SCREENING-LEVEL HAZARD CHARACTERIZATION OF HIGH PRODUCTION VOLUME CHEMICALS

CHEMICAL CATEGORY NAME
Cinnamyl Derivatives

SPONSORED CHEMICALS

Cinnamaldehyde (3-phenyl-2-propenal) **CAS No. 104-55-2**
[9th CI Name: 2-Propenal, 3-phenyl-]

***alpha*-Amylcinnamaldehyde (2-amyl-3-phenyl-2-propenal)** **CAS No. 122-40-7**
[9th CI Name: Heptanal, 2-(phenylmethylene)-]

***alpha*-Hexylcinnamaldehyde (2-hexyl-3-phenyl-2-propenal)** **CAS No. 101-86-0**
[9th CI Name: Octanal, 2-(phenylmethylene)-]

***p*-*t*-Butyl-*alpha*-methyldihydrocinnamaldehyde (3-*p*-*t*-butylphenyl)-2-methylpropanal)** **CAS No. 80-54-6**
[9th CI Name: Benzene propanal, 4-(1,1-dimethylethyl)-*alpha*-methyl-]

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INTERIM

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SCREENING-LEVEL HAZARD CHARACTERIZATION
OF HIGH PRODUCTION VOLUME CHEMICALS

The High Production Volume (HPV) Challenge Program[1] is a voluntary initiative aimed at developing and making publicly available screening-level health and environmental effects information on chemicals manufactured in or imported into the United States in quantities greater than one million pounds per year. In the Challenge Program, producers and importers of HPV chemicals voluntarily sponsor chemicals; sponsorship entails the identification and initial assessment of the adequacy of existing toxicity data/information, conducting new testing if adequate data do not exist, and making both new and existing data and information available to the public. Each complete data submission contains data on 18 internationally agreed to "SIDS" (Screening Information Data Set[1,2]) endpoints that are screening-level indicators of potential hazards (toxicity) for humans or the environment.

The Environmental Protection Agency's Office of Pollution Prevention and Toxics (OPPT) is evaluating the data submitted in the HPV Challenge Program on approximately 1400 sponsored chemicals. OPPT is using a hazard-based screening process to prioritize review of the submissions. The hazard-based screening process consists of two tiers described below briefly and in more detail on the Hazard Characterization website[3].

Tier 1 is a computerized sorting process whereby key elements of a submitted data set are compared to established criteria to "bin" chemicals/categories for OPPT review. This is an automated process performed on the data as submitted by the sponsor. It does not include evaluation of the quality or completeness of the data.

In Tier 2, a screening-level hazard characterization is developed by EPA that consists of an objective evaluation of the quality and completeness of the data set provided in the Challenge Program submissions. The evaluation is performed according to established EPA guidance[2,4] and is based primarily on hazard data provided by sponsors. EPA may also include additional or updated hazard information of which EPA, sponsors, or other parties have become aware. The hazard characterization may also identify data gaps that will become the basis for a subsequent data needs assessment where deemed necessary. Under the HPV Challenge Program, chemicals that have similar chemical structures, properties and biological activities may be grouped together and their data shared across the resulting category. This approach often significantly reduces the need for conducting tests for all endpoints for all category members. As part of Tier 2, evaluation of chemical category rationale and composition and data extrapolation(s) among category members is performed in accord with established EPA[2] and OECD[5] guidance.

The screening-level hazard characterizations that emerge from Tier 2 are important contributors to OPPT's existing chemicals review process. These hazard characterizations are technical documents intended to support subsequent decisions and actions by OPPT. Accordingly, the documents are not written with the goal of informing the general public. However, they do provide a vehicle for public access to a concise assessment of the raw technical data on HPV chemicals and provide information previously not readily available to the public. The public, including sponsors, may offer comments on the hazard characterization documents.

The screening-level hazard characterizations, as the name indicates, do not evaluate the potential risks of a chemical or a chemical category, but will serve as a starting point for such reviews. In 2007, EPA received data on uses of and exposures to high-volume TSCA existing chemicals, submitted in accordance with the requirements of the Inventory Update Reporting (IUR) rule. For the chemicals in the HPV Challenge Program, EPA will review the IUR data to evaluate exposure potential. The resulting exposure information will then be combined with the screening-level hazard characterizations to develop screening-level risk characterizations[4,6]. The screening-level risk characterizations will inform EPA on the need for further work on individual chemicals or categories. Efforts are currently underway to consider how best to utilize these screening-level risk characterizations as part of a risk-based decision-making process on HPV chemicals which applies the results of the successful U.S. High Production Volume Challenge Program and the IUR to support judgments concerning the need, if any, for further action.

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[1] U.S. EPA. High Production Volume (HPV) Challenge Program; http://www.epa.gov/chemrtk/index.htm.
[2] U.S. EPA. HPV Challenge Program – Information Sources; http://www.epa.gov/chemrtk/pubs/general/guidocs.htm.
[3] U.S. EPA. HPV Chemicals Hazard Characterization website (http://www.epa.gov/hpvis/abouthc.html).
[4] U.S. EPA. Risk Assessment Guidelines; http://cfpub.epa.gov/ncea/raf/rafguid.cfm.
[5] OECD. Guidance on the Development and Use of Chemical Categories; http://www.oecd.org/dataoecd/60/47/1947509.pdf.
[6] U.S. EPA. Risk Characterization Program; http://www.epa.gov/osa/spc/2riskchr.htm.

SCREENING-LEVEL HAZARD CHARACTERIZATION
Cinnamyl Derivatives Category

Introduction

The sponsor, The Flavor and Fragrance High Production Volume Consortia (FFHPVC), submitted a Test Plan and Robust Summaries to EPA for the Cinnamyl Derivatives Category on December 27, 2000. EPA posted the submission on the ChemRTK Web site on February 7, 2001 (http://www.epa.gov/chemrtk/pubs/summaries/cinna/c12912tc.htm). EPA comments on the original submission were posted to the website on June 7, 2001. Public comments were also received and posted to the website. The sponsor submitted updated/revised documents on March 15, 2005, which were posted to the ChemRTK website on May 10, 2005. The cinnamyl derivatives category consists of the following chemicals:

Cinnamaldehyde (3-phenyl-2-propenal) [9th CI Name: 2-Propenal,3-phenyl-)]	CAS No. 104-55-2
alpha-Amylcinnamaldehyde (2-amyl-3-phenyl-2-propenal) [9th CI Name: Heptanal,2-(phenylmethylene)-)]	CAS No. 122-40-7
alpha-Hexylcinnamaldehyde (2-hexyl-3-phenyl-2-propenal) [9th CI Name: Octanal,2-(phenylmethylene)-)]	CAS No. 101-86-0
p-t-Butyl-*alpha*-methylhydrocinnamaldehyde (3-*p*-t-butylphenyl)-2-methylpropanal) [9th CI Name: Benzene propanal,4-(1,1-dimethylethyl)-*alpha*-methyl-)]	CAS No. 80-54-6

This screening-level hazard characterization is based primarily on the review of the test plan and robust summaries of studies submitted by the sponsor(s) under the HPV Challenge Program. In preparing the hazard characterization, EPA considered its own comments and public comments on the original submission as well as the sponsor's responses to comments and revisions made to the submission. Structures of the sponsored chemicals are included in the appendix. The screening-level hazard characterization for environmental and human health toxicity is based largely on SIDS endpoints and is described according to established EPA or OECD effect level definitions and hazard assessment practices.

Category Justification

The four members of the cinnamyl derivatives category are naturally occurring substances. Cinnamaldehyde is the predominant constituent of cassia oil and Ceylon cinnamon bark oil. It is a common component of traditional foods; responsible for the spicy aroma strongly reminiscent of cinnamon spice. Cinnamaldehyde, *alpha*-amylcinnamaldehyde and *alpha*-hexylcinnamaldehyde are generally recognized as safe (GRAS) as flavoring substances by the U.S. Food and Drug Administration (US FDA) and as food additives by the World Health Organization (WHO). *p*-t-Butyl-*alpha*-methylhydrocinnamaldehyde is used only in fragrance products, and *alpha*-amylcinnamaldehyde and *alpha*-hexylcinnamaldehyde are also used in fragranced consumer products such as soaps and cosmetics. The grouping into one category is based on similarities in structure and physical-chemical and toxicological properties.

EPA agreed with the sponsor's category approach except that for the human health endpoints, it did not consider the inclusion of *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde adequately justified. In its response to EPA comments on the original test plan, the sponsor provided further justification for the inclusion of *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde in the category by describing studies for the metabolism of saturated and unsaturated phenyl substituted aldehydes. Each of the category members is oxidized to the corresponding acid followed either by conjugation and excretion or by *beta*-oxidation, conjugation and excretion. EPA agrees with the explanation that the inclusion of *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde for the human health endpoints is justified.

