toxicity to Invertebrates

Phenethyl alcohol has been subjected to a 48-hour acute toxicity guideline study with *Daphnia magna*. A 48-hour EC50 of 287 mg/L was reported [BASF AG, 1988a]. The calculated [ECOSAR EPI Suite, 2000] LC50 of 239 mg/L is in the same range as the measured value.

4.3.3 Acute Toxicity to Aquatic Plants

Phenethyl alcohol has been subjected to a 72-hour growth inhibition test with algae (*Scenedesmus subspicatus*). The reported EC50 was 490 mg/L [BASF AG, 1988b]. The model value for the 96-hour EC50 is 146 mg/L [ECOSAR EPI Suite, 2000]. Although the model prediction is more conservative, it is on the same order of magnitude as the measured value.

4.3.4 New Testing Required

None. The acute aquatic toxicity of phenethyl alcohol has been well characterized in fish, invertebrates and plants and indicates a low order of toxicity.

4.4 Human Health Data

4.4.1 Acute Toxicity

Phenethyl alcohol has been subjected to acute oral, dermal, inhalation and intraperitoneal tests in rats, mice, rabbits, and guinea pigs. The rat oral LD50 values range from 1500 mg/kg bw to 2540 mg/kg bw [Jenner *et al.*, 1964; Carpenter *et al.*, 1974; Zaitsev and Rakhmanina, 1974; International Flavors & Fragrances, Inc., 1982; Moreno, 1982a].

The reported dermal LD50 values are in considerable disagreement ranging from 805 mg/kg [Carpenter *et al.*, 1974] to 2535 mg/kg in the rabbit [International Flavors & Fragrances, Inc., 1983] to greater than 5000 mg/kg in the rat [Moreno, 1982b]. The intermediate value, 2535 mg/kg is from the best-documented study and is most consistent with what would be expected based on the dermal penetration in rabbits of 46-56% obtained from a pharmacokinetic study (Hawkins *et al.*, 1987, no robust summary provided) and the oral LD50 values discussed above.

An acute inhalation exposure of phenethyl alcohol aerosol in rats for a 4-hour period followed by a 14-day observation resulted in no deaths and the LC50 was reported to be greater than 4.63 mg/L [Breckenridge *et al.*, 1980].

Based on these data, it is concluded that phenethyl alcohol exhibits a very low acute toxicity.

4.4.2 Genetic Toxicity

4.4.2.1 *In vitro* Genotoxicity

No evidence of mutagenicity was observed when phenethyl alcohol [Florin *et al.*, 1980] was incubated with *Salmonella typhimurium* (SAL) strains TA98, TA100, TA1535 and TA1537 with and without S-9 metabolic activation at concentrations up to and including 3 micromol/plate. No evidence of mutagenicity was reported when *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 were incubated with

concentrations of 1.22 to 10,000 ug/plate of phenylacetic acid. The highest concentration in the study was cytotoxic (100% lethality) [Heck *et al.*, 1989]. In an Ames assay on a structurally related substance, no mutagenicity was observed when phenethyl alcohol, 2-methyl was incubated with *Salmonella typhimurium* TA 1535, TA 100, TA 1537, TA 1538, and TA 98 [Wild *et al.*, 1983]. In a forward mutation assay, no evidence of mutagenicity was reported when L5871Y mouse lymphoma cells were incubated with concentration of 31.3 to 500 ug/ml of phenylacetic acid [Heck *et al.*, 1989]. No increase in a sister chromatid exchange was observed when human whole-blood lymphocyte cultures were exposed to 2-phenethyl alcohol for 72 hours [Norppa and Vainio, 1983]. Also, no increase in unscheduled DNA synthesis was noted when rat hepatocytes were incubated with its principal metabolite phenylacetic acid [Heck *et al.*, 1989].

4.4.2.2 *In vivo* Genotoxicity

In vivo mutagenicity and genotoxicity data exist for two structurally related substances that participate in the same metabolic pathway as phenethyl alcohol. One, is a phenylacetic acid ester, isoeugenol phenylacetate and the other is 2-methyl substituted phenylacetaldehyde. Phenylacetic acid esters undergo hydrolysis prior to absorption. The methyl, ethyl, isopropyl, isoamyl, citronellyl esters of phenylacetic acid are rapidly hydrolyzed *in vitro* in simulated gastric juice and pancreatic juice [Longland *et al.*, 1977] or in a buffered solution of pancreatin [Grundschober, 1977]. Once formed phenylacetic acid is excreted as the glutamine conjugate.

Given the rapid rate of formation of phenylacetaldehyde derivatives from the corresponding phenethyl alcohol derivatives *in vivo* [Bosron and Li, 1980; Pietruszko *et al.*, 1973] and the rapid conversion of phenylacetaldehyde derivatives to phenylacetic acid metabolites [Martini and Murray, 1996], the structurally related aldehyde participates in the same metabolic pathway utilized by phenethyl alcohol.

