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The Flavor and Fragrance High Production Volume Consortia

The Aromatic Consortium

Revised 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. 101-86-0**
***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:

The Flavor and Fragrance High Production Volume Chemical Consortia

1620 I Street, NW, Suite 925

Washington, D.C. 20006

Phone: 202-331-2325

Fax: 202-463-8998

List of Member Companies in the

Aromatic Consortium Supporting

the Cinnamyl Derivatives

BASF

Noveon, Incorporated

Firmenich, Incorporated

Givaudan

Symrise, Incorporated

International Flavor & Fragrances, Inc.

Polarome International

Quest International

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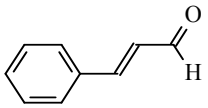
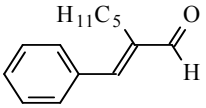


Cinnamaldehyde

3-phenyl-2-propenal

CAS No. 104-55-2



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2-amyl-3-phenyl-2-propenal

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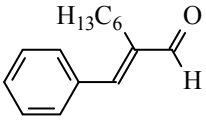

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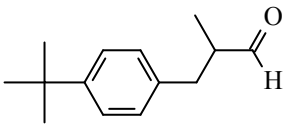


***p*-t-Butyl-*alpha*-methylhydrocinnamaldehyde**

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 revised test plan, category analysis and robust summaries along with new data presented below are the second 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 cinnamaldehyde 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 451,400 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 *trans*- isomer of cinnamaldehyde predominates in nature. On a commercial scale, cinnamaldehyde is prepared almost exclusively from the alkaline condensation of benzaldehyde 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 carboxylic 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 (8/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 mmol/kg 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 (ALD) [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 than 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-(1-phenyl-3-hydroxypropyl)cysteine [Delbressine *et al.*, 1981].

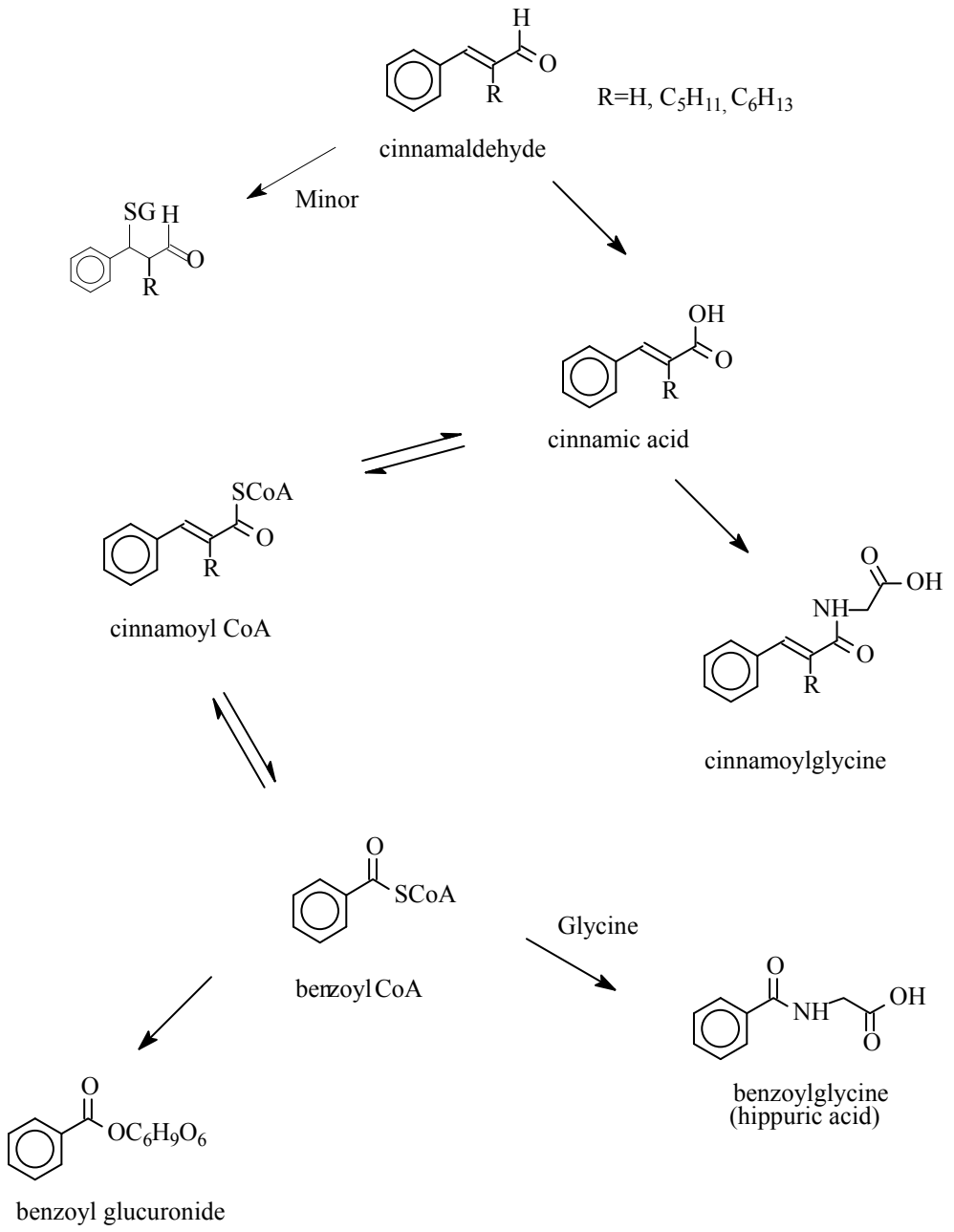
The position and size of the substituent do not significantly affect the pathways of metabolic detoxication of cinnamyl derivatives. Cinnamyl derivatives containing *alpha*-alkyl substituents (e.g.. *alpha*-methylcinnamaldehyde) are extensively metabolized *via beta*-oxidation followed by cleavage to yield mainly the corresponding hippuric acid derivative. A benzoic acid metabolite was isolated from the urine of dogs given either *alpha*-methylcinnamic 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*-methoxycinnamic 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