Summary-Conclusion

The log K_{ow} of cinnamaldehyde indicates that its potential to bioaccumulate is low. The log K_{ow} values of *alpha*-amylcinnamaldehyde, *alpha*-hexylcinnamaldehyde and *p*-*t*-butyl-*alpha*-methylhydrocinnamaldehyde indicate that their potential to bioaccumulate is high. The cinnamyl derivative category members are readily biodegradable, indicating that they are not expected to persist in the environment.

The evaluation of available toxicity data (measured and estimated) for fish, aquatic invertebrates and aquatic plants indicates that the potential acute hazard of cinnamaldehyde and *alpha*-amylcinnamaldehyde to aquatic organisms is moderate. However, the more highly substituted category members, *alpha*-hexylcinnamaldehyde and *p*-*t*-butyl-*alpha*-methylhydrocinnamaldehyde, are estimated to pose high acute hazard to aquatic invertebrates and plants.

The acute oral, dermal and inhalation toxicity for the members of the cinnamyl derivatives category is low. Cinnamaldehyde and *p*-*t*-butyl-*alpha*-methylhydrocinnamaldehyde are eye and skin irritants in experimental animals. Cinnamaldehyde and *alpha*-hexylcinnamaldehyde are skin sensitizers in experimental animals. However, *p*-*t*-Butyl-*alpha*-methylhydrocinnamaldehyde does not cause skin irritation and is not a skin sensitizer based on human repeat insult patch tests. Repeated exposures to all category members via the oral route indicate an effect on body weight and toxicity to multiple organs (forestomach in rats and mice and liver, kidney, testicular atrophy in rats) as well as an effect on spermatogenesis. Evaluation of reproductive organs from the 90-day repeated-dose toxicity studies for *alpha*-hexylcinnamaldehyde and *p*-*t*-butyl-*alpha*-methylhydrocinnamaldehyde showed no effects on reproductive organs. However, limited information on reproductive organs from a 14-day oral toxicity study showed effects on male and female reproductive organs. Developmental toxicity data for cinnamaldehyde suggest that rats are more sensitive to this category of substances than mice. Developmental effects included decreased ossification of the cranium and tympanic bulla, increased incidence of dilated pelvis/reduced papilla in kidney, dilated ureter and incidences of hypoplastic/dysplastic kidneys. The members of the cinnamyl derivatives category did not show mutagenic potential when tested *in vitro* in *Salmonella typhimurium,* excepting a positive test in strain TA100 with cinnamaldehyde. Cinnamaldehyde also induced chromosomal aberrations *in vitro*, but the data were equivocal *in vivo*. The other category members were negative when tested *in vivo* for chromosomal aberrations. Results from 2-year carcinogenicity studies of the related chemical, *trans*-cinnamaldehyde, conducted by the National Toxicology Program, there was no evidence of carcinogenic activity of *trans*-cinnamaldehyde in male and female F344/N rats and $B6C3F_1$ mice.

The potential health hazard of the cinnamyl derivatives category is moderate based on repeated-dose and developmental toxicity. Available data suggest that cinnamaldehyde has potential to be genotoxic.

No data gaps were identified under the HPV Challenge Program.

1. Physical-Chemical Properties and Environmental Fate

A summary of physical-chemical properties and environmental fate data submitted is provided in Table 1 for the purpose of the screening-level hazard characterization, the review and summary of these data was limited to the octanol-water partition coefficient and biodegradation endpoints as indicators of bioaccumulation and persistence, respectively.

Octanol-Water Partition Coefficient

Cinnamaldehyde (CAS No. 104-55-2)
Log K_{ow} = 1.9 (measured)

alpha-Amylcinnamaldehyde (CAS No. 122-40-7)
Log K_{ow} = 4.7 (measured)

alpha-Hexylcinnamaldehyde (CAS No. 101-86-0)
Log K_{ow} = 5.3 (measured)

p-t-Butyl-alpha-methylhydrocinnamaldehyde (CAS No. 80-54-6)
Log K_{ow} = 4.2 (measured)

Biodegradation

Cinnamaldehyde (CAS No. 104-55-2)
In a carbon dioxide evolution method using secondary effluent from an unacclimatized activate as inoculum, 100% cinnamaldehyde had degraded after 28 days.
Cinnamaldehyde is readily biodegradable

alpha-Amylcinnamaldehyde (CAS No. 122-40-7)
(1) In a carbon dioxide evolution method using secondary effluent from an unacclimatized activate as inoculum, 65% *alpha*-amylcinnamaldehyde had degraded after 28 days.
***alpha*-Amylcinnamaldehyde is readily biodegradable**

(2) In a respirometric method using activated sludge as inoculum, 90% *alpha*-amylcinnamaldehyde had degraded after 28 days.
***alpha*-Amylcinnamaldehyde is readily biodegradable**

alpha-Hexylcinnamaldehyde (CAS No. 101-86-0)
(1) In a carbon dioxide evolution method using secondary effluent from an unacclimatized activate as inoculum, 76.5% *alpha*-hexylcinnamaldehyde had degraded after 28 days.
***alpha*-Hexylcinnamaldehyde is readily biodegradable.**

(2) In a respirometric method using activated sludge as inoculum, 97% *alpha*-amylcinnamaldehyde had degraded after 28 days.
***alpha*-Hexylcinnamaldehyde is readily biodegradable**

p-t-Butyl-alpha-methylhydrocinnamaldehyde (CAS No. 80-54-6)
In a respirometric method using activated sludge from a sewage plant as inoculum, 68% *p*-t-butyl-*alpha*-methyldihydrocinnamaldehyde had degraded after 28 days.
***p-t*-Butyl-*alpha*-methylhydrocinnamaldehyde is readily biodegradable**

Conclusion: The log K_{ow} of cinnamaldehyde indicates that its potential to bioaccumulate is low. The log K_{ow} values of *alpha*-amylcinnamaldehyde, *alpha*-hexylcinnamaldehyde and *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde indicate that their potential to bioaccumulate is high. The cinnamyl derivative category members are readily biodegradable, indicating that they are not expected to persist in the environment.

Table 1. Summary of Physical-Chemical Properties and Environmental Fate Data				
Endpoints	**Cinnamaldehyde (104-55-2)**	***alpha*-Amyl cinnamaldehyde (122-40-7)**	***alpha*-Hexyl cinnamaldehyde (101-86-0)**	***p-t*-Butyl-*alpha*-methylhydro-cinnamaldehyde (80-54-6)**
Melting Point (°C)	**-7.5 (m)**	**80 (m)**	44.4 (e)	46.3 (e)
Boiling Point (°C)	**246 (m)**	**284 (m)**	**305 (m)**	**258 (m)**
Vapor Pressure (hPa at 25°C)	**3.5×10^{-2} (m)**	1.6×10^{-3} (e)	**2.7×10^{-4} (m)**	4.8×10^{-3} (e)
Log K_{ow}	**1.9 (m)**	**4.7 (m)**	**5.3 (m)**	**4.9 (m)**
Water Solubility (mg/L at 25°C)	**1420 (m)**	8.5 (e)	2.75 (e)	**33 (m)**
Indirect (OH^-) Photodegradation Half-life ($t_{1/2}$)	3.17 (e)	2.4 (e)	2.33 (e)	3.88 (e)
Stability in Water (Hydrolysis) ($t_{1/2}$)	All category members are expected to be stable in aqueous solution although they may be slowly oxidized to the corresponding cinnamic acid derivative.			
Fugacity (Level III Model)				
Air (%)	0.597	0.575	0.449	0.597
Water (%)	25.6	32.6	25.4	25.6
Soil (%)	68.4	57.3	47.7	68.4
Sediment (%)	5.47 (e)	9.53 (e)	26.5 (e)	5.47 (e)
Biodegradation at 28 days (%)	**100 (m) Readily biodegradable**	**65 (m) Readily biodegradable**	**76.5 (m) Readily biodegradable**	**68 (m) Readily biodegradable**

(m) = measured data (i.e., derived from testing); (e) = estimated data (i.e., derived from modeling)

2. Environmental Effects – Aquatic Toxicity

A summary of aquatic toxicity data submitted for SIDS endpoints is provided in Table 2. The table also indicates where data for tested category members are read-across (RA) to untested members of the category.

