AR 201-12912A

The Flavor and Fragrance High Production Volume Consortia

The Aromatic Consortium

Test Plan for Cinnamyl Derivatives

Cinnamaldehyde (3-phenyl-2-propenal)	CAS No. 104-55-2
alpha-Amylcinnamaldehyde (2-amyl-3-phenyl-2-propenal)	CAS No. 122-40-7
alpha-Hexylcinnamaldehyde (2-hexyl-3-phenyl-2-propenal)	CAS No. 101-86-o
p-t-Butyl-alpha-methyldihydrocinnamaldehyde (3-(*p*-t-butylphenyl)-2-methylpropanal)	CAS No. 80-54-6

FFHPVC Aromatic Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

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BASF

BF Goodrich Company

Bush Boake Allen, Incorporated

Eastman Chemical

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International Flavor & Fragrances, Inc.

KoSa

Polarome

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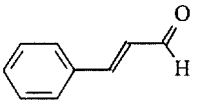
Table of Contents

The HPV Challenge Test Plan for Cinnamyl Derivatives

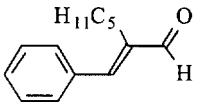
1 Identity of Substances



Cinnamaldehyde

3-phenyl-2-propenal

CAS No. 104-55-2



alpha-Amylcinnamaldehyde

2-amyl-3-phenyl-2-propenal

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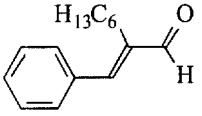

alpha-Hexylcinnamaldehyde

2-hexyl-3-phenyl-2-propenal

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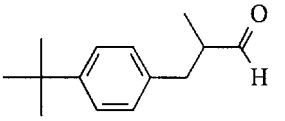


***p-t-Butyl-alpha-*methylhydrocinnamaldehyde**

3-(*p*-t-butylphenyl)-2-methylpropanal

CAS No. 80-54-6

2 Category Analysis

2.1 Introduction

In October of 1999, members of the U.S. flavor and fragrance industries and 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. Twelve (12) 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.

2.2 Background Information

The chemical category designated "Cinnamyl Derivatives" includes cinnamaldehyde, two alkyl-substituted cinnamaldehydes, and one alkyl-substituted dihydrocinnamaldehyde derivative. The four substances are grouped together because of their close structural relationships and the resulting similarities of their physio-chemical and toxicological properties.

In nature, cinnamaldehyde is the predominant constituent of cassia oil and Ceylon cinnamon bark oil. It is responsible for the spicy aroma strongly reminiscent of cinnamon spice. It is common components of traditional foods. Cinnamaldehyde, *alpha*-amylcinnamaldehyde, and alpha-hexylcinnamaldehyde are currently recognized by the U.S. Food and Drug Administration (FDA) as GRAS ("generally regarded as safe") for their intended use as flavoring substances [Hall and Oser, 1965]. p-t-Butyl-alpha-

methylhydrocinnamaldehyde is used only in fragrance products. Quantitative natural occurrence data for cinnarnaldehyde indicates that oral intake occurs predominantly from consumption of cinnamon spice products and cinnamon flavorings [Stofberg and Grundschober, 1987; Stofberg and Kirschman, 1985]. Greater than 38,000 kg [Stofberg and Grundschober, 1987] of cinnamaldehyde is consumed annually as a natural component of food while 45 1,400 kg is consumed as an added flavoring substance in the U.S.A. annually [Lucas *et al.,* 1999].

alpha-Amylcinnamaldehyde and alpha-hexylcinnamaldehyde have a flowery aroma reminiscent of jasmine and are widely used as fragrance ingredients in cosmetics, soaps, detergents and other fragranced consumer products. Because both substances are stable in alkali, they are used in soap perfumes. p-t-Butyl-alpha-methylhydrocinnamaldehyde, commonly recognized as lilial, produces a stable and long lasting pleasant, mild blossom odor popular in soap and cosmetic products with a "lily of the valley" or linden fragrance.

2.3 Structural Classification

The four substances in this group are un-substituted or alkyl-substituted cinnamaldehyde or 2,3-dihydrocinnamaldehyde derivatives. Common structural features among members of this chemical category are that they contain either a 3-phenyl-2-propenal or 3-phenylpropanal backbone. The group includes cinnamaldehyde (3-phenyl-2-propenal), alpha-amylcinnamaldehyde (2-amyl-3-phenyl-2-propenal), alpha-hexylcinnamaldehyde (2-hexyl-3-phenyl-2-propenal) and p-t-butyl-alpha-methylhydrocinnamaldehyde {3-(*p*-t-butylphenyl)-2-methylpropanal}.

2.4 Production of Cinnamyl Derivatives

The *tram-* isomer of cinnamaldehyde predominates in nature. On a commercial scale, cinnamaldehyde is prepared almost exclusively from the alkaline condensation of benzaidehyde and acetaldehyde [Richmond, 1950]. *In* a similar manner, *alpha-*amylcinnamaldehyde and, alpha-hexylcinnamaldehyde are prepared by the condensation of heptanal and octanal, respectively, with benzaldehyde. These aldehydes must be protected from oxidation to the corresponding carboxyhc acid. Therefore, antioxidants

are added as stabilizers. The remaining substance in the chemical category, *p*-t-butyl-alpha-methylhydrocinnamaldehyde is prepared by the condensation of *p*-t-butylbenzaldehyde with propanal. It is also prepared by reduction of *alpha*-methylcinnamaldehyde to yield *alpha-methylhydrocinnamic* alcohol. The alcohol is then alkylated with *tert*-butyl chloride and subsequently oxidized to the aldehyde [Webb, 1981].

2.5 Chemical Reactivity and Metabolism

2.5.1 Absorption, Distribution, and Excretion

Cinnamaldehyde, the *alpha-amyl* and *alpha-hexyl* derivatives and its saturated analog (*p*-t-butyl-alpha-methyldihydrocinnamaldehyde) are rapidly absorbed from the gut, metabolized and excreted primarily in the urine and to a minor extent, in the feces. Rodent and humans studies for cinnamaldehyde and alpha-substituted cinnamaldehydes indicate that cinnamyl derivatives are absorbed, metabolized and excreted as polar metabolites within 24 hours.

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

metabolite, suggesting that saturation of the glycine conjugation pathway occurs at repeated high dose levels of cinnamaldehyde.