None of the two structurally related substances (a phenethyl aldehyde and phenylacetic acid ester) showed any evidence of genotoxicity in well-recognized *in vivo* assays (mouse micronucleus and sex-linked recessive lethal assay). In mammals, substances were

administered orally, by gavage, or by intraperitoneal injection at doses that were significant fractions of the reported lethal dose levels.

No increase in the frequency of sex-linked recessive mutations occurred in a three brood study when *Drosophila melanogaster* were maintained on 10 mM of phenylacetaldehyde, 2-methyl or 25 mM solutions of phenylacetic acid, isoeugenol ester for 3 days [Wild *et al.*, 1983].

In two clastogenicity assays, groups of 10- to 14-week-old NMRI mice were intraperitoneally injected at 0 and 24 hours with 564, 987, or 1,410 mg/kg bw of phenylacetic acid, isoeugenol ester or at 0 hours with 134, 401, or 670 mg/kg bw of phenylacetaldehyde, 2-methyl [Wild *et al.*, 1983]. At 30 hours, the mice were sacrificed and bone marrow smears were prepared using the staining method of Schmid (1976). There was no evidence of micronucleated polychromatic erythrocytes for treated or control groups.

Based on the results of this *in vivo* genotoxicity assays for a structurally related phenethyl aldehyde and phenylacetate ester and the lack of any evidence of genotoxicity for numerous *in vitro* assays with and without metabolic activation for phenethyl alcohol, it is unlikely that phenethyl alcohol would exhibit a significant genotoxic potential *in vivo*. No additional *in vitro* and *in vivo* assays are requested for this chemical category.

Given that the *in vitro* and *in vivo* results consistently demonstrate that the substances exhibit a low order of genotoxic potential, no additional studies are required.

4.4.3 Repeated Dose Toxicity

A 90 day dermal toxicity study has been reported for phenethyl alcohol at daily doses of 250, 500, 1,000 or 2,000 mg/kg bw. The two highest dose groups exhibited a statistically significant lower growth rate than controls but with no significant differences in degree: final body weights (g) 1 g/kg males 482 ± 56 , females 276 ± 16 ; 2 g/kg males 484 ± 43 , females 272 ± 16 . There was also a statistically significant decrease in hemoglobin and white blood cell count in males at the high dose. No significant effects on clinical examination, hematology, and urinalysis were seen. Histopathologic examination was

performed on the adrenals, brain, heart, kidneys, liver, lung, mesenteric lymph node, pituitary, sternum, spinal cord, testes with epididymides, ovaries, spleen, urinary bladder and nerve. No findings were reported upon histopathological examination of any organ or tissue including the sex organs of male and female animals. The no observable adverse effect level (NOAEL) was concluded to be 500 mg/kg bw/day [Owston, *et al.*, 1981]. Based on the high dermal penetration of phenethyl alcohol on rats (70% after 5 daily repeated doses of 140 mg/kg bw; Hawkins *et al.*, 1986, 1990), this translated to an internal dose of 350 mg/kg bw/day.

A 17-week study is available for a phenethyl ester that hydrolyzes to phenethyl alcohol and phenylacetic acid prior to absorption [Longland *et al.*, 1977; Grundschober, 1977]. For 17 weeks, rats were maintained on diets containing 1,000, 2,500 or 10,000 ppm of phenethyl phenylacetate. These dietary levels were calculated to provide an average daily intake of approximately 50, 125 or 500 mg/kg bw/day. At necropsy, no differences were reported in major organ weights between test and control animals including the liver, kidneys, spleen, heart and testes. 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 for tissues prepared from the above mentioned organs [Hagan *et al.*, 1967]. While this study was conducted prior to GLP, it was conducted by the U.S. Food and Drug Administration and can be classified as reliable.

Additionally, a study of phenethyl alcohol in a mixture is available. 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 6,000 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%)¹¹. A control group of 20 rats/sex was maintained on tap water only. Body 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

¹¹Conversions of dose based on FDA, 1993.

intervals in the livers of rats. At study termination, liver, kidney, heart, spleen, and lung were examined histologically. 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 the test and control groups. Histopathological examination revealed no significant abnormalities in any of the organs examined. The authors concluded that the mixture of chemicals containing phenethyl alcohol did not produce any effects in the parameters tested [Johannsen and Purchase, 1969].

4.4.4 Reproductive Toxicity

The lack of toxicity to reproductive organs in subchronic toxicity tests [Owston, *et al.*, 1981; Hagan *et al.*, 1967] (see section 3.4.3), the well-defined NOELs for material (250 mg/kg bw/day) and developmental toxicity in a reproductive/developmental screening study using the phenethyl alcohol metabolite phenylacetic acid, and the lack of toxicity in females in numerous developmental studies at high dose levels of phenethyl alcohol, indicate that phenethyl alcohol exhibits a low order of reproductive toxicity. A reproductive/developmental screening test has been performed for the principal metabolite phenylacetic acid.