The saturated analogues such as 3-phenyl-1-propyl derivatives participate in the same *beta*-oxidation pathways as do cinnamic acid derivatives. Like cinnamic acid, 3-phenyl-1-propanol is oxidized to the corresponding acid which as the CoA ester undergoes beta oxidation and dehydration to yield the corresponding cinnamyl CoA derivative. When ring deuterated 3-phenylpropionic acid is administered orally to a human as a single dose (57 mg), deuterobenzoic acid corresponding to 110% of the dose is isolated from the alkaline hydrolyzed urine collected within 100 minutes of dosing (Pollitt, 1974).

Eleven adult volunteers received single intravenous doses of cinnamic acid, equivalent to 5 mg/kg bw. Analysis of the blood plasma revealed cinnamic acid at 100% of the total dose within 2.5 minutes declining to 0% after 20 minutes. Ninety minutes after dosing, urinalysis revealed mainly the glycine conjugate of benzoic acid (hippuric acid), cinnamoylglucuronide, and benzoylglucuronide present in a ratio of 74:24.5:1.5 (Quarto di Palo and Bertolini, 1961). These data demonstrate that 3-phenylpropionic acid and cinnamic acid are rapidly oxidized to benzoic acid metabolites, and excreted in the urine of humans.

In conclusion, the presence of unsaturation in the side chain or alkyl substituents at the alpha position in the side chain does not significantly affect the primary metabolic pathway of detoxication. For the four substances in this category, it is anticipated that each member will undergo beta-oxidation and cleavage to eventually yield the corresponding benzoic acid derivative that is excreted in the urine mainly as the glycine (hippurate) conjugate.

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 ^{o}C [Merck, 1997], 80 ^{o}C for *alpha*-amylcinnamaldehyde [CRC, 1973], while that of *alpha*-hexylcinnamaldehyde is 4°C [Fenaroli's, 1994]. The calculated [SRC] melting point for cinnamaldehyde is 0.04 ^{o}C while those for *alpha*-amylcinnamaldehyde and *alpha*-hexylcinnamaldehyde are 46°C and 44°C, respectively.

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*-hexylcinnamaldehyde (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 measured vapor pressure of 0.0289 mm Hg [CRC, 1973] for cinnamaldehyde and the calculated vapor pressure of 0.02 mm Hg [FMA] and 0.09 mm Hg (Antoine and Grain Method) [SRC] are in good agreement. The calculated vapor pressure of less than 0.001 mm Hg [FMA] and 0.0012 mm Hg (Modified Grain Method) [SRC] for *alpha*-amylcinnamaldehyde, and 0.00358 mm Hg (Modified Grain Method) for *p*-*t*-butyl-*alpha*-

methylhydrocinnamaldehyde [SRC] are consistent with that of *alpha*-hexylcinnamaldehyde since the increased vapor pressure of the former two aldehydes reflect their decreased molecular weights (14 daltons) compared to the *alpha*-hexyl derivative.