The effect of dose and sex on the disposition of [3-^{14}C]-cinnamaldehyde has been studied in F344 rats or CD1 mice [Peters and Caldwell, 1994]. Greater than 85% of either a 2.0 or 250 mg/kg bw dose of cinnamaldehyde administered to groups of male and female F344 rats (4/group) or CD1 mice (6/group) by intraperitoneal injection was recovered in the urine and feces within 24 hours. Greater than 90% was recovered after 72 hours. When 250 mg/kg bw of [3-^{14}C]-cinnamaldehyde was administered orally to F344 rats, 98% was recovered from the urine (91%) and feces (7%) within 24 hours [Peters and Caldwell, 1994]. The effect of dose on the disposition of [3-^{14}C-d_5]-cinnamic acid in F344 rats and CD1 mice has also been studied. Five dose levels of cinnamic acid in the range from 0.0005 mmol/kg bw (0.072 mg/kg bw) to 2.5 mmolikg bw (370 mg/kg bw) were given orally to groups of F344 rats (4/group) or by intraperitoneal injection to groups of CD1 mice (4/group). After twenty-four (24) hours, 73-88% of the radioactivity was recovered in the urine of rats and 78-93% in the urine of mice. After 72 hours, 85-100% of the radioactivity was recovered from rats mainly in the urine [Caldwell and Nutley, 1986]. In mice, the recovery was 89- 100% within 72 hours. Only trace amounts of radioactivity were present in the carcasses, indicating that cinnamic acid was readily and quantitatively excreted at all dose levels [Nutley et *al.*, 1994]. In summary, it appears that the parent alcohol, aldehyde, and acid undergo rapid absorption, metabolism, and excretion independent of dose (up to 250 mg/kg bw), species, sex, and mode of administration.

In rats, alpha-methylcinnamaldehyde [Kay and Raper, 1924] and p-methylcinnamic acid [Solheim and Scheline, 1973] are rapidly absorbed, metabolized, and excreted in the urine as free and conjugated forms of cinnamic acid or benzoic acid. Based on these studies, cinnamyl derivatives are anticipated to be rapidly absorbed, metabolized, and excreted mainly in the urine within 24 hours.

2.5.2 Oxidation and Conjugation Reactions

The aromatic cinnamaldehyde derivatives are readily oxidized to cinnamic acid derivatives (see Figure 1). Human NAD^+ dependent alcohol dehydrogenase (ADH) catalyzes oxidation of primary alcohols to aldehydes [Pietruszko et *al.*, 1973]. Isoenzyme mixtures of NAD^+ dependent aldehyde dehydrogenase (AID) [Weiner, 1980] catalyze oxidation of aldehydes to carboxylic acids. Aromatic alcohols and aldehydes have been reported to be excellent substrates for ADH [Sund and Theoell, 1963] and ALD [Feldman and Wiener, 1972], respectively. The urinary metabolites of cinnamyl alcohol and cinnamaldehyde are mainly derived from metabolism of cinnamic acid (see Figure 1).

Doses of 2 and 250 mg trans-[3-^{14}C]cinnamaldehyde/kg bw were given by ip. injection to male and female Fischer 344 rats and CD1 mice [Peters and Caldwell, 1994]. Doses of 250 mg/kg bw were also administered via oral gavage to male rats and mice only. In both species, the major urinary metabolites were formed from oxidation of cinnamaldehyde to yield cinnamic acid, which was subsequently *oxidized in the beta-oxidation* pathway. The major urinary metabolite was hippuric acid (71-75% in mice and 73-87% in rats), accompanied by small amounts of metabolites including 3 - hydroxy- 3 -phenylpropionic acid (0.4-4%), benzoic acid (0.4-3%), and benzyl glucuronide (0.8-7.0%). The glycine conjugate of cinnamic acid was formed to a considerable extent only in the mouse (4-13%). To a small extent, glutathione conjugation of cinnamaldehyde competes with the oxidation pathway. Approximately 6-9% of either dose was excreted in 24 hours as glutathione conjugates of cinnamaldehyde. The authors concluded that the excretion pattern and metabolic profile of cinnamaldehyde in rats and mice are not systematically affected by sex, dose size, or route of administration [Peters and Caldwell, 1994].

The toxicokinetic profile of cinnamaldehyde has been investigated in male F344 rats [Yuan and Deiter, 1992]. Plasma levels of cinnamaldehyde (less than 0.1 µg/ml) and cinnamic acid (less tban 1 µg/ml) were not measurable when rats (3-6/group) were administered a single oral dose of 50 mg/kg bw of cinnamaldehyde by gavage in corn oil. At dose levels of 250 and 500 mg/kg bw, plasma levels of cinnamaldehyde and cinnamic acid were ≈1 and less than 10 µg/ml, respectively. The bioavailability of cinnamaldehyde was calculated to be less than 20% at both dose levels. A dose-dependent increase in

hippuric acid, the major urinary metabolite, occurred 6 hours after gavage and continued over the next 18 hours. Only small amounts of cinnamic acid were excreted in the urine either free or as the glucuronic acid conjugate. The urinary hippuric acid recovered over 50 hours accounted for 72-81% over the dose range from 50 to 500 mg/kg bw.

Approximately 15% of an oral dose of 250 mg cinnamaldehyde/kg bw administered to rats by gavage was excreted in the urine as two mercapturic acid derivatives, N-acetyl-S-(1-phenyl-3-hydroxypropyl)cysteine and N-acetyl-S-(1-phenyl-2-carboxyethyl)cysteine, in a ratio of four to one. Approximately 9% of an oral dose of 125 mg cinnamyl alcohol/kg bw was excreted in the urine as N-acetyl-S-(l-phenyl-3-hydroxypropyl)cysteine [Delbressine et *al.*, 198 1].