Acute Toxicity to Fish

Cinnamaldehyde (CAS No. 104-55-2)
Zebrafish (*Brachydanio rerio*) were exposed to cinnamaldehyde at nominal concentrations of 0, 2.8, 3.9, 5.5 and 7.8 mg/L under semi-static conditions for 96 hours. The concentrations were measured analytically.
96-h EC_{50} = 4.3 mg/L

alpha-Amylcinnamaldehyde (CAS No. 122-40-7)
A 96-hour LC_{50} for fish, estimated by ECOSAR, was provided to support evaluation of the acute toxicity of *alpha*-amylcinnamaldehyde. The estimated concentration is consistent with the results obtained in the acute toxicity study cinnamaldehyde, supporting read-across.
96-h LC_{50} = 3.14 mg/L (estimated)

alpha-Hexylcinnamaldehyde (CAS No. 101-86-0)
A 96-hour LC_{50} for fish, estimated by ECOSAR, was provided to support evaluation of the acute toxicity of *alpha*-hexylcinnamaldehyde. The estimated concentration is consistent with the results obtained in the acute toxicity study cinnamaldehyde, supporting read-across.
96-h LC_{50} = 2.36 mg/L (estimated)

p-t-Butyl-alpha-methylhydrocinnamaldehyde (CAS No. 80-54-6)
A 96-hour LC_{50} for fish, estimated by ECOSAR, was provided to support evaluation of the acute toxicity of *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde. The estimated concentration is consistent with the results obtained in the acute toxicity study cinnamaldehyde, supporting read-across.
96-h LC_{50} = 3.19 mg/L (estimated)

Acute Toxicity to Aquatic Invertebrates

Cinnamaldehyde (CAS No. 104-55-2)
(1) *Daphnia magna* were exposed to cinnamaldehyde at nominal concentrations of 0, 2, 3.3, 5.5, 9.0, 15 and 25 mg/L under semi-static conditions for 48 hours with 24-hour renewal. The measured concentrations were 0.00452, 1.91, 3.34, 5.30, 9.57, 13.9 and 25.5 mg/L.
48-h EC_{50} = 3.86 mg/L

(2) *D. magna* were exposed to cinnamaldehyde (65 – 75%) at nominal concentrations of 0, 3.8, 7.5, 15.0, 30.0 and 60.0 mg/L under static conditions for 48 hours.
48-h EC_{50} = 11.5 mg/L

alpha-Amylcinnamaldehyde (CAS No. 122-40-7)
D. magna were exposed to *alpha*-amylcinnamaldehyde at nominal concentrations of 0, 0.2, 0.4, 0.7, 1.4, 2.8 and 5.5 mg/L under semi-static conditions for 48 hours. Measured concentrations were provided for the low and high nominal concentrations at 0- and 48-hour intervals and were 0.022 and 0.05 mg/L for 0.2 mg/L, respectively, and 5.12 and 2.94 mg/L for 5.5 mg/L, respectively.
48-h EC_{50} = 1.1 mg/L

alpha-Hexylcinnamaldehyde (CAS No. 101-86-0)
A 48-hour LC_{50} for *Daphnia*, estimated by ECOSAR, was provided to support evaluation of the acute toxicity of *alpha*-hexylcinnamaldehyde. ECOSAR estimates *alpha*-Hexylcinnamaldehyde to be more toxic than cinnamaldehyde.
48-h LC_{50} = 0.22 mg/L (estimated)

p-t-Butyl-alpha-methylhydrocinnamaldehyde (CAS No. 80-54-6)
A 48-hour LC_{50} for *Daphnia*, estimated by ECOSAR, was provided to support evaluation of the acute toxicity of *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde. ECOSAR estimates *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde to be more toxic than cinnamaldehyde.
48-h LC_{50} = 0.40 mg/L (estimated)

Toxicity to Aquatic Plants

Cinnamaldehyde (CAS No. 104-55-2)
Green algae (*Pseudokirchneriella subcapitata*) were exposed to cinnamaldehyde at nominal concentrations of 0, 0.5, 1.0, 2.0, 4.0 and 8.0 mg/L for 72 hours. The initial mean measured concentrations were 0.523, 1.04, 2.00, 3.80 and 7.03 mg/L. The final measured concentrations were 88 – 105% of the nominal concentrations.
72-h EC_{50} (biomass) = 4.56 mg/L
72-h EC_{50} (growth) = 6.87 mg/L

alpha-Amylcinnamaldehyde (CAS No. 122-40-7)
Green algae (*P. subcapitata*) were exposed to *alpha*-amylcinnamaldehyde at nominal concentrations of 0, 0.095, 0.19, 0.38, 0.75, 1.5 and 3.0 mg/L for 72 hours. The initial mean measured concentrations were 0.0934, 0.154, 0.363, 0.651, 1.39 and 2.75 mg/L. The final measured concentrations were 81 – 98% of the nominal concentrations.
72-h EC_{50} (biomass) = 1.18 mg/L
72-h EC_{50} (growth) = 1.88 mg/L

alpha-Hexylcinnamaldehyde (CAS No. 101-86-0)
A 96-hour LC_{50} for algae, estimated by ECOSAR, was provided to support evaluation of the acute toxicity of *alpha*-hexylcinnamaldehyde. ECOSAR estimates *alpha*-hexylcinnamaldehyde to be more toxic than cinnamaldehyde.
96-h LC_{50} = 0.34 mg/L (estimated)

p-t-Butyl-alpha-methylhydrocinnamaldehyde (CAS No. 80-54-6)
A 96-hour LC_{50} for algae, estimated by ECOSAR, was provided to support evaluation of the acute toxicity of *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde. ECOSAR estimates *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde to be more toxic than cinnamaldehyde.
96-h LC_{50} = 0.83 mg/L (estimated)

Conclusion: The evaluation of available toxicity data (measured and estimated) for fish, aquatic invertebrates and aquatic plants indicates that the potential acute hazard of cinnamaldehyde and *alpha*-amylcinnamaldehyde to aquatic organisms is moderate. However, the more highly substituted category members, *alpha*-hexylcinnamaldehyde and *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde, are estimated to pose high acute hazard to aquatic invertebrates and plants.

Table 2. Summary of Environmental Effects – Aquatic Toxicity Data

Endpoints	**Cinnamaldehyde (104-55-2)**	***alpha*-Amylcinnamaldehye (122-40-7)**	***alpha*-Hexylcinnamaldehye (101-86-0)**	***p*-t-Butyl-*alpha*-methylhydro-cinnamaldehyde (80-54-6)**
Fish 96-h LC_{50} (mg/L)	**4.3 (m)**	No Data 4.3 (RA) 3.14 (e)	No Data 4.3 (RA) 2.36(e)	No Data 4.3 (RA) 3.19 (e)
Aquatic Invertebrates 48-h EC_{50} (mg/L)	**3.86 (m)**	**1.1 (m)**	No Data 1.1 (RA) 0.22 (e)	No Data 1.1 (RA) 0.40 (e)
Aquatic Plants 72-h EC_{50} (mg/L) **(growth)** **(biomass)**	 **6.87 (m)** **4.56 (m)**	 **1.88 (m)** **1.18 (m)**	No Data 1.88 1.18 (RA) 0.34 (e)	No Data 1.88 1.18 (RA) 0.83 (e)

(m) = measured data (i.e., derived from testing); (RA) = Read Across

3. Human Health Effects

A summary of health effects data submitted for SIDS endpoints is provided in Table 3. The table also indicates where data for tested category members are read-across (RA) to untested members of the category.

Acute Oral Toxicity

Cinnamaldehyde (CAS No. 104-55-2)
(1) Guinea pigs (males and females, number not stated) were administered cinnamaldehyde orally. Doses were not included in the robust summary. Coma at higher doses was the only clinical sign of toxicity observed.
LD_{50} = 1160 mg/kg-bw

(2) Osborne-Mendel rats (10/sex/dose) were administered cinnamaldehyde orally. The observed clinical signs of toxicity were reduced activity, diarrhea and scrawny appearance.
LD_{50} = 2220 mg/kg-bw

alpha-Amylcinnamaldehyde (CAS No. 122-40-7)
Osborne-Mendel rats (5/sex/dose) were administered *alpha*-amylcinnamaldehyde orally. The observed clinical signs of toxicity were reduced activity and porphyrin-like deposits around the eyes and nose.
LD_{50} = 3730 mg/kg-bw

alpha-Hexylcinnamaldehyde (CAS No. 101-86-0)
Wistar rats (10 males/dose) were administered 1780, 2670, 4000 and 6000 mg/kg-bw of *alpha*-hexylcinnamaldehyde orally and were observed for 14 days. The observed clinical signs of toxicity were decreased activity, lethargy and anorexia.
LD_{50} = 3100 mg/kg-bw

p-t-Butyl-alpha-methylhydrocinnamaldehyde (CAS No. 80-54-6)
Rats (10/sex/dose) were administered 1.22, 2.47, 5.0 and 10.14 g/kg-bw of *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde orally. The observed clinical signs of toxicity were diarrhea, piloerection, lethargy, flaccidity, ataxia and coma.
LD_{50} = 3700 mg/kg-bw

Acute Dermal Toxicity

Cinnamaldehyde (CAS No. 104-55-2)
(1) Albino rabbits (2/dose) were administered 0.25, 0.50, 1.0, 2.0 and 4.0 mL/kg-bw (approximately 263, 525, 1050, 2100 and 4200 mg/kg-bw) of cinnamaldehyde dermally via intact and abraded skin.
LD_{50} = 620 mg/kg-bw

