

SCREENING-LEVEL HAZARD CHARACTERIZATION

Ionone Derivatives Category

SPONSORED CHEMICALS

Methyl ionone (mixture of isomers) (CASRN 1335-46-2)

***Alpha-iso-Methylionone* (CASRN 127-51-5)**

SUPPORTING CHEMICALS

***alpha-Ionone* (CASRN 127-41-3)**

***beta-Ionone* (CASRN 14901-07-6)**

The High Production Volume (HPV) Challenge Program¹ was conceived as 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 sponsored chemicals; sponsorship entailed the identification and initial assessment of the adequacy of existing toxicity data/information, conducting new testing if adequate data did 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 by developing hazard characterizations (HCs). These HCs consist of an evaluation of the quality and completeness of the data set provided in the Challenge Program submissions. They are not intended to be definitive statements regarding the possibility of unreasonable risk of injury to health or the environment.

The evaluation is performed according to established EPA guidance³ and is based primarily on hazard data provided by sponsors; however, 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. In order to determine whether any new hazard information was developed since the time of the HPV submission, a search of the following databases was made from one year prior to the date of the HPV Challenge submission to the present: (ChemID to locate available data sources including Medline/PubMed, Toxline, HSDB, IRIS, NTP, ATSDR, IARC, EXTOXNET, EPA SRS, etc.), STN/CAS online databases (Registry file for locators, ChemAbs for toxicology data, RTECS, Merck, etc.) and Science Direct. OPPT’s focus on these specific sources is based on their being of high quality, highly relevant to hazard characterization, and publicly available. Additionally,

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. Risk Assessment Guidelines; <http://cfpub.epa.gov/ncea/raf/rafguid.cfm>.

on a case-by-base basis, EPA independently reviews references included in robust summaries, and denotes these specific reviews by including the reference information in the study summary in parentheses.

OPPT does not develop HCs for those HPV chemicals which have already been assessed internationally through the HPV program of the Organization for Economic Cooperation and Development (OECD), and for which Screening Initial Data Set (SIDS) Initial Assessment Reports (SIAR) and SIDS Initial Assessment Profiles (SIAP) are available. These documents are presented in an international forum that involves review and endorsement by governmental authorities around the world. OPPT is an active participant in these meetings and accepts these documents as reliable screening-level hazard assessments.

These hazard characterizations are technical documents intended to inform 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.

<p>Chemical Abstract Service Registry Numbers (CASRN)</p>	<p><u>Sponsored Chemicals</u> 1335-46-2 127-51-5</p> <p><u>Supporting Chemicals</u> 127-41-3 14901-07-6</p>
<p>Chemical Abstract Index Name</p>	<p><u>Sponsored Chemicals</u> Ionone, methyl-3-Buten-2-one, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-</p> <p><u>Supporting Chemicals</u> <i>alpha</i>-Ionone <i>beta</i>-Ionone</p>
<p>Structural Formula</p>	<p>See Section 1</p>
<p style="text-align: center;">Summary</p> <p>The ionone derivatives category is composed of two substances that are grouped together due to their core structure being based on the parent terpene ionone, which occurs in nature as three olefinic isomers (alpha, beta and gamma). The two members of the ionone derivatives category differ in that one is a methyl ionone and the other is an isomethylionone; however, both are commercially sold as mixtures of several isomers. The members of the ionone derivatives category are liquid mixtures at room temperature with moderate water solubilities and moderate vapor pressures. These substances are expected to have moderate mobility in soil. Volatilization of the members of the ionone derivatives category is considered moderate based on estimated Henry's Law constants. The rate of hydrolysis is considered negligible. The rate of atmospheric photooxidation is considered rapid. The members of the ionone derivatives category are expected to have low persistence (P1) and moderate bioaccumulation potential (B2).</p> <p>The acute oral and dermal toxicities of the ionone derivatives category to rats and mice, and rabbits, respectively, are low. In single-dose 90-day oral (dietary) repeated-dose studies in rats with CASRN 127-51-5 and the supporting chemical, CASRN 127-41-3, no treatment-related effects were observed. The NOAEL for systemic toxicity for CASRN 127-51-5 is 3.55 - 4.10 (male – female) mg/kg/day (only dose tested) and for CASRN 127-41-3 is 11.1 – 11.8 (female-male) mg/kg/day (only dose tested). Similarly, in a single-dose 90-day dietary study in rats, the supporting chemical, CASRN 14901-07-6, showed no treatment-related effects. The NOAEL for systemic toxicity is 11.4 – 11.6 (male – female) mg/kg/day (only dose tested). In another 90-day repeated-dose toxicity study in rats, the supporting chemical, CASRN 14901-07-6 showed no treatment-effects; the NOAEL for systemic toxicity is 106-108 mg/kg/day (highest dose tested). No treatment-related effects were observed on the reproductive organs of rats in the 90-day repeated-dose studies described above. The</p>	