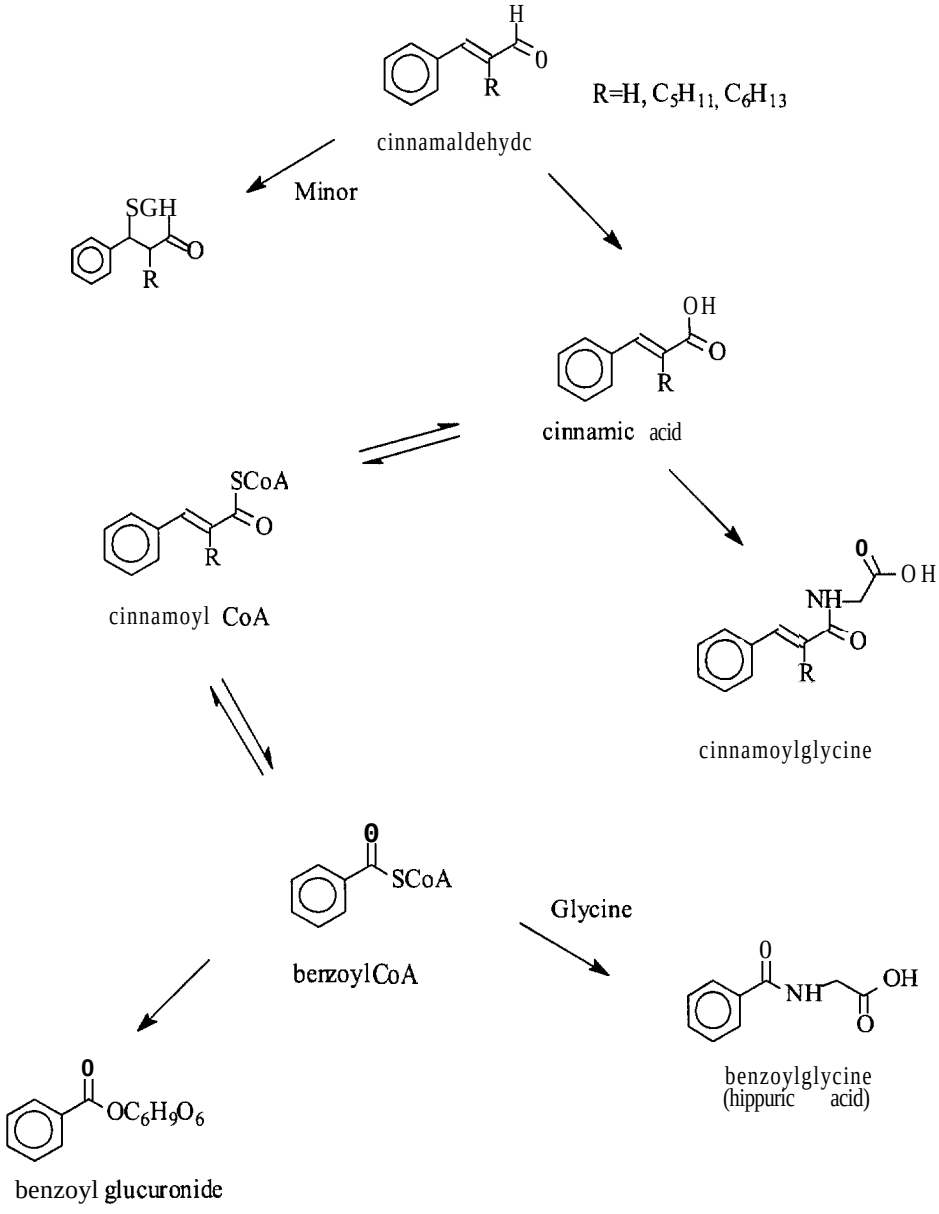
The position and size of the substituent do not significantly affect the pathways of metabolic detoxication of cinnamyl derivatives. Cinnamyl derivatives containing *alpha*-alkyl substituents (e.g. 2*alpha*-methylcinnamaldehyde) are extensively metabolized *via* beta-oxidation followed by cleavage to yield mainly the corresponding hippuric acid derivative. A benzoic acid metabolite was isolated from the urine of dogs given either *alpha-methylc*innamic acid or alpha-methylphenylpropionic acid [Kay and Raper, 1924]. These studies suggest that alpha-methylcinnamaldehyde undergoes oxidation to benzoic acid while higher homologues are excreted primarily unchanged or as the conjugated form of the cinnamic acid derivative.

para (*p*-) Ring substituents (e.g. 3-(*p*-isopropylphenyl)propionaldehyde and *p*-methylcinnamaldehyde) do not significantly impact metabolism via beta-oxidation. In male albino rats, p-methoxyc innamic acid has been shown to be metabolized primarily to p-methoxybenzoic acid and its corresponding glycine conjugate [Solheim and Scheline, 1973]. Similar results were reported with 3,4-dimethoxycinnamic acid (which is meta and para substituted) [Solheim and Scheline, 1976]. The structurally related substance *p*-tolualdehyde is metabolized to p-methylbenzoic acid without any apparent oxidation of the methyl group [Williams, 1959]. Based on these observations, it may be concluded that the presence of side-chain alkyl substituents and ring substituents do not alter the principal metabolic detoxication pathway for cinnamyl derivatives. Each of the four

cinnamyl derivatives is oxidized to the corresponding acid followed either by conjugation and excretion or by beta-oxidation, conjugation and excretion

Figure 1

Metabolism of Cinnamaldehyde Derivatives



3 Test Plan

3.1 Chemical and Physical Properties

3.1.1 Melting Point

The melting point of cinnamaldehyde is reported to be –7.5°C [Merck, 1997] while that of alpha-hexylcinnamaldehyde is 4°C [Fenaroli's, 1994]. The calculated [SRC] melting points (0.04 to 46°C) are significantly higher than experimental values.

3.1.2 Boiling Point

The increase in experimental boiling points in going from cinnamaldehyde (246°C [Merck, 1997] and 250°C [FMA]), alpha-amylcinnamaldehyde (284°C [FMA]), alpha-hexylc innamaldehyde (304°C [FMA]), to p-t-butyl-alpha-methylhydrocinnamaldehyde (258°C [Arctander, 1969]) is consistent with an increase in molecular weight and alkyl group branching. Boiling points calculated by the Stein and Brown Method produce the same trend in boiling points for cinnamaldehyde (227°C), alpha-amylcinnamaldehyde (305°C), alpha-hexylcinnamaldehyde (319°C), and *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde (280°C) but the difference in boiling point between cinnamaldehyde and the three alkyl-substituted cinnamaldehyde derivatives is greater than experimentally determined values.

3.1.3 Vapor Pressure

The reported vapor pressure for alpha-hexylcinnamaldehyde, 0.0002 mm Hg [Vuilleumier, 1995] is in good agreement with calculated vapor pressures of less than 0.001 [FMA] and 0.00048 mm Hg (Modified Grain Method) [SRC]. The calculated vapor pressure of less than 0.001 mm Hg [FMA] and 0.0012 mm Hg (Modified Grain Method) [SRC] for *alpha-amylc* innamaldehyde, and 0.00358 mm Hg (Modified Grain Method) for *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde [SRC] are consistent with that of alpha-hexylcinnamaldehyde (, since their increased vapor pressure reflect their decreased molecular weights (14 daltons less than the *alpha-hexyl* derivative). Cinnamaldehyde, having the lowest molecular weight, exhibits a proportionately higher

calculated vapor pressure of 0.02 mm Hg [FMA] and 0.09 mm Hg (Antoine and Grain Method) [SRC].