Four groups of 10 virgin Crl CD rats were administered oral dose levels of 0, 250, 500, or 1,000 mg/kg bw of phenylacetic acid by gavage once daily, 7 days prior to cohabitation, through cohabitation (maximum of 7 days), gestation, delivery, and a 4-day post-parturition period. The duration of the study was 39 days [Vollmuth *et al.*, 1995]. Maternal indices monitored included twice-daily clinical observation, measurement of body weights, food consumption, duration of gestation, and fertility parameters (mating and fertility index, gestation index, and number of offspring per litter). Offspring indices included daily observation, clinical signs, examination for gross external malformations, and measurement of body weight.

At 250, 500 and 1,000 mg/kg in dams, a significant (P less than 0.05) decrease in body weight and absolute and relative food consumption was reported during the premating period. Clinical signs of toxicity and a statistically significant increase in mortality was recorded in the mid- and high dose groups, but not in the low dose dams. Necropsy of

dams showed gross lesions in the mid- and high-dose groups. Measurements of mating success and fertility were similar for controls, low-dose and mid-dose groups. No changes in fertility index, averages for duration of cohabitation or gestation, gestation index, implantation sites, litter size, or pup sex ratios were seen at any dose levels. The only reproductive parameter affected was a decrease in the number of females mated per number of females pregnant at the 1000 mg/kg bw level. Based on the toxicity and increased dam mortality at the two highest dose levels and a decrease in mating index in the high-dose group, the maternal reproductive effects were reported at 500 and 1,000 mg/kg bw/day. The dose level of 250 mg/kg bw/day had no adverse effects on maternal toxicity and the level of 500 mg/kg bw/day had no effect on reproductive performance of female Sprague-Dawley rats [Vollmuth *et al.*, 1995].

4.4.5 Developmental/Teratogenicity Toxicity

Screening studies performed by one group of investigators during the 1980's reported that low dose levels of phenethyl alcohol and phenylacetic acid produce teratogenic effects resembling Fetal Alcohol Syndrome [Mankes *et al.*, 1983]. (Mankes, 1984 and 1985 were presentation abstracts and no robust summaries were prepared). 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 do not cause any visible anomalies in embryonal development [Bottomley *et al.*, 1987]. More recent comprehensive studies conducted with high dose levels of phenethyl alcohol given either by oral [Bottomley *et al.*, 1987] and dermal [Palmer *et al.*, 1986] routes of exposure have demonstrated that this group of substances exhibits a very low order of developmental toxicity.

In the original studies [Mankes *et al.*, 1983, 1984 and 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 to 15 of gestation. The average birth weight was decreased in the lowest and highest dose levels and pup size of all treated groups were significantly lower than those of the control group. The birth weight change was not dose-related in that birth weights were greater in the mid-dose group than in the control group. Mean litter size was greater in the high dose group (13) than in either the two lower doses (9) or controls (12). Also,

embryo lethality did not occur in the high dose group but 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). In particular, they noted significant differences in crown rump length and “variant ossifications” of the skull, limbs, sternum, rib, and tail. Other malformations included mainly ocular malformation, neural tube defects, and hydronephrosis [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 LD50 were administered to pregnant Long Evans rats. Intrauterine growth retardation (birth weight reductions) and embryo lethality were reported at all dose levels. These observations were inconsistent with those of the original study.

In response to the published data by Mankes *et al.*, (1983), the effects of dietary administration of microencapsulated phenethyl alcohol on pregnancy of the rat was studied [Bottomley *et al.*, 1987] according to a protocol essentially the same as OECD 414. The test diet containing nominal 0 (control), 1,000, 3,000, or 10,000 ppm (approximately 0, 50, 150, or 500 mg/kg bw) was made available to the rats during days 6 to 15 of pregnancy. Spray-dried gum Arabic, the microencapsulant, was used as a placebo control and was also added to the lower concentrations so that the total inclusion level remained constant for all groups at 5%. The animals were killed on day 20 post coitum and *in utero* development assessed by determination of litter values and examination of the fetuses for structural malformations or anomalies. 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 1,000, 3,000 and 10,000 ppm, respectively. Daily clinical observations revealed no evidence of toxicity to dams. The treatment of the dam with phenethyl alcohol by dietary inclusion of 799 mg/kg had a negligible detrimental effect on *in utero* development. Although there was clear evidence of impaired weight gain and decreased food consumption in dams in the high-dose group following the initial 2 days of exposure to the test material, fetal development was virtually unaffected, the only possible

exception being a marginal delay in the ossification process, an event that the authors indicated is usually transient and self-correcting during postnatal maturation.