supporting chemical, CASRN 14901-07-6, did not cause reproductive effects in a two-generation single dose dietary reproduction study in rats; the NOAEL for reproductive/maternal/developmental toxicity is 8-10 mg/kg/day (only dose tested). In an oral prenatal developmental toxicity study in rats with CASRN 127-51-5, no maternal or fetal toxicity was observed; the NOAEL for maternal and developmental toxicity is 30 mg/kg/day (highest dose tested). CASRNs 1335-46-2 and 127-51-5, were negative in bacteria *in vitro* and the supporting chemical, CASRN 127-41-3, was negative in a mouse *in vivo* micronucleus test.

For CASRN 1335-46-2, the 96-h LC₅₀ for fish is 3.04 mg/L, the 48-h EC₅₀ for aquatic invertebrates is 2.65 mg/L and the 72-h EC₅₀ for aquatic plants is 2.89 mg/L for biomass and 7.47 mg/L for growth rate. There are no data for the chronic toxicity to aquatic invertebrates.

Chronic toxicity to aquatic invertebrates was identified as a data gap under the HPV Challenge Program.

The sponsor, The Flavor and Fragrance High Production Volume Consortia, submitted a test plan and robust summaries to EPA for Ionone Derivatives on March 26, 2002. EPA posted the submission on the ChemRTK HPV Challenge website on April 19, 2002 (<http://www.epa.gov/oppt/chemrtk/pubs/summaries/ionederv/c13660tc.htm>). EPA comments on the original submission were posted to the website on August 20, 2002. Public comments were also received and posted to the website. The sponsor submitted responses to the EPA comments and updated/revised documents on August 18, 2004, which were posted to the ChemRTK website on September 20, 2004.

Category Justification

The ionone derivatives category consists of two substances, methyl ionone (mixture of isomers) (CASRN 1335-46-2) and *alpha-iso*-methylionone (CASRN 127-51-5), which consist of methylated derivatives of the parent terpene ionone. Chemically, ionone is 1-(2,6,6-trimethylcyclohexen-1-yl)-1-butene-3-one. It occurs in nature as three isomers, *alpha*, *beta* and *gamma*, which differ only in the presence of a double bond at the 1, 2 or 2 (exocyclic) position of the cyclohexane ring, respectively. *n*-Methylionone exhibits the same ring double bond isomers as ionone, but contains an additional methyl group at the terminal (4-) position of the butenone side chain. The category member, CASRN 1335-46-2, is a mixture of *alpha*-, *beta*- and *gamma*-*n*-methylionone. *iso*-Methylionone also exhibits the same ring double bond isomers as ionone, but contains an additional methyl group at the 2- position of the butenone side chain. The category member, CASRN 127-51-5, is also a mixture of isomers because the sponsor uses this CASRN to refer to the commercial product recognized as *alpha-iso*-methylionone rather than the pure isomer.