3.1.4 Octanol/Water Partition Coefficients

The calculated log Kow values [SRC] of 4.33 for *alpha*-amylcinnamaldehyde, 4.82 for alpha-hexylcinnamaldehyde, and 4.36 for p-t-butyl-alpha-methylhydrocinnamaldehyde follow the same trend but are slightly lower than experimental values of 4.7 [Givaudan-Roure, 1994a], 5.3 [Givaudan-Roure, 1994d], and 4.2 [Givaudan-Roure, 1994b], respectively determined by OECD guideline 117. Experimental values show a slightly higher lipophilic character *(i.e.,* higher log Kow) than are estimated by the model [SRC]. The experimental log Kow for the more polar, lower molecular weight aldehyde, cinnamaldehyde, is also expected to be slightly lower than the calculated log Kow of 1.82 [SRC].

3.1.5 Water Solubility

The water solubilities of 33 mg/L [Givaudan-Roure, 1995] obtained according to OECD 105 guideline and less than 100 mg/L [Givaudan-Roure, 1994b] and 200 mg/L [BBA, 1990] reported using other experimental procedures for *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde are an order of magnitude greater than the calculated solubility of 7.8 mg/L (KOWWIW). Other calculated solubilities of 8.5 mg/L for *alpha*-amylcinnamaldehyde, 2.75 mg/L for *alpha-hexyl*cinnamaldehyde, and 2150 mg/L for cinnamaldehyde are expected to be 5-10 times less than experimentally measured water solubilities. Because of the wide discrepancies between measured and calculated values for water solubility, it is recommended that water solubilities be measured using OECD guidelines for cinnamaldehyde and p-t-butyl-alpha-methylhydrocinnamaldehyde.

3.1.6 New Testing Required

Measurement of water solubility is recommended for cinnamaldehyde and *p*-t-butyl-alpha-methylhydrocinnamaldehyde.

3.2 Environmental Fate and Pathways

3.2.1 Photodegradation

The calculated photodegradation half lives (AOPWIN) of the four cinnamaldehyde derivatives are in the range from 2.33 to 3.88 hrs. Structurally, 3 of the 4 substances in this category *are alpha,beta*-unsaturated aldehydes. These substances have an oxidizable aldehyde function and an allylic position (C_4) labile to attack by hydroxy radical species in the gas phase. The known chemical reactivity of these substrates supports short photodegradation half-lives predicted by the model.

3.2.2 Stability in Water

No hydrolysis is possible for any of these 4 cinnamaldehyde derivatives. All four are expected to be relatively stable in aqueous solution, although they may be slowly oxidized to the corresponding cinnamic acid derivative in aqueous media.

3.2.3 Biodegradation

Studies for alpha-amylcinnamaldehyde, alpha-hexylcinnamaldehyde, and *p-t*-butyl-alpha-methylhydrocinnamaldehyde demonstrate these materials to be readily biodegradable. Biodegradation of alpha-amylcinnamaldehyde was 70.5% and 90% after 28 days using OECD test guidelines 301B [Quest, 1996] and 301F [Givaudan-Roure, 1992a], respectively. Similarly, alpha-hexylcinnamaldehyde was 76.5% and 97% biodegraded after 28 days using OECD test guidelines 301B [Quest, 1994] and 301F [Givaudan-Roux-e, 1992b], respectively and *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde was 84% and 96% biodegraded after 28 days using test OECD guideline 301F [Givaudan-Roure, 1994c; BBA, 1990]. The three cinnamyl derivatives met the 10 day window criteria for biodegradability. Although no biodegradation study is available for cinnamaldehyde, this substance, like the other three cinnamaldehyde derivatives contains an oxidizable aldehyde function. There is no reason to suspect that cinnamaldehyde will not be readily biodegradable using either test OECD guideline 301B or 301F.

3.2.4 Fugacity

Transport and distribution in the environmental were modeled using Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11 [Mackay and Donald, 199 1]. The principal input parameters into the model are molecular weight, melting point, vapor pressure, water solubility, and log Kow. Where measured values were available, these were used but where they were not, calculated data from the EPIWIN series of programs were used. Based on the comparable physiochemical properties of the four aldehydes, it is not unexpected that the four would exhibit similar distribution in the environment. The significance of these calculations must be evaluated in the context that the substances in this chemical category are readily oxidized in the environment to corresponding carboxylic acids. The aldehydes have been shown to be readily and/or ultimately biodegradable, and the remainder would be expected to behave similarly in the environment. Since the model does not account the effects of biodegradation, the relevance of fugacity calculations for these substances is highly questionable.

3.2.5 New Testing Required

None

3.3 Ecotoxicity

3.3.1 Acute Toxicity to Fish

Only ECOSAR calculated values are available. The 96-hour LC50 for cinnamaldehyde is calculated to be 11.9 mg/L while the alkyl substituted homologues *alpha*-amylcinnamaldehyde and alpha-hexylcinnamaldehyde, being more lipophilic, are calculated to have LC50 values about one third of that for cinnamaldehyde (3.14 mg/L and 2.36 mg/L, respectively). The remaining substance *p-t-butyl-alpha-methylhydrocinnamaldehyde* possessing the same molecular weight as *alpha*-amylcinnamaldehyde and is also an alkyl substituted cinnamaldehyde is calculated to have approximately the same LC50 (LD50=3.19 mg/L). Because of the lack of fish acute toxicity data on this group, the QSAR algorithm should be validated by conducting LC50 assays with cinnamaldehyde andp-t-butyl-alpha-methylhydrocinnamaldehyde.

3.3.2 Acute Toxicity to Aquatic Invertebrates

Only an ECOSAR calculated value is available for cinnamaldehyde at 8.1 mg/L (48-hour Daphnia LC50). It does not differ significantly from that for fish. The Daphnia 48-hour LC50s for the more lipophilic substances alpha-amylcinnamaldehyde and *alpha*-hexylcinnamaldehyde are calculated to be 0.416 and 0.224 mg/L, respectively. The calculated LC50 for *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde is in the same range, 0.403 mg/L. Because of the lack of data on this chemical category, the QSAR algorithm should be validated by conducting tests on cinnamaldehyde and p-t-butyl-alpha-methylhydrocinnamaldehyde.