Litter findings revealed no significant differences between test and control groups on the number of pregnant animals, early or late embryonic death, implantations, corpora lutea, mean sex ratio, pre- or post-implantation loss. There was no evidence of total litter loss. Mean litter weight in the high dose group was slightly less than that of the control group while mean fetal weight was higher than that of the control group. These changes were a reflection of the slightly reduced litter size in the high-dose group. Malformations were limited to three pups in the control groups and 2 pups in the mid-dose group. No more than one malformed pup was found in a single litter. The authors concluded that, at 83 and 266 mg/kg, phenethyl alcohol did not elicit any overt response in the dam and embryofetal development and morphology was unaffected [Bottomley *et al.*, 1987].

The effect of phenethyl alcohol on pregnancy of rats was studied following a similar protocol to OECD 414. Phenethyl alcohol was applied topically at the dose of 0, 0.14, 0.43 or 1.40 ml/kg during day 6 to 15 of pregnancy. The doses are approximately equal to 0, 140, 430, and 1400 mg/kg bw, 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. 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 developmental 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 dose 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 dosages, the slight differences in morphological changes between the 0.43 ml/kg dose and controls (cervical rib(s) thoracic vertebral irregularities), the authors concluded that the 0.14 ml/kg dose level (143 mg/kg bw) is the NOEL for developmental toxicity in the rat [Palmer *et al.*, 1986].

In order to better clarify the fetal NOAEL in the previous study, a limited developmental study was conducted by a similar protocol, but looking particularly at the cervical rib bud and thoracic vertebrae effects, pregnant rats were treated dermally with 70, 140, 280, 430 or 700 mg/kg bw/day on days 6 to 16 of pregnancy. Cervical rib buds were statistically significantly higher than controls at 700 mg/kg only and there were no significant incidences of vertebrae effects. However, significant and dose-related skin irritation was seen in the dams at all dose groups and delayed ossification (judged to be reversible) was seen in fetuses of all groups. The only statistically significant difference from controls in the two lower dose groups was incomplete ossification of the pelvis but with no dose correlation. These effects may have been secondary to the dermal irritation. No clear no observable effect level (NOEL) for dams or fetuses can be concluded from this study, however, the minor effects seen in the two lower doses could lead to a conclusion of a fetal NOAEL of 140 mg/kg bw [Christian *et al.*, 1988].

Metabolic and Kinetic Criteria in the Hazard Assessment of Phenethyl Alcohol

The principal developmental effect was decreased ossification as reported in the gavage [Magnova and Seitzer, 1973; Mankes *et al.*, 1983] and dermal studies [Palmer, 1986]. These results are reminiscent of those reported for high plasma levels of carboxylic acids such as phenylacetic acid [Brown, 1987; Magnova and Seitzer, 1973]. The significant disparity between effect levels *via* the gavage, dermal, and dietary mode of administration were resolved through a series of pharmacokinetic studies on phenethyl alcohol and phenylacetic acid, the principal *in vivo* metabolite [Hawkins D.R. and Mayo B.C., 1986; Hawkins *et al.*, 1986, 1990]. The developmental effects were closely correlated with the peak plasma levels and area under the curve concentrations of phenylacetic acid and not phenethyl alcohol. In addition, the peak plasma levels were related to mode of administration in the order gavage>dermal>dietary. At the

approximately the same dose levels, the peak plasma levels and area under the curve concentrations are both less than one-tenth of that produced by the gavage route. The conclusion of these studies is that formation of high *in vivo* concentrations of phenylacetic acid is responsible for the observed teratogenic effects and that dietary NOAEL is 266 mg/kg for developmental toxicity.

Phenylacetic acid

In the reproduction/developmental screening test discussed in the section on reproductive toxicity, four groups of 10 virgin Crl CD rats were administered oral dose levels of 0, 250, 500, or 1,000 mg/kg bw of phenylacetic acid by gavage once daily, 7 days prior to cohabitation, through cohabitation (maximum of 7 days), gestation, delivery, and a 4-day post-parturition period. The duration of the study was 39 days [Vollmuth *et al.*, 1995]. Offspring indices monitored included daily observation, clinical signs, examination for gross external malformations, and measurement of mortality (number of stillborns), viability (pups dying on days 1-4), body weight and body weight gain. The only effects reported occurred at the 1000 mg/kg bw/day level. A statistically significant decrease in viability, a non-significant decrease in body weight gain and slight skeletal malformations were reported at the highest dose level. The dose level of 500 mg/kg bw/day had no adverse effects on the development of the offspring of female Sprague-Dawley rats.

4.4.6 New Testing Required

None.