Support for the proposed category is based on similar structures and metabolic pathways for the sponsored chemicals. As indicated above, essentially the only structural difference between the mixture of *n*-methylionone isomers and CASRN 127-51-5 is the position of the methyl group on the butenone side chain. As discussed in the test plan, studies of orally administered *alpha*- and *beta*-ionone (CASRN 127-41-3 and CASRN 14901-07-6, respectively) in animals suggest that ionones are metabolized by (1) allylic hydroxylation of the ring followed by oxidation of the hydroxyl group to 3-oxo derivatives and (2) reduction of the ketone function to the corresponding secondary alcohol. Combinations of these detoxification reactions result in the formation of multiple polar metabolites, which are excreted in the urine unchanged or conjugated with glucuronic acids. Similar metabolism of ionones is expected to occur in humans; this is supported by human metabolism studies of retinoids and carotenoids that possess ionone fragments. The toxicity data on the category members and parent ionones are reasonably concordant, and thereby strengthen the justification for the proposed category. Based on the close structural similarity and expected similar metabolism, the existing testing can be reasonably extrapolated between the category members for human health endpoints. However, testing is needed to determine if the same holds true for environmental effects. All aquatic toxicity acute were conducted and included in the revised test plan. In conclusion, the basis for grouping CASRN 1335-46-2 and CASRN 127-51-5 in the category is acceptable.

Justification for Supporting Chemicals

The sponsor provided data for *beta*-ionone (CASRN 14901-07-6) as a supporting chemical for physical-chemical properties and environmental effects.

Data for *alpha*-ionone (CASRN 127-41-3), *beta*-ionone (CASRN 14901-07-6), *gamma*-ionone (CASRN 79-76-5), *gamma-n*-methylionone (CASRN 64070-16-2), *delta-n*-methylionone (CASRN 7784-98-7) and pseudoionone (CASRN 141-10-6) were provided as supporting chemicals for human health endpoints. The sponsor's use of CASRN 127-41-3, CASRN 14901-07-6, and CASRN 79-76-5 as supporting chemicals is based on the close structural similarity and expected similar metabolism of these ionone isomers to the sponsored chemicals. As noted in the sponsor's responses to EPA comments, this indicates that the toxicologic potential of any of the ionones and *n*-methylionone and *iso*-methylionone derivatives is similar, and toxicity data support this conclusion. CASRN 64070-16-2 is one of the isomers comprising the category member CASRN 1335-46-2. EPA was concerned that information on CASRN 7784-98-7 and CASRN 141-10-6 were included in the original test plan without providing structures and reasons for inclusion. As indicated in the sponsor's responses to EPA's comments and in the revised test plan, information on CASRN 7784-98-7 is considered relevant to the category members due to structural similarity. This isomer contains the ring double bond at the 3- position of the cyclohexane ring, and is therefore related to the other three *n*-methylionone isomers. Although the structure of CASRN 141-10-6 is somewhat different from the sponsored chemicals because it is the open-chain version of ionone, it is an α,β -unsaturated methyl ketone (the most reactive part of the molecule) of the same molecular weight, and with similar physicochemical properties and to the three *n*-methylionone isomers and CASRN 127-51-5. Therefore, CASRN 141-10-6 could be expected to exhibit biological activities similar to the sponsored chemicals. EPA agrees that the use of data for these supporting chemicals is appropriate and data for CASRNs 127-41-3 and 14901-07-6 have been used for the human health endpoints as read-across to the sponsored chemicals, where appropriate.

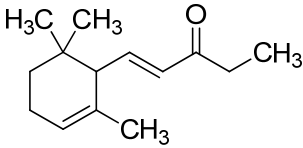
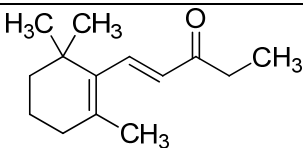
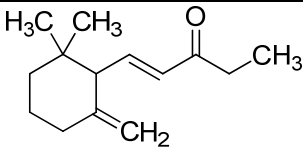
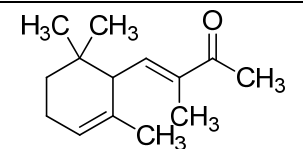
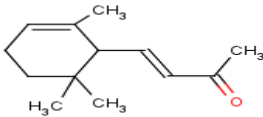
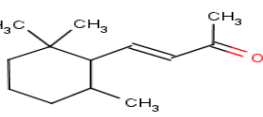
1. Chemical Identity

1.1. Identification and Purity

The structures of the sponsored substances and supporting chemicals are provided in Table 1.