3.3.3 Acute Toxicity to Aquatic Plants

The only study of algae toxicity indicates that a 50 uM solution of cinnamaldehyde inhibits the growth of green algae by 35% after 80 hours and 5% after 160 hours [Dedonder, 1971]. ECOSAR calculated 48-hour EC50 values for cinnamaldehyde (8.1 mg/L), alpha-amylcinnamaldehyde (0.871 mg/L), alpha-hexylcinnamaldehyde (0.343 mg/L), and *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde (0.827 mg/L) are consistent with calculated values for acute fish and aquatic invertebrate toxicity cited above. The QSAR algorithm should be validated by conducting tests on cinnamaldehyde and *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde because of the lack of data on this group. Assuming the measured values for cinnamaldehyde and *p-t-butyl-alpha*-methylhydrocinnamaldehyde in fish, Daphnia, and green algae are greater than calculated values; it will not be necessary to conduct this test on the other two members of this chemical category.

3.3.4 New Testing Required

- Acute toxicity to fish by OECD guideline 203 for cinnamaldehyde and *p*-t-butyl-*alpha-metbylbydrocinnamic* aldehyde. (Due to limited solubility of these substances, LC50 will be carried out only up to the solubility limit of the substance in a static-renewal test.)

- Acute toxicity to Daphnia by OECD guideline 202 for cinnamaldehyde and *p*-t-butyl-alpha-methylhydrocinnamaldehyde.

- Acute toxicity to algae according to OECD guideline 201 for cinnamaldehyde and p-t-butyl-alpha-methylhydrocinnamaldehyde.

3.4 Human Health Data

3.4.1 Acute Toxicity

Oral LD50 values have been reported for the four substances in this chemical category. In rats, LD50 values are in the range of 2220-3400 mg/kg, demonstrating that the oral acute toxicity of these substances is extremely low [Denine and Palanker, 1973; Jenner *et al.*, 1964; Keating, 1972; Levenstein and Wolven, 1972; Levenstein, 1975; Levenstein, 1976; Moreno, 197 1; Moreno, 1972; Moreno, 1973; Moreno, 1974; Moreno, 1975; Moreno, 1976; Moreno, 1977a; Moreno, 198 1; Moreno, 1982; Opdyke, 1974; Russell, 1973; Schafer *et al.*, 1983; Weir and Wong, 1971; Wohl, 1974; Zaitsev and Rakhmanina, 1974]. Lowest LD50 values are reported for cinnamaldehyde (LD50=1160 mg/kg) while LD50 values for the alkyl-substituted derivatives are in the range from 3100 mg/kg to 3730 mg/kg. LD50 values in the range from approximately 2318 to 3400 mg/kg have been reported in mice [Draize *et al.*, 1948; Harada and Ozaki, 1972; Levenstein, 1975; Schafer and Bowles, 1985; Zaitsev and Rakhmanina, 1974].

Dermal acute toxicity shows a similar trend for the four substances in this chemical category. Dermal LD50 values range from a low of 590 ul/kg for cinnamaldehyde to more than 2000 mg/kg for alpha-amylcinnamaldehyde, more *than 3000* mg/kg for *alpha*-hexylcinnamaldehyde, and more than 5000mgikg for *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde [Moreno, 197 1; Moreno, 1973b; Moreno, 1977b; Shelanski, 1973; Draize *et al.*, 1948; Zaitsev and Rakhmanina, 1974].

3.4.2 Genetic Toxicity

3.4.2.7 In vitro

Cinnamaldehyde (*trans* and unspecified stereochemistry), alpha-amylcinnamaldehyde, alpha-hexylcinnamaldehyde, and p-t-butyl-alpha-methylhydrocinnamaJdehyde were *inactive in Salmonella typhimurium,* including strains TA92, TA94, TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA2637. The assays were

performed at concentrations ranging up to the level of cytotoxicity, both in the absence and presence of metabolic activation (S9 fraction) obtained from the livers of Aroclor 1254 or methylcholanthrene-induced Sprague-Dawley rats or Syrian hamsters [Azizan and Blevins, 1995; Dillon et *al.,* 1992; Eder *et al.,* 1980; Eder *et al.,* 1982a; Eder *et al.,* 1982b; Eder *et al.,* 1991; Florin *et al.,* 1980; Fujita and Sasaki, 1987; Ishidate *et al.,* 1984; Kasamaki et *al.,* 1982; Lijinsky and Andrews, 1980; Mamett *et al.,* 1985; Neudecker *et al.,* 1983; Sekizawa and Shibamoto, 1982; Tennant *et al.,* 1987; Wild *et al.,* 1983; Wagner, 1999; Givaudan-Roure, 1984].

Some weakly equivocal- to-positive results were reported for cinnamaldehyde in *Salmonella typhimurium* strain TA100 using the pm-incubation method [Dillon *et al.,* 1992; Ishidate *et al.,* 1984]. However, the majority of similar studies in strain TA100, including a recent study using a prolonged pre-incubation time (120 minutes), and others using the standard plate incorporation method, did not find any evidence of mutagenicity [Azizan and Blevins, 1995; Eder *et al.,* 1982a, Eder *et al.,* 1982b; Eder *et al.,* 1991; Kasamaki *et* al., 1982; Lijinsky and Andrews, 1980; Neudecker *et al.,* 1983; Sasaki and Endo, 1978; Sekizawa and Shibamoto, 1982; Wagner and Twarszik, 1999; Givaudan-Roure, 1984].

Mutation assays *in Escherichia coli* strains WP2 *uvrA* were negative for cinnamaldehyde and *p*- t-butyl-alpha-methylhydrocinnamaldehyde [Yoo, 1986; Sekizawa and Shibamoto, 1982; Wagner, 1999]. Cinnamaldehyde produced equivocal to positive results in the forward mutation assay in L5178Y mouse lymphoma cells both with and without metabolic activation, but the reports describing these tests did not provide sufficient details on the methodology, test concentrations, or cytotoxic effects to adequately evaluate the results [Palmer, 1984; Rudd *et al.,* 1983]. In L1210 mouse lymphoma cells, DNA strand breaks were observed only at cytotoxic concentrations of cinnamaldehyde [Eder *et al.,* 1993].