4.5 Test Plan Table

Chemical	Physical-Chemical Properties				
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility
CAS No. 60-12-8 Phenethyl alcohol	A, Calc	A, Calc	A, Calc	A, Calc	A, Calc

Chemical	Environmental Fate and Pathways			
	Photodegradation	Stability in Water	Biodegradation	Fugacity
CAS No. 60-12-8 Phenethyl alcohol	Calc	NA	A, Calc	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants
CAS No. 60-12-8 Phenethyl alcohol	A, Calc	A, Calc	A, Calc

Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity <i>In Vitro</i>	Genetic Toxicity <i>In Vivo</i>	Repeat Dose Toxicity	Reproductive Toxicity	Developmental Toxicity
CAS No. 60-12-8 Phenethyl alcohol	A	A	R	A	A,R	A, R

LEGEND

Symbol	Description
R	Endpoint requirement fulfilled using data for structurally related substances, SAR
T	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
O	Other

5 REFERENCES FOR TEST PLAN AND ROBUST SUMMARIES

- Ambrose A.M., Power F.W. and Sherwin C.P. (1933) Further studies on the detoxication of phenylacetic acid. *J. Biol. Chem.* **101**, 669-675.
- AOPWIN EPI Suite (2000) US Environmental Protection Agency.
- BASF AG (1988a) Labor Oekologie, unpublished data (0107/88).
- BASF AG (1988b) Labor Oekologie, unpublished data (1010/88).
- BASF AG (1988c) Abteilung Toxikologie, unpublished data (87/410).
- Bauer K. and D. Garbe (1985) Common Flavor and Fragrance Materials
Verlagsgesellschaft mbH, D-6940, Weinheim, Federal Republic of Germany.
- BIOWIN EPI Suite (2000) US Environmental Protection Agency.
- Block W. (1953) On the physiology of C[14]-radioactive mescaline in animals. Part IV. Comparative studies with C[14]-mescaline and C(14)-*beta*-phenylethylamine. *Zeitschrift fur Naturforschung*, **8B**, 440-444.
- Bosron W.F. and Li T.K. (1980) Alcohol dehydrogenase. In *Enzymatic Basis of Detoxication* Edited by W.B. Jakoby vol. 1, pp. 231-248, Academic Press, Orlando FL.
- Bottomley A. M., Ratcliffe H. E., John D. M., Anderson A., Dawe I. S. (1987) Effect of Dietary Administration of Micro-Encapsulated Phenylethyl Alcohol on Pregnancy of the Rat (Embryotoxicity Study). Unpublished report.
- Bray H. G., Neale F. C. and Thorpe W. V. (1946) The Fate of Certain Organic Acids and Amines in the Rabbit. *Biochemical Journal*, **40**, 134-139.
- Bray H.G., James S.P., and Thorpe W.V. (1958) Metabolism of some omega-halogenoalkylbenzenes and related alcohols in the rabbit. *Biochemical Journal*, **70**, 570-579.
- Breckenridge C., Collins C.J., Qureshi S. and Procter B.G. (1980) The acute toxicity of inhaled phenyl ethyl alcohol in the albino rat. Unpublished report to RIFM.
- Brown N.A. (1987) Teratogenicity of carboxylic acids: Distribution studies in whole embryo culture. *Pharmacokinetica and Teratogenesis*, **II**, 154-163.
- Caldwell J. (1987) Human disposition of [14C]-ORP/178. Private communication to FEMA. Unpublished report.

- Carpenter C.P., Weil, C.S., and Smyth, H.F. (1974) Range-finding toxicity data: List VIII. *Toxicology and Applied Pharmacology*, **28**, 313-319.
- Christian M.S. and Hoberman A.M. (1988) Dosage-range developmental toxicity (embryo/fetal toxicity and teratogenicity) study of 2-phenylethylalcohol (PEA) administered dermally to presumed pregnant mice. Unpublished report to RIFM.
- CRC Handbook of Chemistry and Physics (1986) 67th edition, Robert C. Weast, editor, The Chemical Rubber Co Press, Inc. Boca Raton, Florida.
- El Masry A.M., Smith J.N. and Williams R.T. (1956) Studies in detoxication. 69. The metabolism of alkylbenzenes: n-propylbenzene and n-butylbenzene with further observations on ethylbenzene. *Biochemical Journal*, **64**, 50-57.
- ECOSAR EPI Suite (2000) US Environmental Protection Agency, OPPT Risk Assessment Division (G. Cash & V. Nabholz, April 2001).
- Fassett D.W. (1963) Toxicity of phenethyl alcohol. *Industrial Hygiene and Toxicology*, 1476-1477.
- Florin I., Rutberg L., Curvall M. and Enzell C. (1980) Screening of tobacco smoke constituents for mutagenicity using the Ames test. *Toxicology*, **18**, 219-232.
- Gocke E., King, K.Eckhardt and D.Wild (1981) Mutagenicity of fragrance ingredients licensed by the European Communities *Mutation Research*, 90, 91-109
- Gregus Z., Fekete T., Varga F. and Klaassen C.D. (1993) Dependence of glycine conjugation on availability of glycine: Role of the glycine cleavage system. *Xenobiotica*. **23**(2),141-153.
- Grundschober F. (1977) Toxicological assessment of flavouring esters. *Toxicology*. **8**, 387-390.
- Hagan E. C., Hansen W. H., Fitzhugh O. G., Jenner P. M., Jones W. I., Taylor J. M., Long E. L., Nelson A. A. and Brouwer J. B. (1967) Food Flavourings and Compounds of related Structure. II. Subacute and Chronic Toxicity. *Food and Cosmetic Toxicology*, **5**, 141-157.
- Hall R.L. (1960) Recent progress in the consideration of flavoring ingredients under the food additives amendment. *Food Technology*, **14**(10), 488-495.
- Hawkins D.R., Elsom L.F., Girkin R., Bigs, S.R. and Williams S.G.F. (1988) The percutaneous absorption and disposition of (14)C-2-phenylethanol in rabbits. Unpublished report to RIFM.
- Hawkins D.R., Elsom L.F., Girkin R., Huckstep M. and De-Salis C.M. (1987) The dermal absorption of (14)C-2-phenylethanol in man following a single topical application. Unpublished report to RIFM.