(2) New Zealand white rabbits (4/sex/dose) were administered 0.59, 0.83, 1.00, 1.23 and 1.50 mL/kg-bw (approximately 620, 872, 1050, 1292 and 1575 mg/kg-bw) of cinnamaldehyde dermally. Deaths occurred by day 3 and were preceded by the following clinical signs of toxicity: decreased feces, lethargy, ataxia and rales. Necropsy of the dead animals indicated effects on the lungs, liver, kidneys, treated skin and gastrointestinal tract, as well as brown staining in the anogenital region. Yellow staining of the nose/mouth area was observed. Clinical signs observed in the survivors were diarrhea, decreased feces, emaciation, ataxia and limited mobility due to severe skin reaction and abnormalities of the skin and intestines. The uterus was larger than normal.
LD_{50} ~1240 mg/kg-bw

(3) Albino rabbits (6/sex) administered 5000 mg/kg-bw of cinnamaldehyde dermally all died overnight after dosing.
LD_{50} < 5000 mg/kg-bw

(4) New Zealand White rabbits (5/sex) were administered 1000 mg/kg-bw of cinnamaldehyde dermally for 24 hours. Dermal responses were recorded on days 7 and 14 post-exposure. At necropsy, treated skin abnormalities (not stated) were observed in all animals. Abnormalities in the liver and kidney were observed.
LD_{50} > 1000 mg/kg-bw

alpha-Amylcinnamaldehyde (CAS No. 122-40-7)
Rabbits (4/sex) were administered 2000 mg/kg-bw *alpha*-amylcinnamaldehyde dermally. There was no evidence of toxicity at the dose tested.
LD_{50} > 2000 mg/kg-bw

alpha-Hexylcinnamaldehyde (CAS No. 101-86-0)
Rabbits (two females) were administered *alpha*-hexylcinnamaldehyde dermally for 24 hours and observed for 7 days. No animals died at any dose. Moderate erythema and occasional sloughing was observed, which was attributed to poor animal handling techniques.
LD_{50} > 3000 mg/kg

p-t-Butyl-alpha-methylhydrocinnamaldehyde (CAS No. 80-54-6)
(1) Rabbits (10/sex/dose) were administered *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde dermally route of exposure. No animals died at any dose. The observed clinical signs of toxicity were erythema (mild to moderate) and edema (mild to moderate).
LD_{50} > 5 g/kg

(2) Albino New Zealand rabbits (3/sex/dose) were administered 5 mL/kg of *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde dermally. The rabbits were observed for 14 days post-exposure. The observed clinical signs of toxicity were moderate erythema and thickened, wrinkled skin in all test animals, persisting through day 9. Subcapsular (agonal) hemorrhages of the kidneys were found at necropsy in most of the test animals.
LD_{50} > 5 mL/kg

Acute Inhalation Toxicity

alpha-Hexylcinnamaldehyde (CAS No. 101-86-0)
Sprague-Dawley rats (5/sex/dose) were administered a single 4-hour exposure to aerosol containing *alpha*-hexylcinnamaldehyde via inhalation at a nominal concentration of 5 mg/L and were observed for 14 days post-exposure. The mean measured concentration was 2.12 mg/L. No deaths occurred.
LC_{50} > 2.12 mg/L

Repeated-Dose Toxicity

Cinnamaldehyde (CAS No. 104-55-2)
(1) Osborne-Mendel rats (male and female, number not stated) were administered cinnamaldehyde via diet at 1000, 2500 and 10,000 ppm (~ 100, 250 and 1000 mg/kg-bw/day, respectively) for 16 weeks. At 10,000 ppm, hepatic cell swelling and hyperkeratosis of the squamous portion of the stomach was noted.
LOAEL ~ 1000 mg/kg-bw/day (based on hepatic cell swelling and hyperkeratosis)
NOAEL ~ 250 mg/kg-bw/day

(2) Rats (10/sex/dose) were administered cinnamaldehyde via diet at 50, 100 and 200 mg/kg-bw/day for 12 weeks. No differences were observed between treated and control groups. No adverse effects were observed on growth, food intake, efficiency of food utilization or other physiological criteria.
NOAEL = 200 mg/kg-bw/day (based on no effects at highest dose tested)

(3) F344 rats (male and female, number not stated) were administered cinnamaldehyde via gavage at 0, 235, 470, 940, 1880 and 3750 mg/kg-bw/day for 14 days. All rats at the two highest doses died or were sacrificed moribund in the first 7 days of dosing. Body weight and clinical parameters were comparable to controls. Males treated with 470 mg/kg-bw/day had minimal to moderate forestomach hyperplasia.
LOAEL = 470 mg/kg-bw/day (based on forestomach hyperplasia)
NOAEL = 235 mg/kg-bw/day

(4) B6C3F1 mice (male and female) were administered cinnamaldehyde by via gavage at 656, 1310, 2620, 5250 and 10,500 mg/kg-bw/day for 21 days. All mice at the two highest doses died within the first two days. Mortality was also observed in the 2620 mg/kg-bw/day treatment group. Body weight and clinical parameters were comparable to controls. At ≥ 1310 mg/kg-bw/day, forestomach hyperplasia and nephropathy were observed.
LOAEL = 1310 mg/kg-bw/day (based on forestomach hyperplasia and nephropathy)
NOAEL = 656 mg/kg-bw/day

(5) F344 rats (male and female) were administered cinnamaldehyde microcapsules in the diet at 0, 0.625, 1.25, 2.5, 5.0 or 10% (~ 0, 625, 1250, 2500, 5000 or 10,000 mg/kg-bw/day, respectively) for 14 days. Treatment of rat with microencapsulated cinnamaldehyde resulted in marked dose-dependent depression of body weight, hypoplastic changes in reproductive organs and accessory sex glands (seminal vesicles and prostates of males and uteri of females) and hyperplasia of the forestomach mucosa.
LOAEL ~ 625 mg/kg-bw/day (based on decreased body weight, affects on reproductive organs and hyperplasia of the forestomach mucosa)
NOAEL = Not established

(6) B6C3F1 mice (male and female) were administered cinnamaldehyde microcapsules in the diet at 0, 0.625, 1.25, 2.5, 5.0 or 10% (~ 0, 937.5, 1875, 3750, 7500, 15,000 mg/kg-bw/day, respectively) for 21 days. Treatment of mice with microencapsulated cinnamaldehyde resulted in dose-dependent depression of body weight and hyperplasia of the forestomach epithelium at highest dose (10%) characterized by a focal thickening of the stratified squamous epithelium, accompanied by hyperkeratosis.
LOAEL ~ 938 mg/kg-bw/day (based on decreased body weight)
NOAEL = Not established

(7) Rats (5/dose) were administered cinnamaldehyde via gavage at 100, 500 and 1000 mg/kg-bw/day for up to 17 days. All rats in the high-dose group died after two doses. Two out of five animals died after 10 doses at 500 mg/kg-bw/day. Decreased weight gain was observed at all doses. The hematology and clinical chemistry data were within normal ranges. Organ weights in the two lower dose groups were normal. At necropsy, cyanosis, dark liver and kidneys and chemical odor in the abdominal cavity were found at 1000 mg/kg-bw/day. Gastric erosion and staining were observed in one animal at 500 mg/kg-bw/day. Histopathological evaluation indicated chronic interstitial nephritis and tubular nephrosis with cast formation in the lumen of the tubules in two of five 1000 mg/kg-bw/day animals. Desquamation enteritis was also observed in one of the mid- and high-dose animals.
LOAEL = 100 mg/kg-bw/day (based on decreased body weight gain)
NOAEL = Not established

alpha-Amylcinnamaldehyde (CAS No. 122-40-7)
(1) CFE rats (15/sex/dose) were administered *alpha*-amylcinnamaldehyde via diet at 0, 80, 400 and 4000 ppm (males: 0, 6.1, 29.9 and 287.3 mg/kg-bw/day, respectively; females: 0, 6.7, 34.9 and 320.3 mg/kg-bw/day, respectively) for 14 weeks. There was an increase in the relative liver and kidney weights at the highest dose tested that were not associated with any histopathological changes. Body weight and other clinical parameters were comparable to controls.
LOAEL = 287 (males) and 320 (females) mg/kg-bw/day (based on increased relative liver and kidney weights)
NOAEL = 30 (males) and 35 (females) mg/kg-bw/day

(2) FDRL strain rats (15/sex/dose) were administered *alpha*-amylcinnamaldehyde via diet at 2% (~2000 mg/kg-bw/day) for 12 weeks. No treatment-related effects were observed in the parameters tested (hematology and clinical chemistry).
LOAEL ~ 2000 mg/kg-bw/day (based on no treatment-related effects on hematological or clinical parameters, only dose tested)
NOAEL = Not established

alpha-Hexylcinnamaldehyde (CAS No. 101-86-0)
(1) Sprague-Dawley rats (5/sex/dose) were administered *alpha*-hexylcinnamaldehyde percutaneously at 125, 250, 500 and 1000 mg/kg-bw/day for 90 days. Treatment-related effects were evident at all concentrations tested, with mortality at the highest dose. Dose-dependent dermal irritation characterized by erythema, cracking, dryness and sloughing were observed. Females exhibited increased food consumption and inconsistent changes in hematological parameters (hemoglobin, hematocrit, erythrocyte count, SGOT and SGPT). There was also a consistent elevation in white blood cell counts. In males, there was reduced lymphocyte count. Reduced serum glucose and increased BUN were observed in both sexes. Dose-dependent irritation of the gastrointestinal tract and treated skin were observed. Females showed increased liver and kidney weights with corresponding histopathologic changes (hepatic vacuolization and single cell degeneration). Other macroscopic changes related to irritant effects were also observed (gastric ulceration, necrotizing dermatitis, hyperkeratosis).
LOAEL = 125 mg/kg-bw/day (based on increased liver and kidney weights and irritant effects)
NOAEL = Not established