Tests for the induction of sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells exposed to cinnamaldehyde produced negative results at low concentrations and weakly positive results at concentrations approaching cytotoxic levels, suggesting

only weak SCE activity [Galloway et *al.*, 1987; Sasaki et *al.*, 1987]. A dose-dependent increase in SCE was reported only when cultures were pre-treated with mitomycin C [Sasaki *et al.*, 1987]; however, in the absence of SCE activity by cinnamaldehyde alone, the activity in conjunction with mitomycin contributes little to the evaluation of the potential SCE activity. Cinnamaldehyde was reported to induce chromosome aberrations at low concentrations (i.e., less than 15 ug/ml) in Chinese hamster fibroblasts and B241 cells tested with and without metabolic activation [Ishidate *et al.*, 1984; Kasamaki *et al.*, 1982; Kasamaki and Urasawa, 1985]. However, higher concentrations were negative in CHO cells, both with and without metabolic activation in a well-conducted, repeated assay [Galloway et *al.*, 1987]. Negative results were obtained with cinnamaldehyde in the mutation assay in Chinese hamster V79 cells [Fiorio and Bronzetti, 1994].

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

3.4.2.2 *In vivo*

An increase in the frequency of sex-linked recessive lethal mutations (SRLM) was reported when *Drosophila melanogaster* was injected with 20,000 ppm cinnamaldehyde. However, no increase in the frequency of mutations occurred when *Drosophila melanogaster* were fed 800 ppm cinnamaldehyde for three days. Reciprocal translocations were not observed in either assay [Woodruff *et al.*, 1985]. The was no evidence of SLRM when *Drosophila melanogaster* were maintained on 10 mM solutions of either alpha-amylcinnamaldehyde or alpha-hexylcinnamaldehyde [Wild *et al.*, 1983].

In mammalian test systems, there was no evidence of an increase in unscheduled DNA synthesis in hepatocytes when rats or mice were administered 1000 mg

cinnamaldehyde/kg bw by oral gavage [Mirsalis *et* al., 1989]. In the rodent micronucleus assay, the frequency of micronuclei was not increased when rats or mice were given 1700 mg/kg bw or 1100 mg/kg bw, respectively, of cinnamaldehyde by oral gavage [Mereto *et al.,* 1994] or when mice were administered 500 mg/kg bw by intraperitoneal injection [Hayashi *et al.* 1984, 1988]. The frequency of micronucleated bone marrow cells in mice that had been exposed to X-rays decreased after 500 mg cinnamaldehyde was administered by intraperitoneal injection [Sasaki et *al.,* 1990].

In one study [Mereto et *al.,* 1994], an increase in micronucleated cells was reported in rat and mouse hepatocytes, and in rat (but not in mouse) forestomach cells after oral gavage dosing with cinnamaldehyde up to 1,100 mg/kg/bw (rats) or 1,700 mg/kg/bw (mice). No increase in liver or forestomach micronuclei were observed at dose levels ≤ 850 mg/kg bw. No DNA fragmentation was observed in the rat hepatocytes or gastric mucosa cells. An increase in the incidence and size of GGT-positive foci was in reported hepatocytes of rats pretreated with *N*-nitrosodiethylamine and then administered 500 mg cinnamaldehyde/kg bw/day by oral gavage for 14 days [Mereto *et al.,* 1994].

The positive in *vivo* findings with cinnamaldehyde in the rat forestomach and in the liver of both rats and mice are inconsistent with negative results observed in the standard bone marrow assays and are observed at dose levels that result in significant toxicity. It has been reported that cinnamaldehyde given at oral doses of ≥ 500 mg/kg bw results in the depletion of hepatocellular glutathione levels [Swales and Caldwell, 1991; 1992; 1993]. Therefore, increases in micronuclei were reported at dose levels (1100 and 1700 mg/kg bw) that appear to affect cellular defense mechanisms (i.e., glutathione depletion). Based on the fact the micronuclei formation is dose-dependent; it appears that induction of micronuclei is a threshold phenomenon, which occurs at extremely high levels of intake. Also, the bolus doses resulting from gavage administration likely produce much greater exposures to both the forestomach and liver, as compared to dietary or dermal administration, The author [Mereto *et al.,* 1994] acknowledged these facts and concluded that the data did not justify the conclusion that ciunamaldehyde was clastogenic. As a result of the apparent threshold for micronuclei induction and the lack of activity in the remainder of the *in vivo* studies, the results obtained with bolus, high-dose exposures

occurring in the liver and forestomach are not considered relevant to the safety of cinnamaldehyde at normal exposure levels.

The conclusion that cinnamaldehyde and the three other cinnamyl derivatives are not mutagenic, is based on the results of three *in vivo* mouse micronucleus assays in which there was no evidence of an increase in the incidence of micronuclei when NMIR or ICR mice were given oral doses of 1213 mg/kg bw of alpha-amylcinnamyl alcohol [Wild *et al.,* 1983], 756 mg/kg bw of alpha-hexylcinnamaldehyde [wild *et al.,* 1983], or 600 mg/kg of *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde [Gudi and Krsmanovic, 2000].

3.4.2.3 Conclusion

Cinnamaldehyde and its alkyl-substituted derivatives lack direct mutagenic or genotoxic activity, as indicated by the negative results obtained in bacterial test systems. Evidence of genotoxic activity was observed in isolated mammalian cells, with the cinnamyl compounds producing chromosome aberrations and/or mutations in the respective test systems regardless of the presence or absence of metabolic activation; however, the reported *in vitro* activity did not translate into mutagenic, clastogenic, or genotoxic activity *in vivo.*

3.4.3 Repeat Dose Toxicity

Oral and/or dermal repeat-dose studies are available for each of the 4 substances in this chemical category.

Groups (IO/sex/group) of male and female Osborne-Mendel rats were maintained on a diet containing either 0 (control), 1000, 2500, or 10,000 ppm (approximately equivalent to 50, 125, or 500 mg/kg bw/day, respectively) cinnamaldehyde for a total of 16 weeks. Measurement of body weight and food intake recorded weekly showed no significant difference between test and control animals at any dose level. At termination, hematological examinations revealed normal values. At necropsy, no differences were reported between major organ weights of test and control animals. Gross examination of the tissue of all animals was unremarkable. Histopathological examination of 6-8 animals, equally represented by gender, in the high-dose group revealed a slight hepatic

cellular swelling and a slight hyperkeratosis squamous epithelium of the stomach [Hagan et *al.*, 1967].