- Hawkins D.R., Elsom L.F., Girkin R. and Jackson R. (1986) Dermal absorption and disposition of (14)C-2-phenylethanol in rats. Unpublished report to RIFM.
- Hawkins D.R., Redrup M.J., Brindley C.J. and Williams S.G.P (1990) Plasma and urine concentrations and pharmacokinetics of phenylacetic acid and phenylethanol in the rat following single doses of phenylethanol administered *via* different routes. Unpublished report.
- Hawkins D.R. and Mayo B.C. (1986) Plasma kinetics of [14C]-ORP/178 in the rat. Private communication to FEMA. Unpublished report.
- Heck J. D., Vollmuth, T. A., Cifone, M. A., Jagannath, D. R., Myhr B., and R.D. Curren (1989) An evaluation of food flavoring ingredients in a genetic toxicity screening battery. *The Toxicologist*, **9(1)**, 257.
- Hijikata Y. (1922) The Influence of Putrefaction Products on Cellular Metabolism. II. On the Influence of Phenylacetic and Phenylproionic acids on the Distribution of Nitrogen in the Urine. *J Biol. Chem.* **51**, 141-154.
- International Flavors & Fragrances, Inc. (1982) Acute oral toxicity study of phenethyl alcohol in rats. Unpublished report.
- International Flavors & Fragrances, Inc. (1983) Acute dermal toxicity test of phenethyl alcohol in rabbits. Unpublished report.
- International Organization of the Flavour Industry (IOFI) (1995) European inquiry on volume use. Private communication to Flavour and Extract Manufacturers Association (FEMA).
- James M.O., Smith R.L. and Robert L. (1973) Conjugation of phenylacetic acid in phenylketonurics. *European Journal of Clinical Pharmacology*, **5(4)**, 243-246.
- James M.O., Smith R.L., Williams R.T. and Reidenberg M. (1972) The conjugation of phenylacetic acid in man, sub-human primates and some non-primate species. *Proceedings Royal Society London, B*, **182(1066)**, 25-35.
- Jenner P. M., Hagan E. C., Taylor J. M., Cook E. L. and Fitzhugh O. G. (1964) Food Flavours and Compounds of Related Structure. I. Acute Oral Toxicity. *Food and Cosmetics Toxicology*, **2**, 327-343.
- JECFA Joint FAO/WHO Expert Committee on Food Additives (2003) Safety Evaluation of Phenethyl Alcohol, Aldehyde, Acid, and Related Acetals and Esters Used as Flavouring Agents (2002-2).
- Johannsen E. and Purchase I.F.H. (1969) Kaffircorn malting and brewing studies. XXI: The effect of the fusel oils of Bantu beer on rat liver. *S.A. Medical Journal (Supplement- S.A. Journal of Nutrition)*, **43(12)**, 326-328.