(2) Sprague-Dawley rats (5/sex/dose) were administered 25 mg/kg-bw/day *alpha*-hexylcinnamaldehyde dermally for 90 days. One male rat died on day 14 of a lung infection that was not considered treatment-related. Body weights, clinical chemistry parameters and organ pathology in treated and control animals were unremarkable.
NOAEL = 25 mg/kg-bw/day (based on no effects at only dose tested)

(3) Sprague-Dawley rats (2/male/dose) were administered *alpha*-hexylcinnamaldehyde percutaneously at 150, 375, 750, 1500 and 3000 mg/kg-bw/day for 28 days. Erythema and eschar formation, dermatitis and hyperkeratosis occurred at all doses. Other clinical effects were hyperirritability, depressed food intake, hematological changes (clotting times and cell counts), clinical chemistry effects (increases in BUN, SAP, SGPT and SGOT and decreases in glucose), thickened skin and erythema of the dermis and epidermis and body emaciation. Organ effects observed at all doses included congested lungs, gastrointestinal tract irritation, decreases in the absolute and relative thymus and spleen weights and focal dilation of the tubules in the kidneys.
LOAEL = 150 mg/kg-bw/day (based on effects on lungs, gastrointestinal tract, thymus, spleen and kidneys)
NOAEL = Not established

p-t-Butyl-alpha-methylhydrocinnamaldehyde (CAS No. 80-54-6)
(1) Beagle dogs (6/sex/dose) were administered *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde orally via gelatin capsules at 4.4, 22.3 and 44.6 mg/kg-bw/day for 91 days. No adverse effects on body weight, behavior, hematological, clinical parameters or histopathology were observed at any dose.
NOAEL = 44.6 mg/kg-bw/day (based on no effects at highest dose tested)

(2) Beagle dogs (three females) were administered 200 mg/kg-bw/day *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde orally via gelatin capsules for 91 days. No treatment-related adverse effects on body weight, behavior, hematological, clinical parameters or histopathology were observed.
NOAEL = 200 mg/kg-bw/day (highest dose tested)

(3) Beagle dogs (two males) were administered *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde orally via gelatin capsules at 400 µL/kg-bw/day (approximately 371 mg/kg-bw/day) for 22 – 50 days. One dog showed increased enzyme levels (GPT and GLDH) from week 4 onward. Changes in seminiferous epithelium were comparable to controls. Otherwise, no treatment-related clinical effects or changes in body weights were observed.
NOAEL = 371 mg/kg-bw/day (based on no effects at only dose tested)

(4) Rhesus monkeys (two males) were administered *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde orally at 100 mg/kg-bw/day for 5 days. The testes and epididymes were examined microscopically. No changes in body weight or testes were noted.
NOAEL = 100 mg/kg-bw/day (based on no effects at only dose tested)

(5) Albino rats (males) were administered *p*-*t*-butyl-*alpha*-methylhydrocinnamaldehyde dermally at 250, 500, 1000 and 2000 mg/kg-bw/day for 5 days. The testes and epididymes were examined. Changes in body weight and testicular atrophy occurred at the highest dose.
LOAEL = 2000 mg/kg-bw/day (based on changes in body weight and testicular atrophy)
NOAEL = 1000 mg/kg-bw/day

(6) Albino rats (males) were administered *p*-*t*-butyl-*alpha*-methylhydrocinnamaldehyde orally route at 25, 50 and 100 mg/kg-bw/day for 5 days. The testes and epididymes were examined. Changes in body weight and testicular atrophy occurred at 50 and 100 mg/kg-bw/day.
LOAEL = 50 mg/kg-bw/day (based on changes in body weight and testicular atrophy)
NOAEL = 25 mg/kg-bw/day

(7) Outbred rats (14/sex/dose) were administered *p*-*t*-butyl-*alpha*-methylhydrocinnamaldehyde via gavage at 2, 5, 25 and 50 mg/kg-bw/day for 90 days. No treatment-related changes in body weight or hematological parameters were observed. Females showed hair loss at 50 mg/kg-bw/day. Both sexes in the 25 and 50 mg/kg-bw/day groups showed a decrease in plasma cholinesterase activity and an increase in plasma cholesterol. In males treated with 50 mg/kg-bw/day, spermatoceles and testicular atrophy were observed.
LOAEL = 25 mg/kg-bw/day (based on changes in clinical chemistry and the appearance of spermatoceles and testicular atrophy)
NOAEL = 5 mg/kg-bw/day

(8) Rats (8 males/dose) were administered *p*-*t*-butyl-*alpha*-methylhydrocinnamaldehyde via gavage at 25, 50, 100, 200 and 400 mg/kg-bw/day for 5 consecutive days. There was a decrease in body weight in the 400 mg/kg-bw/day group. The following effects were observed: shaggy fur, hunched posture, hematuria and paresis of the foreleg. At necropsy, delineation of hepatic lobules, small prostate and seminal vesicles and reddened testes were seen. Testes weight decreased in rats treated with ≥ 100 mg/kg-bw/day. Disturbances in spermatogenesis and spermiogenesis were noted at 25 mg/kg-bw/day, which was preceded by the formation of detectable spermatoceles.
LOAEL = 25 mg/kg-bw/day (based on the appearance of spermatoceles and disturbances in spermatogenesis and spermiogenesis)
NOAEL = Not established

Reproductive Toxicity

The sponsor did not submit reproductive toxicity data for the category chemicals. Evaluation of effects on reproductive organs from the repeated-dose toxicity studies and available developmental toxicity studies addressed the reproductive toxicity endpoint for the purposes of the HPV Challenge Program.

Cinnamaldehyde (CAS No. 104-55-2)

(1) In the 14-day repeated-dose toxicity study in F344 rats described previously, histological evaluation was performed on the male and female reproductive organs from each dose group and the control group. Hypoplastic changes in reproductive organs and accessory sex glands were observed in both sexes (reduction in size of reproductive organs and secondary sex glands, seminal vesicles and prostates of males and uteri of females).

(2) In a screening-level reproductive toxicity study that resembles the developmental toxicity study described below, Charles River SPF CD-1 mice (10 females/group) were administered *trans*-cinnamaldehyde by gavage at a concentrations corresponding to 250, 445, 790, 1405 and 2500 mg/kg-bw/day on days 7 – 14 of gestation. All mice administered the high dose died within 3 days of exposure. Of the mice administered 1405 mg/kg-bw/day, 70% survived to termination. The clinical signs preceding death were languidity, dyspnea and prostration. In the surviving animals, statistically significant ($p < 0.05$) body weight changes were observed at 1405 mg/kg-bw/day. No clinical signs of toxicity were observed in the offspring.
LOAEL (systemic toxicity) = 1405 mg/kg-bw/day (based on mortality and body weight changes)
NOAEL (systemic toxicity) = 790 mg/kg-bw/day

alpha-Hexylcinnamaldehyde (CAS No. 101-86-0)
In the 90-day repeated-dose toxicity study in Sprague-Dawley rats described previously, histological evaluation was performed on the male and female reproductive organs from each dose group and the control group. No gross or microscopic lesions were observed in the reproductive organs for either sex at any dose.

p-t-Butyl-alpha-methylhydrocinnamaldehyde (CAS No. 80-54-6)
In the 90-day repeated-dose toxicity study in Beagle dogs described previously, histological evaluation was performed on the male and female reproductive organs from each dose group and the control group. In the high dose males, the presence of spermatoceles and testicular atrophy were seen. No treatment-related effects on the female reproductive system were observed.