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

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

Groups of male and female rats (CFE strain; 15/sex/group) were maintained on a diet containing 0 (control), 80, 400, or 4000-ppm alpha-amylcinnamaldehyde for 14 weeks. Additional groups of 5 male and 5 female rats were maintained on diets containing 400 and 4000 ppm alpha-amylcinnamaldehyde for 2 and 6 weeks. The respective mean dietary intakes over the 14-week period were reported to be 0, 6.1, 29.9 and 287.3 mg/kg bw/day for males and 0, 6.7, 34.9, and 320.3 mg/kg bw/day for females. Measurement of bodyweight, food and water consumption revealed no significant differences between treated and control groups. Hematological examinations (hemoglobin content, hematocrit, erythrocyte and leucocyte counts, and individual leucocyte counts) and blood chemistry determinations conducted at 2, 6, and 14 weeks revealed normal values. Reticulocyte counts performed only on control and the high dose groups showed no significant differences. Urinanalysis performed during the final week of treatment revealed no difference in cell content and renal concentration tests for test and control groups. Measurement of organ weights at autopsy revealed a statistically significant increase in relative liver weight in males ($p<0.01$) and females ($p<0.05$) at the 4000 ppm dietary level after 14 weeks, increased stomach weights in males at the 400 ppm level after 6 weeks, and increased relative kidney weight in males ($p<0.01$) at 4000 ppm after 14 weeks. The relative organ weight increases were not associated with any evidence of histopathology. Microscopic examination of prepared tissues from all major organs revealed no evidence of histopathological changes that could be associated with administration of the test material in the diet [Carpanini et al., 1973].

Groups of male and female rats (15/sex) were maintained on a diet containing alpha-amylcinnamaldehyde at levels calculated to result in the approximate daily intake of 6.1 mg/kg bw for males and 6.6 mg/kg bw for females for a total of 90 days. Bodyweight measurements, food consumption, and observations of general condition were recorded regularly. Hematological and clinical chemistry examinations were conducted on 8 rats of each sex at week 6 and again on all animals at week 12 of experimentation. Neither measurements of growth, hematology, clinical chemistry, nor histopathology at necropsy revealed any evidence of toxic effects [Oser et al., 1965].

Groups of male and female Sprague-Dawley rats (Ysex) received a 25 mg/kg dose of alpha-hexylcinnamaldehyde applied topically to the back daily for 9 days. Bodyweight measurements and observations of general condition were recorded regularly. At termination, hematological and clinical chemistry examinations, urinalysis, and liver and kidney weights were measured. Microscopic examination of liver, kidney, skin and spinal cord were conducted. Neither measurements of growth, hematology, clinical chemistry, nor histopathology revealed any evidence of toxic effects [Moreno, 1981].

Dose levels of 0, 125, 250, 500, cr 1000 mg/kg bw of alpha-hexylcinnamaldehyde were administered percutaneously to the backs of groups of albino rats (15/sex/group) daily for 90 days. Clinical observations and weekly body weight measurements showed a decreased survival in the 1000 mg/kg dose level and significantly decreased body weights in both sexes at 500 and 1000 mg/kg dosed groups. Hematological and clinical chemistry examinations conducted at week 6 and again on all animals at study termination revealed elevated white cell counts and segmented neutrophils in the two highest dose group of males and reduced lymphocyte counts only at the highest dose. In females, elevated white blood cell counts were reported in the three highest dosed groups, but only the 250 mg/kg group showed significantly reduced lymphocytes. Gross examination revealed irritation to the application site and gastrointestinal mucosa. Liver and kidney weights of females were significantly increased at 250, 500, and 1000 mg/kg dose levels. Histopathological examination revealed that the 1000 mg/kg dose level was associated with hepatic hydropic vacuolization and single cell degeneration, splenic lymphoid fibrosis, focal gastric ulceration, necrotizing dermatitis, and increased myeloid-erythroid upon bone marrow examination. A NOAEL of 125 mg/kg was reported [Lough *et al.*, 1980].

In a study designed to evaluate the toxicity to the male and female reproductive systems, groups of SPF Fu albino male and female rats (14/sex/group) were given oral doses of 0, 2, 5, 25, or 50 mg/kg bw of p-t-butyl-alpha-methylhydrocinnamaldehyde daily by gavage in rape seed oil for 13 weeks. A satellite group at 50 mg/kg bw/day was maintained for an additional 4 weeks post treatment. Relative and absolute liver weights were increased in males marginally at 25 mg/kg bw/day and more significantly at 50 mg/kg bw/day. Females showed increased absolute and relative liver weight at 25 and 50 mg/kg bw/day

and increased absolute and relative adrenal weights at 50 mg/kg bw/day. However, these organ weight effects were reversible, in that after 4 weeks post treatment these was no difference between absolute and relative organ weights in treatment and control groups. Effects on spermatogenesis and spermiogenesis included, induction of spermatoceles in the cauda epididymidis, possible obstruction of the epididymal ducts, and significant number of Sertoli cell-only tubules in the 50 mg/kg bw/day group only. An NOAEL level for testicular effects was of 25 mg/kg bw/day [Givaudan-Roure, 1990c].

The study was repeated when 6 groups of albino Fu male (14/group) rats were given the same dose levels of p-t-butyl-alpha-methylhydr~innamaldehyde daily by oral gavage for 13 weeks. An additional 50 mg/kg bw/day dose group was observed for 4 weeks post-treatment. Testes and epididymides of all male rats were subjected to microscopic examination. Treatment related histopathology revealed increased density of Leydig cells, spermatoceles and testicular atrophy in males, again only in the 50 mg/kg bw/day group [Givaudan-Roure, 1990d].

To determine if the testicular effects were species specific to the rat, groups of Beagle dogs (3/sex/group) were administered capsules containing 0, 4.4, 22.3, or 44.6 mg/kg bw p-t-butyl-alpha-methylhydrocinnamaldehyde daily for 13 weeks. There was no evidence of toxicity in either sex based on daily observations, weekly measurement of body weights and food intake, hematological and clinical chemistry examination, urinanalysis, organ weight measurement, and complete histopathology evaluation [Givaudan-Roure, 1990b]. In a Pweek pilot study, 2 male beagle dogs were given oral doses of *p*-t-butyl-alpha-methyklihydrocinnamaldehyde at increasing dose level beginning at 50 ul/kg/day for the first week and reaching 400 ul/kg/day from weeks 48. At week 9, the dose was increased to 600 ul/kg/day. Histopathological examination revealed no significant changes to the any of the tissue, including the testes, evaluated [Givaudan-Roure, 1990e]. In a similar study, 3 female Beagle dogs were given capsules containing 200 mg/kg bw *p*-t-butyl-alpha-methylhydrocinnamaldehyde daily for 13 weeks. Again no adverse effects were observed [Givaudan-Roure, 1990f].