- Kay H.D. and Raper H.S. (1922) Mode of oxidation of fatty acids with branched chains. II. The fate in the body of hydratropic, tropic, atrolactic and atropic acids together with phenylacetaldehyde. *Biochemistry Journal*, **16**, 465-474.
- Klimisch H. J., Andreae, M., and U. Tillman (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Journal of Regulatory Toxicology and Pharmacology*, **25**, 1-5.
- Koss F.W. and Lamprecht W. (1968) Experimental Investigations in Choleresis. *European Journal of Pharmacology* **4**(2), 215-223.
- KOWWIN EPI Suite (2000a) US Environmental Protection Agency.
- KOWWIN EPI Suite (2000b) US Environmental Protection Agency (Hansch C. *et al.*, 1995).
- Klyosov A. A. (1996) Kinetics and Specificity of Human Liver Aldehyde Dehydrogenases Toward Aliphatic, Aromatic and Fused Polycyclic Aldehydes. *Biochemistry*, **35**(14), 4457-4467.
- Ko G.K.W., Raghupathy E. and McKean C.M. (1973) UDP-Galactose: Glycoprotein galactosyl transferase and UDP-N-acetylgalactosamine: Protein N-acetylgalactosaminyl transferase activities of human cerebrospinal fluid. *Canadian Journal of Biochem.* **51**(11), 1460-1469.
- Longland R.C., Shilling W.H. and Gangolli S.D. (1977) The hydrolysis of flavouring esters by artificial gastrointestinal juices and rat tissue preparations. *Toxicology*, **8**, 197-204.
- Lucas C.D., Putnam J.M., and Hallagan J.B. (1999) Flavour and Extract Manufacturers Association (FEMA) of the United States 1995 Poundage and Technical Effects Update Survey. Washington D.C. Self-published.
- Maarse H., Visscher C.A., Willemsens L.C. and Boelens M.H. (2000) Volatile Components in Food-Qualitative and Quantitative Data. Centraal Instituut Voor Voedingsonderzoek TNO. Zeist, The Netherlands.
- Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a) Assessing the fate of new and existing chemicals: a five stage process. *Environmental Toxicology and Chemistry*, **15**(9), 1618-1626.
- Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. *Environmental Toxicology and Chemistry*, **15**(9), 1627-1637.
- Maganova N.B. and Zaitsev A.N. (1973) Study of the Embryotoxic Action of Some Synthetic Food Flavourings. *Vopr Pitan*, **32**(4), 50-54.

- Mankes R. F., LeFevre R., Bates H. and Abraham R. (1983) Effects of Various Exposure Levels of 2-Phenylethanol on Fetal Development and Survival in Long-Evans Rats. *Journal of Toxicology and Environmental Health*, **12**, 235-244.
- Mankes R. F., LeFevre R. and Abraham R. (1984) Reproductive Effects of Some Solvent Alcohols: Evaluation of Structure-Activity Relationships in 2-Substituted Ethanols. *Journal of the American College of Toxicology*, **3(2)**, 166.
- Mankes R. F., LeFevre R., Renak R., Fiesher J., and Abraham R. (1985) Reproductive effects of some solvent alcohols with differing partition coefficients. *Teratology*, **31(3)**, 67A.
- Martini R. and Murray M. (1996) Rat Hepatic Microsomal Aldehyde Dehydrogenase. Identification of 3- and 4-Substituted Aromatic Aldehydes as Substrates of the Enzyme. *Chemistry Research in Toxicology*, **9**, 268-276.
- Merck Index (1996) 12th edition, Susan Budavari, editor, Merck & Co. Inc. Whitehouse Station, NJ.
- Moldave K. and Meister A. (1957) Enzymic acylation of glutamine by phenylacetic acid. *Biochim. Biophys. Acta*. **24**, 654-655.
- Moreno O.M. (1982a) Acute toxicity studies in rats. Private communication to FEMA.
- Moreno O.M. (1982b) Acute toxicity studies in rats. Private communication to FEMA.
- MPBPVP EPI Suite (2000a) US Environmental Protection Agency.
- MPBPVP EPI Suite (2000b) US Environmental Protection Agency (Daubert T.E. and Danner, R.P., 1989)
- Norppa H. and Vainio H. (1983) Induction of Sister-Chromatid Exchanges by Styrene Analogues in Cultured Human Lymphocytes. *Mutation Research*, **116**, 379-387.
- Nour-Eldin F. (1968) Phenols and blood coagulation. *J. Biomed. Mater. Res.* **2(1)**, 23-42.
- Opdyke D.L.J. (1975) Monographs on fragrance raw materials, *Fd. Cosmet. Toxicol.*, **33**, (Supplement) 903.
- Owston E., Lough R. and Opdyke D.L. (1981) A 90-day study of phenylethyl alcohol in the rat. *Fd and Cosmet Toxicol*, **19(6)**, 713-715.
- Palmer A.K., Bottomley, A. M., Ratcliffe, H.E. Clark, R., and John, D. M. (1986). Effect of Phenylethyl Alcohol (PEA) on Pregnancy of the Rat. Huntingdon Research Center. Unpublished report to RIFM.
- Pietruszko R., Crawford K. and Lester D. (1973) Comparison of substrate specificity of alcohol dehydrogenases from human liver, horse liver, and yeast towards saturated and 2-enoic alcohols and aldehydes. *Archives of Biochemistry and Biophysics*, **159**, 50-60.