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 1.9 [CRC, 1973], slightly higher than the calculated log Kow of 1.82 [SRC].

3.1.5 Water Solubility

The measured water solubility of cinnamaldehyde of 1420 mg/L [SRC] is slightly less than the calculated value of 2150 mg/L [SRC]. 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). Based on the difference in measured and calculated data for the *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde and the small difference in molecular weight between that substance and the *alpha*-amyl and *alpha*-hexyl derivatives, it is anticipated that the measured solubilities of *alpha*-amylcinnamaldehyde and *alpha*-hexylcinnamaldehyde will be significantly higher than the calculated values of 8.5 mg/L and 2.75 mg/L, respectively. It is estimated that the *alpha*-amyl and *alpha*-hexyl derivatives will exhibit water solubility in the range of 25-35 mg/L and 5-10 mg/L, respectively.

3.1.6 New Testing Required

None

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 hours. 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-Roure, 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]. In addition, *p*-*t*-butyl-*alpha*-methylhydrocinnamaldehyde was more than 50% oxidized in air in 16 days in an OECD 111 test [Givaudan-Roure, 1995b] suggesting that the substance has approximately a two-week lifetime in the environment. The three cinnamyl derivatives met the 10 day window criteria for biodegradability. Cinnamaldehyde was 100% biodegraded after 21 days in an

OECD 301B test [Haarmann and Reimer, 2001]. In conclusion, all of the members of this chemical category are readily biodegradable.

3.2.4 Fugacity

The transport, distribution and persistence in the environment were modeled using the EQC Fugacity Model III [MacKay *et al*., 1996]. Based on input parameters of molecular weight, vapor pressure, log Kow, water solubility, melting point, and boiling point, it was calculated that the three alpha-substituted cinnamyl derivatives are distribution mainly to the soil was (47.7 and 68.4%) and to water (25.4 and 44.8 %). This is consistent with molecular polarity of the substances. Persistence in the environment was relatively short (364 and 632 hours) which is consistent with the ready biodegradability of these substances. Cinnamaldehyde is also distributed mainly to soil (54.35) and water (44.8%) with a short persistence in the environment (279 hours). Based on these calculations, the members of this category are distributed mainly to the water and soil with relatively short (*i.e*., days to weeks) persistence in these environmental compartments.

Transport and distribution in the environmental were modeled using Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11 [Mackay and Donald, 1991]. 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

The acute toxicity of cinnamaldehyde in Zebra fish/*Brachydanio rerio* has been determined in a 96-hour semi-static toxicity test [Caspers, 1993]. The measured 96-hour NOEC=2.8 mg/L, the LC50=4.3 mg/L, and the LC100=5.5 mg/L. The calculated 96-hour LC50 for cinnamaldehyde is 11.9 mg/L, slightly higher but in the same order of magnitude as the measured value. 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). Based on the fish acute toxicity data for cinnamaldehyde and the measured LC50 values for aquatic invertebrates (see below), the acute LC50 value for cinnamaldehyde is 4.3 mg/L while the three alkyl-substituted cinnamyl derivatives are expected to exhibit LC50 values of approximately 1 to 1.5 mg/L.

3.3.2 Acute Toxicity to Aquatic Invertebrates

The acute 48-hour EC50 and LC50 for cinnamaldehyde in *Daphnia magna* under semi-static conditions were 3.86 and 4.22 mg/L, respectively. The NOEC for cinnamaldehyde in *Daphnid magna* is 1.91 mg/L [Ward, 2003a]. In a second test using cinnamaldehyde (Assay; 65-75%), the acute 48-hour EC50 in *Daphnia magna* under static conditions was 11.5 mg/L [Barth and Winkler, 2001]. 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.

In a third test in *Daphnia magna*, the acute 48-hour EC50 for *alpha*-amylcinnamaldehyde in *Daphnia magna* under static conditions was 1.1 mg/L, respectively. The NOEC in Daphnid magna was 0.4 mg/L [Caspers, 1993]. 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, much below the measured value.

The calculated LC50 for *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde is in the same range, 0.403 mg/L. based on the experimental data for on cinnamaldehyde and *alpha*-amylcinnamaldehyde the EC50 values for members of this group should be in the range from 1-4 mg/L with cinnamaldehyde exhibiting the highest value.