The commercial product associated with CASRN 127-51-5 is a mixture of *alpha*, *beta* and *gamma* isomers of both *iso*-methylionone and *n*-methylionone, with *alpha-iso*-methylionone being the major isomer [*alpha-iso*-methylionone (55-65%), *beta-iso*-methylionone (4-7%), *alpha-n*-methylionone (22-32%), *beta-n*-methylionone (1-5%) and *gamma-n*-methylionone (1-5%)].

Test substance purity was not provided in the Robust Summaries. Exceptions are noted.

Table 1: Ionone Derivatives Category Structures for Sponsored and Supporting Chemicals		
Chemical Abstract Index Name	CASRN	Structure
SPONSORED CHEMICALS		
Ionone, methyl-	1335-46-2	 .alpha.-methylionone, ¹
		 .beta.-methylionone ¹
		 .gamma.-methylionone ¹
3-Buten-2-one, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-	127-51-5	 Representative Structure ²
SUPPORTING CHEMICALS		
<i>alpha</i> -Ionone	127-41-3	
<i>beta</i> -Ionone	14901-07-6	

¹ Composition of ionone, methyl- is specified as a mixture of alpha, beta, and gamma methylionones. The exact isomeric ratio is not indicated in the sponsor's submission. Individual components have their own CAS registry numbers; alpha-methylionone (CASRN 127-42-4) on TSCA, betamethylionone (CASRN 127-43-5) on TSCA, gamma-methylionone (CASRN 64070-16-2) not on TSCA.

² The commercial product recognized as alpha-isomethylionone (3-buten-2-one, 3-methyl-4-[2,6,6-trimethyl-2-cyclohexen-1-yl])- (CASRN 127-51-5) is a mixture of alpha, beta, and gamma isomers of both isomethylionone and n-methylionone with alpha-isomethylionone being the major isomer: alpha-isomethylionone (55–65%), beta-isomethylionone (4–7%), alpha-n-methylionone (22–32%), beta-n-methylionone (1–5%), and gamma-n-methylionone (1–5%)

1.2. Physical-Chemical Properties

The physical-chemical properties of the members of the ionone derivatives category are summarized in Table 2.

The members of the ionone derivatives category are liquid mixtures with moderate water solubilities and moderate vapor pressures.

Property	Ionone, methyl-	3-Buten-2-one, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-
	Value	Value
CASRN	1335-46-2	127-51-5
Molecular Weight	206.33	206.33
Physical State	Liquid, yellow color ²	Liquid, slightly yellow color ³
Melting Point	<25°C (based on physical state)	<25°C (based on physical state)
Boiling Point	266.2°C at 749 mmHg (mixture, measured) ⁴ ; 238°C (mixture, measured); 255°C at 760 mmHg (alpha, extrapolated) ⁵ ; 97°C at 2.7 mm Hg (alpha, measured) ⁶ ; 261°C at 760 mmHg (beta, extrapolated) ⁵ ; 102°C at 2.7 mm Hg (beta, measured) ⁶ ; 271°C at 760 mmHg (gamma, estimated) ⁷	238°C (mixture, measured); 130-131°C at 9.8 mm Hg (measured) ⁶ ; 257°C at 760 mmHg (extrapolated) ⁵
Vapor Pressure	0.005 mmHg at 20°C (mixture, measured); 0.027 mmHg at 25°C (alpha, estimated) ⁵ ; 0.019 mmHg at 25°C (beta, estimated) ⁵ ; 0.015 mmHg at 25°C (gamma, estimated) ⁷	0.006 mmHg at 20°C (measured)
Water Solubility	90 mg/L at 25°C (mixture, measured); 5.8 mg/L at 25°C (alpha, estimated) ⁷ ; 4.4 mg/L at 25°C (beta, estimated) ⁷ ; 4.9 mg/L at 25°C (gamma, estimated) ⁷	16 mg/L at 20°C (measured); 9.0 mg/L (measured) ⁸
Dissociation Constant (pK _a)	Not applicable	Not applicable
Henry's Law Constant	2.4×10 ⁻⁴ atm·m ³ /mole (alpha, estimated) ⁷ ; 2.3×10 ⁻⁴ atm·m ³ /mole (beta, estimated) ⁷ ; 2.0×10 ⁻⁴ atm·m ³ /mole (gamma, estimated) ⁷	1.0×10 ⁻⁴ atm·m ³ /mole (estimated) ⁷