Developmental Toxicity

Cinnamaldehyde (CAS No. 104-55-2)
(1) Pregnant Sprague-Dawley rats were administered cinnamaldehyde via gavage on days 7 – 17 of gestation at concentrations of 0, 5, 25 and 250 mg/kg-bw/day. Decreased weight gain between days 7 and 20 was observed in the presence of decreased food intake at all doses. Developmental effects observed at all doses included decreased ossification of the cranium and tympanic bulla, increased incidence of dilated pelvis/reduced papilla in kidney, dilated ureter and incidences of hypoplastic/dysplastic kidneys.
LOAEL (maternal toxicity) = 5 mg/kg-bw/day (based on reduced weight gain in dams)
NOAEL (maternal toxicity) = Not established
LOAEL (developmental toxicity) = 5 mg/kg-bw/day (based on reduced cranial ossification and kidney effects)
NOAEL (developmental toxicity) = Not established

(2) Pregnant CD-1 mice were exposed to cinnamaldehyde via gavage on days 6 – 13 of gestation to 1200 mg/kg-bw/day. No signs of maternal toxicity were observed (mortality, changes in body weight or delivery of viable litter) when compared to control group. The number of stillborn per litter, percent survival, birth weight and body weight gain were unremarkable when compared to controls.
NOAEL (maternal/developmental toxicity) = 1200 mg/kg-bw/day (based on no treatment-related effects at the only dose tested)

(3) Charles River SPF CD-1 mice (10 females/group) were administered *trans*-cinnamaldehyde by gavage at concentrations corresponding to 250, 445, 790, 1405 and 2500 mg/kg-bw/day on days 7 – 14 of gestation. All mice administered the high dose died within 3 days of exposure. Of the mice administered 1405 mg/kg-bw/day, 70% survived to termination. The clinical signs preceding death were languidity, dyspnea and prostration. In the surviving animals, statistically significant ($p < 0.05$) body weight changes were observed at 1405 mg/kg-bw/day. No clinical signs of toxicity were observed in the offspring.
LOAEL (maternal toxicity) = 1405 mg/kg-bw/day (based on mortality and body weight changes)
NOAEL (maternal toxicity) = 790 mg/kg-bw/day
NOAEL (developmental toxicity) = 2500 mg/kg-bw/day (based on no effects at the highest dose tested)

Genetic Toxicity – Gene Mutation

In vitro
Cinnamaldehyde (CAS No. 104-55-2)
(1) In 10 separately conducted assays, *Salmonella typhimurium* strains TA 98, TA100, TA102, TA104, TA1535, TA1537 and/or TA1538, *Escherichia coli* WP2 uorA and *Bacillus subtilis* H17 Rec+ or M45 Rec or Chinese hamster V79 cells were exposed to cinnamaldehyde at concentrations ranging from 0.05 to 5000 µg/plate in the presence an absence of metabolic activation. In all of these assays, cinnamaldehyde was negative. Positive and negative controls were included in most assays.
Cinnamaldehyde was not mutagenic in these assays.

(2) *S. typhimurium* strains (TA97a, TA100, TA102 and TA104) were exposed to *trans*-cinnamaldehyde at concentrations of 25, 50, 100, 200 and 300 µg/plate with and without metabolic activation. A positive control was used, but the response was not provided in the robust summary. *trans*-Cinnamaldehyde exhibited a weak mutagenic response in TA100 with mouse liver S9 mix.
***trans*-Cinnamaldehyde was weakly mutagenic in TA100 in this assay.**

(3) *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to cinnamaldehyde at concentrations of 100 and 500 µg/plate (other concentrations not mentioned) with and without metabolic activation. Mutagenic activity was observed in TA100 in the presence and absence of metabolic activation.
Cinnamaldehyde was mutagenic in TA100 with and without metabolic activation in this assay.

***alpha*-Hexylcinnamaldehyde (CAS No. 101-86-0)**
S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to *alpha*-hexylcinnamaldehyde in the presence and absence of metabolic activation at concentrations up to 3600 µg/plate. Positive controls were run, but the responses were not provided in the robust summary. No mutagenic activity was observed in any of the strains tested.
***alpha*-Hexylcinnamaldehyde was not mutagenic in this assay.**

***alpha*-Amylcinnamaldehyde (CAS No. 122-40-7)**
S. typhimurium TA97, TA98, TA100, TA102, TA1535, TA1537 or TA1538 were exposed to *alpha*-amylcinnamaldehyde at several concentrations up to 3600 µg/plate. Positive controls were run, but the responses were not provided in the robust summary. No mutagenic activity was observed with any of the strains tested.
***alpha*-Amylcinnamaldehyde was not mutagenic in this assay.**

***p*-t-Butyl-*alpha*-methylhydrocinnamaldehyde (CAS No. 80-54-6)**
(1) *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to *p*-t-*b*utyl-*alpha*-methylhydrocinnamaldehyde in the presence and absence of metabolic activation at concentrations ranging from 0.0078 – 0.125 µL/plate. Positive controls were run, but the response was not provided in the robust summary. No mutagenic activity was observed in any of the strains tested.
***p*-t-Butyl-*alpha*-methylhydrocinnamaldehyde was not mutagenic in this assay.**

(2) *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 were exposed to *p*-t-*b*utyl-*alpha*-methylhydrocinnamaldehyde in the presence and absence of metabolic activation at concentrations ranging from 2.5 – 750 µg/plate (without activation) and 250 µg/plate with activation. Information on positive controls was not provided in the robust summary. Cytotoxicity was observed at 667 µg/plate (with metabolic activation) and 333 µg/plate (without metabolic activation). No mutagenic activity was observed in any of the strains tested.
***p*-t-Butyl-*alpha*-methylhydrocinnamaldehyde was not mutagenic in this assay.**

Genetic Toxicity – Chromosomal Aberrations

In vitro
Cinnamaldehyde (CAS No. 104-55-2)
(1) Chinese hamster B241 cells were exposed to *trans*-cinnamaldehyde at several concentrations up to 10 nM in the presence and absence of metabolic activation. *trans*-Cinnamaldehyde induced chromatid breaks, chromosomal breaks, chromatid exchange, ring or dicentric chromosomes, fragmentation, translocation and pulverization.
***trans*-Cinnamaldehyde induced chromosomal aberrations in this assay.**

(2) Chinese hamster fibroblast (CHL) cells were exposed to cinnamaldehyde at three concentrations up to 0.015 mg/mL without metabolic activation.
Cinnamaldehyde induced chromosomal aberrations in this assay.

(3) Chinese hamster B241 cells were exposed to cinnamaldehyde at unspecified concentrations with and without metabolic activation between the 5th and 8th passages. Severe chromosomal aberrations were observed: chromatid breaks, chromosomal breaks, chromatid exchange, ring or dicentric chromosomes, fragmentation, translocation and pulverization.
Cinnamaldehyde induced chromosomal aberrations in this assay.

In vivo
Cinnamaldehyde (CAS No. 104-55-2)
(1) Male ddY mice were administered cinnamaldehyde via intraperitoneal injection at doses of 125, 250, 500 and 1000 mg/kg-bw. At 500 mg/kg-bw more than one animal died and at 1000 mg/kg-bw all animals died. Mice were sacrificed 18, 24, 30, 48 and 72 hours post-dosing. Femoral marrow cells were smeared, fixed and stained. One hundred polychromatic erythrocytes were scored and the number of micronucleated polychromatic erythrocytes was recorded. Micronucleated polychromatic erythrocytes (MNPCEs) did not increase at any dose or any sampling time. There was no evidence of genotoxicity.
Cinnamaldehyde did not induce chromosomal aberrations in this assay.

(2) Male ddY mice were irradiated with X-ray at 200 rad and then were administered cinnamaldehyde via gavage at 250, 313 and 500 mg/kg-bw. There was a dose-dependent decrease in MNPCEs. Cinnamaldehyde reduced the frequency of X-ray induced micronuclei without toxicity of the test chemical to the bone marrow.
Cinnamaldehyde did not induce chromosomal aberrations in this assay.

(3) In two separate assays, B6C3F1 mice (10/sex/dose) and F344/N rats were administered microencapsulated cinnamaldehyde in the diet at concentrations of 0, 4100, 8200, 16,500 and 33,000 ppm (~ 0, 615, 1230, 2475 and 4950 mg/kg, respectively) for mice and 0, 4100, 8200, 16,500 and 33,000 ppm (~ 0, 410, 820, 1650 and 3300 mg/kg, respectively) for rats. The frequency of micronuclei per 1000 polychromatic erythrocytes and 2000 MNPCEs was evaluated in blood from up to five animals per group. No mutagenic activity was detected.
Cinnamaldehyde did not induce chromosomal aberrations in these assays.