3.3.3 Acute Toxicity to Aquatic Plants

Two acute algae toxicity studies has been recently performed. In one study, green algae (*Selenastrum capricornutum*) exposed to various concentrations of cinnamaldehyde exhibited a 72-hr EC50=6.87 mg/L based on average specific growth rate [Ward, 2003b]. The 72-hr EC50 was 4.56 mg/L calculated based upon the number of cells/mL or 4.07 mg/L based on the area under the growth curve. The 72-hr NOEC was 2.00 mg/L based on number of cells/mL. ECOSAR calculated 48-hour EC50 values for cinnamaldehyde was in the same range (8.1 mg/L). In the second study, green algae (*Selenastrum capricornutum*) exposed to *alpha*-amylcinnamaldehyde exhibited a 72-hr EC50=1.88 mg/L based on average specific growth rate [Ward, 2003c]. The 72-hr EC5 was 1.18 mg/L calculated based upon the number of cells/mL or 1.24 mg/L based on the area under the growth curve. The 72-hr NOEC was 0.154 mg/L based on number of cells/mL. Similar to cinnamaldehyde, ECOSAR calculated 48-hour EC50 values for *alpha*-amylcinnamaldehyde was in the same range (0.871 mg/L). The ECOSAR 48-hour EC50 values for *alpha*-hexylcinnamaldehyde (0.343 mg/L), and *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde (0.827 mg/L) are consistent with calculated values for *alpha*-amylcinnamaldehyde. The calculated and measured values are also consistent with those for acute fish and aquatic invertebrate toxicity cited above.

The only other 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]. Based on the good agreement between the measured and calculated values, it is estimated that the EC50 values for the three alkyl substituted cinnamaldehyde derivatives should be between 1 and 1.5 mg/L while the that for cinnamaldehyde is 4-5 mg/L.

3.3.4 New Testing Required

None

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, 1971; Moreno, 1972; Moreno, 1973; Moreno, 1974; Moreno, 1975; Moreno, 1976; Moreno, 1977a; Moreno, 1981; 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 5000mg/kg for *p*-*t*-butyl-*alpha*-methylhydrocinnamaldehyd*e* [Moreno, 1971; Moreno, 1973b; Moreno, 1977b; Shelanski, 1973; Draize *et al.*, 1948; Zaitsev and Rakhmanina, 1974].

3.4.2 Genetic Toxicity

3.4.2.1 In vitro

Cinnamaldehyde (*trans* and unspecified stereochemistry), *alpha*-amylcinnamaldehyde, *alpha*-hexylcinnamaldehyde, and *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde 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; Marnett *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 pre-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]. There 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 cinnamaldehyde 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]. Also, there was no evidence of an increased incidence of micronuclei in the polychromatic erythrocytes isolated from male and female of mice maintained on diets containing 0, 4100,8200,16,500, and 33,000 ppm microencapsulated cinnamaldehyde for 90 days [NTP, 2003].

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 (10/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), 50, 100, or 200 mg/kg 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, 1958b].

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].

In a 2-year bioassay on trans-cinnamaldehyde (NTP, 2002), groups of 50 F344/N rats and B6C3F1 mice of both sexes were administered diets containing 0, 1000, 2100, or 4100 ppm of trans-cinnamaldehyde in modified corn starch and sucrose microcapsules. The microcapsules were coated with modified corn starch. The dietary load of microencapsulated trans-cinnamaldehyde was maintained at 1.25%. A vehicle control group (50/sex) received placebo microcapsules (1.25%) in the diet and an untreated control (50/sex) was maintained on the standard NTP-2000 feed. Analysis of the diet every 9 to 12 weeks demonstrated that the diet was homogeneous throughout the study. The dietary levels were estimated to provide an average daily intake of 0, 50, 100 or 200 mg/kg bw of trans-cinnamaldehyde in rats and 125, 270, or 540 mg/kg bw of trans-cinnamaldehyde in mice.

Food and water was made available *ad libitum* to animals housed either individually (male mice), 2 to 3 per cage (male rats) of 5 per cage (female rats and mice). All animals were observed twice daily and body weights were recorded initially, on days 8 and 36, and then every 4 weeks to completion of the study. Complete necropsies and histopathological examinations were performed on all animals at the conclusion of the study. The urine of randomly selected male and female rats (10/sex/group) from each treated group was collected and analyzed for hippuric acid, the principal metabolite of *trans*-cinnamaldehyde.

Survival in male rats at the highest feeding level (4100 ppm) was greater than that for the vehicle control group. Mean body weight in males in the 4100 ppm group and in the 2100 ppm group after week 94 were less than that of the vehicle control group. Throughout the study, the rate of hippuric acid excretion reported as the hippuric acid/creatinine ratio was proportional to dose, supporting the conclusion that the primary metabolic pathway was

not saturated over the 2 years of exposure in rats. There was no increase in the incidence of either non-neoplastic or neoplastic lesions in any group of treated male or female rats.