- Power F.W. and Sherwin C.P. (1927) The detoxication of putrefactive products by the human body. *Arch Int. Med.* **39**, 60-66.
- Quest International Ltd. (1994). The ultimate biodegradability of phenylethyl alcohol in the sealed vessel test. Unpublished report.
- Richter D. (1938) Elimination of amines in man. *The Biochemical Journal*, **32**, 1763-1769.
- Sandler M., Ruthven C.R.J., Goodwin B.L., Lees A. and Stern G.M. (1982) Phenylacetic acid in human body fluids: High correlation between plasma and cerebrospinal fluid concentration values. *J Neurol. Neurosurg. Pshychiat.*, **45(4)**, 366-368.
- Sangster S.A. and Lindley M.G. (1986) Metabolism and excretion of ORP/178 in man. Private communication to FEMA. Unpublished report.
- Sangster J. (1989) Octanol-water partition coefficients of simple organic compounds. *J Phys. Chem. Ref. Data*, **18(3)**, 1111-1229.
- Seakins J.W.T. (1971) The determination of urinary phenylacetylglutamine as phenylacetic acid: Studies on its origin in normal subjects and children with cystic fibrosis. *Clinica Chimica Acta.* **35(1)**, 121-131.
- Shiple G.J. and Sherwin C.P. (1922) Fate of phenylacetyl derivatives of the amino acids in the animal organism. *J. Biol. Chem.* **53**, 463.
- Simpson V. J., Baker R. and Deitrich R. A. (1985) Inducible Aldehyde Dehydrogenases from Rat Liver Cytosol. *Toxicology and Applied Pharmacology*, **79**, 193-203.
- Stewart G.A. (1962) Pharmacological Studies on Oral Hypoglycaemic Agents. *Anglo_Ger. Med. Rev.* **1(4)**, 334-347. Ref. No. 690.
- Stein W.H., Paladini A.C., Hirs C.H. and Moore S. (1954) A phenylacetylglutamine as a constituent of normal human urine. (Letter to the Editor). *J.of the Chem. Soc.-Perkin Transaction* **76(7-12)**, 2848-2849.
- Stofberg J. and Grundschober, F. (1987) Consumption ratio and food predominance of flavoring materials. *Perfumer and Flavorist*, **12**, 27.
- Tashian R.E. (1960) Effect of phenylalanine metabolites on urinary excretion of indole-3-acetic acid and 5-hydroxyindole-3-acetic acid in man. *Proc. Soc. of Exp. Biol. Med.* **103**, 407-410.
- Teuchy H., Quatacker G., and Van Sumere C. F. (1971) Quantitative Investigation of the Hippuric Acid Formation in the Rat After Administration of Some Possible Aromatic and Hydroaromatic Precursors. *Arch. Internal. Physiol. And Biochim.* **79**, 573-587.
- Thierfelder H. and Schempp E. (1917) Behaviour of benzoylpropionic acid, phenethyl alcohol and phenoxyacetic acid in the body of men and dogs. *Arch. Ges. Physiol.* **167**, (280-288).

- Tulane V. J. and Lewis H. B. (1933) Studies in the synthesis of hippuric acid in the animal organism. IX. A comparative study of the rate of synthesis and excretion of hippuric and phenacetic acids by the rabbit. *Journal of Biological Chemistry*, **103**, 151-160.
- Vollmuth T.A., Bennett, M.B., Hoberman, A.M. and Christian, M.S. (1995) An Evaluation of Food Flavoring Ingredients Using an In Vivo Reproductive and Developmental Toxicity Screening Test. *Teratology*, **41(5)**, 597.
- Vuilleumier C., Flament I., and Sauvegrain P. (1995) Headspace analysis study of evaporation rate of perfume ingredients applied to skin. *International Journal of Cosmetic Science*, **17**, 61-76.
- Wagreich H., Kamin H. and Harrow B. (1940) On the detoxication of phenylacetic acid by glucuronic acid in humans. *Pro. Soc. Exp. Biol. Med.* **43(3)**, 468-470.
- Wild D., King, M.T., Gocke, E. and Eckhardt, K. (1983) Study of artificial flavouring substances for mutagenicity in the salmonella/microsome, base and micronucleus tests. *Fd Chem Toxicol.*, **21(6)**, 707-719.
- Williams R.T. (1959) Detoxication mechanisms- The metabolism and detoxication of drugs, toxic substances and other organic compounds. 2nd ed. Chapman and Hall Ltd., London. pp. 796.
- WSKOWIN EPI Suite (2000a) US Environmental Protection Agency.
- WSKOWIN EPI Suite (2000b) US Environmental Protection Agency (Vivandi S.C., *et al.*, 1981).
- Zaitsev A. N. and Rakhmanina, N. L. (1974) Some Data on the Toxic Properties of Phenylethyl and Cinnamyl Alcohols. *Voprosy pitaniia*, **6**, 48-53.