(4) Male Sprague-Dawley rats were administered a single oral dose of cinnamaldehyde at 1100 mg/kg-bw in a DNA fragmentation/alkaline elution assay. The animals were sacrificed 3 hours post-exposure. No DNA fragmentation in the liver or gastric mucosa was observed.
Cinnamaldehyde did not induce chromosomal aberrations in this assay.

alpha-Amylcinnamaldehyde (CAS No. 122-40-7)
Male and female NMRI mice were administered *alpha*-amylcinnamaldehyde (route not specified) at 0, 405, 809 and 1213 mg/kg-bw and were sacrificed 30 hours post-exposure. The frequency of MNPCEs was evaluated in bone marrow. No mutagenic activity was observed.
***alpha*-Amylcinnamaldehyde did not induce chromosomal aberrations in this assay.**

alpha-Hexylcinnamaldehyde (CAS No. 101-86-0)
Male and female NMRI mice were administered *alpha*-hexylcinnamaldehyde (route not specified) at 0, 324, 540 and 756 mg/kg-bw and were sacrificed 30 hours post-exposure. The frequency of MNPCEs was evaluated in bone marrow. No mutagenic activity was observed.
***alpha*-Hexylcinnamaldehyde did not induce chromosomal aberrations in this assay.**

p-t-Butyl-alpha-methylhydrocinnamaldehyde (CAS No. 80-54-6)
Male and female ICR mice were administered *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde via intraperitoneal injection at doses of 150, 300 and 600 mg/kg-bw. Mice were sacrificed 48 and 72 hours post-dosing. Femoral marrow cells were smeared, fixed and stained. Approximately 2000 polychromatic erythrocytes were scored and the number of MNPCEs was recorded. MNPCEs were not increased at any dose or any sampling time except in males at 600 mg/kg-bw. Given that this was only observed in one mouse and fell within the range of historical controls, it is not considered biologically relevant.
***p-t*-Butyl-*alpha*-methylhydrocinnamaldehyde did not induce chromosomal aberrations in this assay.**

Genetic Toxicity – Other

In vivo
Cinnamaldehyde (CAS No. 104-55-2)
(1) Unscheduled DNA synthesis (UDS) or S-phase-synthesis (SPS) was evaluated in male Fischer 344 rats. The animals were administered oral doses of 50, 200 or 1000 mg/kg-bw.
No induction of UDS or SPS was observed.
Cinnamaldehyde did not induce unscheduled DNA synthesis or S-phase-synthesis in this assay.

(2) UDS or SPS was evaluated in male and female B6C3F1 mice. The animals were administered oral doses of 50, 200 or 1000 mg/kg-bw. No induction of UDS or SPS was observed.
Cinnamaldehyde did not induce unscheduled DNA synthesis or S-phase-synthesis in this assay.

alpha-Amylcinnamaldehyde (CAS No. 122-40-7)
In a BASC test with *Drosophila melanogaster*, male and female flies were orally exposed to 10 mM of *alpha*-amylcinnamaldehyde. No mutagenic activity was observed.
***alpha*-Amylcinnamaldehyde was not mutagenic in this assay.**

alpha-Hexylcinnamaldehyde (CAS No. 101-86-0)
In a BASC test with *D. melanogaster*, male and female insects were orally exposed to *alpha*-hexylcinnamaldehyde at 10mM. No mutagenic activity was observed.
***alpha*-Hexylcinnamaldehyde was not mutagenic in this assay.**

Additional Information

The skin and eye irritation and sensitization studies on animals and in humans described below were submitted to EPA under TSCA and the summaries are available in the TSCATS database (http://www.syrres.com/esc/tscats.htm).

Skin Irritation

Cinnamaldehyde (CAS No. 104-55-2)
Cinnamaldehyde is considered a strong skin irritant in guinea pigs.
Cinnamaldehyde was strongly irritating to guinea pig skin.

p-t-Butyl-alpha-methylhydrocinnamaldehyde (CAS No. 80-54-6)
(1) White Vienna rabbits (five males and one female) were exposed to 0.5 mL *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde via skin patch to abraded and intact skin for 24 hours and were observed for 48 hours post-exposure.
***p-t*-Butyl-*alpha*-methylhydrocinnamaldehyde was severely irritating to rabbit skin in this assay.**

(2) White Vienna rabbits (males and females) were exposed to 0.5 g *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde via skin patch under semi-occlusive conditions for 4 hours and were observed for 8 days post-exposure.
***p-t*-Butyl-*alpha*-methylhydrocinnamaldehyde was irritating to rabbit skin in this assay.**

(3) The skin irritation properties of *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde were evaluated in a repeated insult patch test in humans (70 male and female participants).
***p-t*-Butyl-*alpha*-methylhydrocinnamaldehyde was not irritating to human skin in this assay.**

Eye Irritation

Cinnamaldehyde (CAS No. 104-55-2)
Cinnamaldehyde was a moderate eye irritant in rabbits; the irritation was reduced after rinsing the eyes.
Cinnamaldehyde was moderately irritating to rabbit eyes in this study.

p-t-Butyl-alpha-methylhydrocinnamaldehyde (CAS No. 80-54-6)
White Vienna rabbits (four males and two females) were exposed to a single 0.1 mL ocular instillation of *p*-t-butyl-*alpha*-methylhydrocinnamaldehyde and were observed for 15 days post-exposure.
***p-t*-Butyl-*alpha*-methylhydrocinnamaldehyde was irritating to rabbit eyes in this study.**

Sensitization

Cinnamaldehyde (CAS No. 104-55-2)
Cinnamaldehyde is considered a moderate skin sensitizer in guinea pigs.
Cinnamaldehyde was a moderate skin sensitizer in guinea pigs in this study.

alpha-Hexylcinnamaldehyde (CAS No. 101-86-0)
(1) In a local lymph node assay, contact hypersensitivity was tested with mice (5/group) treated with 1.5, 10 or 50% *alpha*-hexylcinnamaldehyde by direct epicutaneous application onto each ear for 3 consecutive days. Approximately 70 hours following final application, the animals were injected intravenously with radiolabeled thymidine to label proliferating cells. Five hours post-injection, the animals were sacrificed and cell suspensions were prepared. No clinical signs were observed in the naïve control mice. All other mice (including carrier controls) exhibited rough hair coat, with darkening of the hair on top of the head and around the ears. Other clinical signs included hyperirritability, hair loss and irritation of the ears.
***alpha*-Hexylcinnamaldehyde was a contact sensitizer in this assay.**

(2) In a skin sensitization study, Hartley-derived albino guinea pigs (10/sex/dose) were topically treated with 20% of *alpha*-hexylcinnamaldehyde in acetone, once per week for 3 consecutive weeks. When challenged 2 weeks later with 2.5% *alpha*-hexylcinnamaldehyde in acetone, a positive response was elicited in 70% of the test animals.
***alpha*-Hexylcinnamaldehyde was a skin sensitizer in guinea pigs in this study.**

p-t-Butyl-alpha-methylhydrocinnamaldehyde (CAS No. 80-54-6)
The skin sensitization properties of *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde were evaluated in a repeated insult patch test in humans (70 male and female participants).
***p-t*-Butyl-*alpha*-methylhydrocinnamaldehyde was a skin sensitizer in humans in this study.**

Carcinogenicity
Carcinogenicity studies have been conducted on the related chemical, *trans*-cinnamaldehyde (CAS No. 14371-10-9)

trans-Cinnamaldehyde (CAS No. 14371-10-9)
(1) In a National Toxicology Program carcinogenicity bioassay, groups of 50 male and 50 female F344/N rats were fed diets containing 1000, 2100 or 4100 ppm microencapsulated trans-cinnamaldehyde for 2 years. Dietary concentrations of 1000, 2100 or 4100 ppm delivered average daily doses of approximately 50, 100 or 200 mg/kg to males and females. There were no neoplasms or nonneoplastic lesions that were attributed to exposure to trans-cinnamaldehyde.
Under the conditions of this 2-year feed study, there was no evidence of carcinogenic activity of *trans*-cinnamaldehyde in male or female F344/N rats.

(2) In a National Toxicology Program carcinogenicity bioassay, groups of 50 male and 50 female B6C3F$_1$mice were fed diets containing 1000, 2100 or 4100 ppm microencapsulated trans-cinnamaldehyde for 2 years. Dietary concentrations of 1000, 2100 or 4100 ppm delivered average daily doses of approximately 50, 100 or 200 mg/kg to males and females. There were no neoplasms or nonneoplastic lesions that were attributed to exposure to trans-cinnamaldehyde.
Under the conditions of this 2-year feed study, there was no evidence of carcinogenic activity of *trans*-cinnamaldehyde in male or female B6C3F$_1$ mice.

Conclusion: The acute oral, dermal and inhalation toxicity for the members of the cinnamyl derivatives category is low. Cinnamaldehyde and *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde are eye and skin irritants in experimental animals. Cinnamaldehyde and *alpha*-hexylcinnamaldehyde are skin sensitizers in experimental animals. However, *p-t*-Butyl-*alpha*-methylhydrocinnamaldehyde does not cause skin irritation and is not a skin sensitizer based on human repeat insult patch tests. Repeated exposures to all category members via the oral route indicate an effect on

body weight and toxicity to multiple organs (forestomach in rats and mice and liver, kidney, testicular atrophy in rats) as well as an effect on spermatogenesis. Evaluation of reproductive organs from the 90-day repeated-dose toxicity studies for *alpha*-hexylcinnamaldehyde and *p-t*-butyl-*alpha*-methylhydrocinnamaldehyde showed no effects on reproductive organs. However, limited information on reproductive organs from a 14-day oral toxicity study showed effects on male and female reproductive organs. Developmental toxicity data for cinnamaldehyde suggest that rats are more sensitive to this category of substances than mice. Developmental effects included decreased ossification of the cranium and tympanic bulla, increased incidence of dilated pelvis/reduced papilla in kidney, dilated ureter and incidences of hypoplastic/dysplastic kidneys. The members of the cinnamyl derivatives category did not show mutagenic potential when tested *in vitro* in *Salmonella typhimurium,* excepting a positive test in strain TA100 with cinnamaldehyde. Cinnamaldehyde also induced chromosomal aberrations *in vitro*, but the data were equivocal *in vivo*. The other category members were negative when tested *in vivo* for chromosomal aberrations. Results from 2-year carcinogenicity studies of the related chemical, *trans*-cinnamaldehyde, conducted by the National Toxicology Program, there was no evidence of carcinogenic activity of *trans*-cinnamaldehyde in male and female F344/N rats and B6C3F_1 mice.