In mice, there was no dose-related decrease in survival for either sex of B6C3F1 mice. Mean body weight of the 2100 and 4100 ppm groups was generally less than that for the vehicle control group. Although squamous cell papillomas {1(M) and 3(F)} and carcinoma {1(M) and 1(F)} were reported in the 2100 ppm group (4% in males and 8% in females), the incidence of these lesions was within the historical control range (0-6%) for animals maintained on an NTP 2000 diet. Also there was no significant increase in this type of lesion in the higher dose group (4100 ppm). Although there was no evidence of a statistically significant increase in the incidence of neoplasms in any group treated with *trans*-cinnamaldehyde, there was a statistically significant decrease in the incidence of hepatocellular ademonas and carcimonas in male mice in the 2100 and 4100 ppm groups and a negative trend in female mice compared to the vehicle control group. NTP researchers had previously correlated (Haseman *et al.*, 1997) the decreased incidence of liver neoplasms with decreased body weights in previous NTP studies using the NTP2000 diet. The NTP Board of Scientific Counselors Technical Report Review Subcommittee met for a peer review of the recently issued draft NTP Technical Report on *trans*-cinnamaldehyde (NTP, 2002). The Subcommittee concluded: *"Under the conditions of these 2-year feed studies there was no evidence of carcinogenic activity of trans-cinnamaldehyde in male or female F344/N rats exposed to 1000, 2100, or 4100 ppm. There was no evidence of carcinogenic activity of trans-cinnamaldehyde in male or female B6C3F1 mice exposed to 1000, 2100, or 4100 ppm."*

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 (5/sex) 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, or 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*-methylhydrocinnamaldehyde 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 9-week pilot study, 2 male beagle dogs were given oral doses of *p*-t-butyl-alpha-methyldihydrocinnamaldehyde at increasing dose level beginning at 50 ul/kg/day for the first week and reaching 400 ul/kg/day from weeks 4-8. 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 0-48 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 0.025 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

Repeat dose studies cited above indicated high-dose effects to the reproductive organs of rodents, mainly rats, but not in other species including dogs and Rhesus monkeys. Development of a pharmacokinetic model [Hawkins *et. al.*, 1994] demonstrated that reproductive effects occurred at levels of exposure at least two orders of magnitude above human exposure to *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde. Based on these studies, *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde (BMHCA) does not present a significant potential for reproductive toxicity to humans.

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 sternebrae 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 nor 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 6-13 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 Cinnamyl Derivatives-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	A, Calc	A, Calc	A,Calc
CAS No. 122-40-7 *alpha*-Amylcinnamaldehyde	NA	A	A, Calc	A	Calc
CAS No. 101-86-0 *alpha*-Hexylcinnamaldehyde	NA	A	A	A	Calc
CAS No. 80-54-6 *p*-t-Butyl-*alpha*-methyldihydrocinnamaldehyde	NA	A	Calc	A	A, Calc

Chemical	Environmental Fate and Pathways			
	Photodegradation	**Stability in Water**	**Biodegradation**	**Fugacity**
CAS No. 104-55-2 Cinnamaldehyde	Calc	Calc	A	Calc
CAS No. 122-40-7 *alpha*-Amylcinnamaldehyde	Calc	Calc	A	Calc
CAS No. 101-86-0 *alpha*-Hexylcinnamaldehyde	Calc	Calc	A	Calc
CAS No. 80-54-6 *p*-t-Butyl-*alpha*-methyldihydrocinnamaldehyde	Calc	Calc	A	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	**Acute Toxicity to Aquatic Invertebrates**	**Acute Toxicity to Aquatic Plants**
CAS No. 104-55-2 Cinnamaldehyde	A, Calc	A, Calc	A, Calc
CAS No. 122-40-7 *alpha*-Amylcinnamaldehyde	A, Calc	A, Calc	A, Calc
CAS No. 101-86-0 *alpha*-Hexylcinnamaldehyde	Calc	Calc	Calc
CAS No. 80-54-6 *p*-t-Butyl-*alpha*-methyldihydrocinnamaldehyde	Calc	Calc	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. 101-86-0 *alpha*-Hexylcinnamaldehyde	A	A	A	A	R	R
CAS No. 80-54-6 *p*-t-Butyl-*alpha*-methyldihydrocinnamaldehyde	A	A	A	A	A	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
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
O	Other

3.6 References for Test Plan and Robust Summaries

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