Finally, 2 rhesus monkeys were given 100 mg/kg bw of *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde administered in baby food daily for 5 days. Microscopic examination of the epididymides and testes failed to reveal any evidence of toxicity [Givaudan-Roure, 1990g]. The testicular and epididymal changes occurring in rats administered 50 mg/kg bw of p-t-butyl-alpha-methylhydrocinnamaldehyde by gavage 5 days per week for 90 days was not observed at 25 mg/kg bw and lower dose levels. Daily doses of 100 mg/kg bw for 5 days did not cause these effects in male mice, male guinea pigs, or male monkeys. Likewise no effects were observed after daily administration of 45 mg/kg bw to male (3) and female (3) dogs (beagles) 5 days per week for 90 days.

Plasma pharmacokinetic studies were performed after oral administration of 25 or 100 mg/kg bw of p-t-butyl-alpha-methylhydrocinnamaldehyde to rats. Peak plasma levels of 14.3 ug equivalents/ml at 3.5 hours and 52 ug equivalents/ml at 1.75 hours were achieved with the low and high dose, respectively, in male rats. The O-48 hour Area Under the Curve (AUC) was 122 ug.hr/ml and 937 ug.hr/ml, respectively [Hawkins et. *al.*, 1995]. Peak plasma levels and AUC were also measured after dermal administration 16 mg of *p*-t-butyl-alpha-methylhydrocinnamaldehyde to humans. This dose was estimated to be approximately equivalent to a high-level exposure encountered in a cosmetic application. Peak plasma levels never exceeded the detection limit of 0.0.25 ug/ml and a theoretical "upper limit" AUC was estimated to be 0.3 ug.hr/ml [Hawkins et. *al.*, 1994]. Based on a comparison of peak plasma levels and AUC for humans and male rats, it was concluded that the adverse effect levels were at least 3 orders of magnitude greater than levels of exposure in humans under conditions of use. Also, no effect levels in rats occurred at dose levels at least 2 orders of magnitude greater than estimated human exposure.

3.4.4 Reproductive Toxicity

Reproductive studies on cinnamyl derivatives have concentrated on the parent alcohol, aldehyde, and acid. Rats were administered 5, 25, or 250 mg/kg bw/day cinnamaldehyde by gavage in olive oil on days 7 to 17 of gestation. A control group was included; however, it was not stated whether or not the controls received the olive oil vehicle. The number of dams treated per group was 15, 14, 16 and 15 for the control, low-, mid-, and high-dose groups, respectively. Fetal abnormalities observed included: poor cranial

ossification in all dose groups; increased incidences of dilated pelvis/reduced papilla in the kidney as well as dilated urethras in the low- and mid-dose groups; and an increase in the number of fetuses with two or more abnormal stemebrae in the mid-dose group. These effects are associated with apparent maternal toxicity as evidenced by a dose related decrease in weight gain at the two highest dose levels [Mantovani *et al.*, 1989].

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

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

In an additional study by the same authors, female rats were orally administered 0, 5, or 50 mg cinnamic acid/kg bw once daily for the entire course of pregnancy. On day 20 of gestation, 50% of the females from all groups were terminated and the fetuses removed for examination. Fetal body weight measurements, number of survivors, bone development, and hepatic nucleic acids were determined and no significant differences between test and control animals were noted. The remaining females from both treated and control groups delivered normally on days 22-23 of gestation. Neither measurements

of offspring bodyweight, size, survival number, nor general development at birth or one month following revealed any significant differences between test and control animals [Zaitsev and Maganova, 1975].

3.4.5 Developmental Toxicity

In an *in vivo* developmental toxicity assay, 50 time-mated CD-1 female mice received single oral doses of 1200 mg/kg of cinnamaldehyde in corn oil on days 613 of gestation. Female body weights were measured on days 6- 15 of gestation and 3 days postpartum. Endpoints monitored included litter size, birth weight, neonatal growth, and survival to 3 days postpartum. Based on the measured parameters there was no significant difference between test and control groups [Hardin et al., 1987].

3.4.6 New Testing Required

Based on the consistent low acute oral and dermal toxicity in 29 studies, the "weight of evidence" that these substances exhibit no significant genotoxic potential in standardized *in vitro* and *in vivo* assays, the lack of any significant toxicity at dose levels many orders of magnitude greater than estimated levels of human exposure, and the lack of any reproductive or developmental effects in the absence of high-dose maternal toxicity, it is concluded that no additional testing is necessary for this chemical category.

3.5 Test Plan Table

Chemical	Physical-Chemical Properties				
	Melting Point	Boiling Point	Vapor Pressure	**Partition Coefficient**	**Water Solubility**
CAS No. 104-55-2 Cinnamaldehyde	NA	A	Calc	Calc	T,Calc
CAS No. **122-40-7** alpha-Amylcinnamaldehyde	NA	A	Calc	A	Calc
CAS No. 101-86-o alpha-Hexylcinnamaldehyde	NA	A	A	A	Calc
CAS No. 80-54-6 p-t-Butyl-alpha-methyldihydrocinnamaldehyde	NA	A	Calc	A	T, A

Chemical	Environmental Fate and Pathways			
	Photodegradation	Stability in Water	Biodegradation	Fugacity
CAS No. 104-55-2 Cinnamaldehyde	Calc	Calc	R	Calc
CAS No. **122-40-7** alpha-Amylcinnamaldehyde	Calc	Calc	A	Calc
CAS No. 101-86-o alpha-Hexylcinnamaldehyde	Calc	Calc	A	Calc
CAS No. 80-54-6 p-t-Butyl-alpha-methyldihydrocinnamaldehyde	Calc	A	A	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants
CAS No. 104-55-2 Cinnamaldehyde	Test, Calc	Test, Calc	Test, Calc
CAS No. 122-40-7 alpha-Amylcinnamaldehyde	Calc	Calc	Calc
CAS No. 101-86-o alpha-Hexylcinnamaldehyde	Calc	Calc	Calc
CAS No. 80-54-6 p-t-Butyl-alpha-methyldihydrocinnamaldehyde	Test, Calc	Test, Calc	Test, Calc

Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity *In Vitro*	Genetic Toxicity *In Vivo*	Repeat Dose Toxicity	Repro-ductive Toxicity	Develop mental Toxicity
CAS No. 104-55-2 Cinnamaldehyde	A	A	A	A	A	A
CAS No. 122-40-7 alpha-Amylcinnamaldehyde	A	A	A	A	R	R
CAS No. 101-86-o alpha-Hexylcinnamaldehyde	A	A	A	A	R	R
CAS No. 80-54-6 p-t-Butyl-alpha-methyldihydrocinnamaldehyde	A	A	A	A	R	R

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
N R	Not required per the OECD SIDS guidance
N A	Not applicable due to physical/chemical properties
0	Other

4 References for Test Plan and Robust Summaries

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