The potential health hazard of the cinnamyl derivatives category is moderate based on repeated-dose and developmental toxicity. Available data suggest that cinnamaldehyde has potential to be genotoxic.

Table 3. Summary of Human Health Data

Endpoints	**Cinnamaldehyde (104-55-2)**	***alpha*-Amyl cinnamaldehye (122-40-7)**	***alpha*-Hexyl cinnamaldehye (101-86-0)**	***p-t*-Butyl-*alpha*-methylhydro-cinnamaldehyde (80-54-6)**
Acute Oral Toxicity LD_{50} (mg/kg-bw)	**1160 – 2220**	**3730**	**3100**	**3700**
Acute Dermal Toxicity LD_{50} (mg/kg-bw)	**620 – < 5000**	**> 2000**	**> 3000**	**> 5000**
Acute Inhalation Toxicity LC_{50} (mg/L)	—*	—*	**> 5**	—*
Repeated-Dose Toxicity Oral NOAEL/LOAEL (mg/kg-bw/day)	**NOAEL = 235 LOAEL = 470 (14-d)** **NOAEL = 656 LOAEL = 1310 (21-d)**	**NOAEL = 30 LOAEL = 300**	No Data NOAEL = 30 LOAEL = 300 (RA)	**NOAEL = 5 LOAEL = 25**
Repeated-Dose Toxicity Dermal NOAEL/LOAEL (mg/kg-bw/day)	No Data NOAEL = NE LOAEL = 125 (RA)	No Data NOAEL = NE LOAEL = 125 (RA)	**NOAEL = NE LOAEL =125**	No Data NOAEL = NE LOAEL = 125 (RA)
Reproductive Toxicity NOAEL/LOAEL (mg/kg-bw/day)	Limited information in reproductive toxicity study. However, 14-d oral toxicity study showed effects on male and female reproductive organs.	No Data	No Data **90-d dermal toxicity study showed no effects on reproductive organs.**	No Data **90-d oral toxicity study showed effects on testes.**

Measured data in bold; RA = Read Across; NE = Not established; — indicates endpoint not addressed for this chemical; * indicates endpoint not necessary for this chemical

Table 3. Summary of Human Health Data				
Endpoints	**Cinnamaldehyde (104-55-2)**	***alpha*-Amyl cinnamaldehye (122-40-7)**	***alpha*-Hexyl cinnamaldehye (101-86-0)**	***p*-*t*-Butyl-*alpha*-methylhydro-cinnamaldehyde (80-54-6)**
Developmental Toxicity NOAEL/LOAEL (mg/kg-bw/day) **Maternal/ Developmental Toxicity**	**NOAEL = NE LOAEL = 5**	No Data NOAEL = NE LOAEL = 5 (RA)	No Data NOAEL = NE LOAEL = 5 (RA)	No Data NOAEL = NE LOAEL = 5 (RA)
Genetic Toxicity – Gene Mutation *In vitro*	**Positive in TA100 only**	**Negative**	**Negative**	**Negative**
Genetic Toxicity – Chromosomal Aberrations *In vitro*	**Positive**	—*	—*	—*
Genetic Toxicity – Chromosomal Aberrations *In vivo*	**Equivocal**	**Negative**	**Negative**	**Negative**
Genetic Toxicity – Other Unscheduled DNA synthesis	**Negative**	—*	—*	—*
S-phase-synthesis	**Negative**	—*	—*	—*
Additional Information – Skin Irritation	**Severe**	—*	—*	**Severe**
Additional Information – Eye Irritation	**Moderate**	—*	—*	**Moderate**
Additional Information – Sensitization	**Positive**	—*	**Positive**	**Negative**
Additional Information – Carcinogenicity	No evidence of carcinogenic activity of *trans*-cinnamaldehyde in male and female F344/N rats and $B6C3F_1$ mice.	—*	—*	—*

Measured data in bold; RA = Read Across; NE = Not established; — indicates endpoint not addressed for this chemical;
* indicates endpoint not necessary for this chemical

4. Hazard Characterization

The log K_{ow} of cinnamaldehyde indicates that its potential to bioaccumulate is low. The log K_{ow} values of *alpha*-amylcinnamaldehyde, *alpha*-hexylcinnamaldehyde and *p*-*t*-butyl-*alpha*-methylhydrocinnamaldehyde indicate that their potential to bioaccumulate is high. The cinnamyl derivative category members are readily biodegradable, indicating that they are not expected to persist in the environment.

The evaluation of available toxicity data (measured and estimated) for fish, aquatic invertebrates and aquatic plants indicates that the potential acute hazard of cinnamaldehyde and *alpha*-amylcinnamaldehyde to aquatic organisms is moderate. However, the more highly substituted category members, *alpha*-hexylcinnamaldehyde and *p*-*t*-butyl-*alpha*-methylhydrocinnamaldehyde, are estimated to pose high acute hazard to aquatic invertebrates and plants.

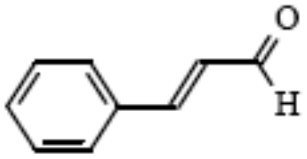
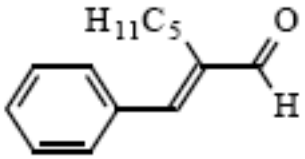
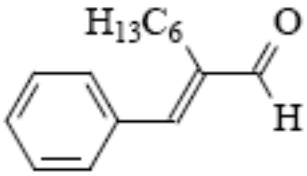
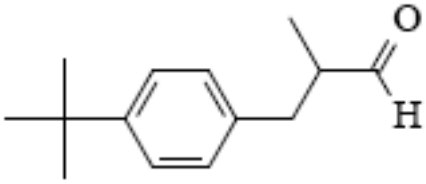
The acute oral, dermal and inhalation toxicity for the members of the cinnamyl derivatives category is low. Cinnamaldehyde and *p*-*t*-butyl-*alpha*-methylhydrocinnamaldehyde are eye and skin irritants in experimental animals. Cinnamaldehyde and *alpha*-hexylcinnamaldehyde are skin sensitizers in experimental animals. However, *p*-*t*-Butyl-*alpha*-methylhydrocinnamaldehyde does not cause skin irritation and is not a skin sensitizer based on human repeat insult patch tests. Repeated exposures to all category members via the oral route indicate an effect on body weight and toxicity to multiple organs (forestomach in rats and mice and liver, kidney, testicular atrophy in rats) as well as an effect on spermatogenesis. Evaluation of reproductive organs from the 90-day repeated-dose toxicity studies for *alpha*-hexylcinnamaldehyde and *p*-*t*-butyl-*alpha*-methylhydrocinnamaldehyde showed no effects on reproductive organs. However, limited information on reproductive organs from a 14-day oral toxicity study showed effects on male and female reproductive organs. Developmental toxicity data for cinnamaldehyde suggest that rats are more sensitive to this category of substances than mice. Developmental effects included decreased ossification of the cranium and tympanic bulla, increased incidence of dilated pelvis/reduced papilla in kidney, dilated ureter and incidences of hypoplastic/dysplastic kidneys. The members of the cinnamyl derivatives category did not show mutagenic potential when tested *in vitro* in *Salmonella typhimurium,* excepting a positive test in strain TA100 with cinnamaldehyde. Cinnamaldehyde also induced chromosomal aberrations *in vitro*, but the data were equivocal *in vivo*. The other category members were negative when tested *in vivo* for chromosomal aberrations. Results from 2-year carcinogenicity studies of the related chemical, *trans*-cinnamaldehyde, conducted by the National Toxicology Program, there was no evidence of carcinogenic activity of *trans*-cinnamaldehyde in male and female F344/N rats and $B6C3F_1$ mice.

The potential health hazard of the cinnamyl derivatives category is moderate based on repeated-dose and developmental toxicity. Available data suggest that cinnamaldehyde has potential to be genotoxic.

5. Data Gaps

No data gaps were identified under the HPV Challenge Program

Appendix

Cinnamyl Derivatives Category Members		
CAS No.	**Chemical Name**	**Structure**
SPONSORED CHEMICALS		
104-55-2	**Cinnamaldehyde**	 C_9H_8O
122-40-7	***alpha*-Amylcinnamaldehyde**	 $C_{14}H_{18}O$
101-86-0	***alpha*-Hexylcinnamaldehyde**	 $C_{15}H_{20}O$
80-54-6	***p-t*-Butyl-*alpha*-methylhydrocinnamaldehyde**	 $C_{14}H_{20}O$