	Ionone, methyl-	3-Buten-2-one, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-
Log K _{ow}	4.6 (mixture, measured); 4.78 (alpha, estimated) ⁷ ; 4.91 (beta, estimated) ⁷ ; 4.86 (gamma, estimated) ⁷ ;	4.6 (measured) ^{1,9}

¹ The Flavor and Fragrance High Production Volume Chemical Consortia, The Terpene Consortium.

August 18, 2004. Revised Robust Summary and Test Plan for Ionone Derivatives. Available online from: <http://www.epa.gov/chemrtk/pubs/summaries/ionederv/c13660tc.htm> as of June 3, 2010.

² Ashford, Robert D. 1994. Ashford's Dictionary of Industrial Chemicals: Properties, Production, Uses. London, England: Wavelength Publ, Ltd.

³ Lewis, R.J. Sr. 2007. Hawley's Condensed Chemical Dictionary 15th Edition. John Wiley & Sons, Inc.: New York, NY. 2007.

⁴ The test plan reported this boiling point was for alpha-iso-methylionone; however, the robust summary indicated that it was for ionone, methyl- CASRN 1335-46-2.

⁵ NOMO5. 1987. Programs to Enhance PC-Gems Estimates of Physical Properties for Organic Compounds. The Mitre Corp. Class 4 was used for the boiling point extrapolation of the representative structure shown in the appendix.

⁶ Fahlbusch et al. 2002. Flavors and Fragrances. Ullmann's Encyclopedia of Industrial Chemistry. VCH Publishers: New York, NY. Available online from <http://mrw.interscience.wiley.com/emrw/9783527306732/home/> as of June 7, 2010.

⁷ U.S. EPA. 2010. Estimation Programs Interface Suite™ for Microsoft® Windows, v4.00. U.S. Environmental Protection Agency, Washington, DC, USA. Available online from: <http://www.epa.gov/opptintr/exposure/pubs/episuitedi.htm> as of June 3, 2010.

⁸ Simonich, S.L. et al. 2000. Trace Analysis of Fragrance Materials in Wastewater and Treated Wastewater. Environ Sci Technol 34:959–965. Although the CASRN number indicated in this reference is 127-51-5, the chemical name used is *g*-methyl ionone, which is indicated by the sponsor as representing only 1–5% of 3-buten-2-one, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-. This data is shown but not used for estimation purposes.

⁹ The sponsor indicated that the main isomer in the test substance for determining log K_{ow} is alpha-isomethylionone at a purity of 87.8%.

2. General Information on Exposure

2.1. Production Volume and Exposure

The Ionone Derivatives category chemicals had an aggregated production and/or import volume in the United States between 1 million pounds and 10.5 million pounds in calendar year 2005.

- CASRN 127-51-5: <500,000 pounds;
- CASRN 1335-46-2: 1 to <10 million pounds;

CASRN 127-51-5:

No industrial processing and uses and commercial and consumer uses were reported.

CASRN 1335-46-2:

Non-confidential information in the IUR indicated that the industrial processing and uses of the chemical include other basic organic chemical manufacturing as odor agents. Non-confidential commercial and consumer uses of this chemical include soaps and detergents, and “other.”

2.2. Environmental Exposure and Fate

The environmental fate properties of the members of the ionone derivatives category are summarized in Table 3.

The members of the ionone derivatives category are expected to have moderate mobility in soil. A mixture of isomers composed of 55–65% alpha-isomethylionone (ionone, methyl-, CASRN 1335-46-2) and other isomers was found to be readily biodegradable (99.1% degradation in 31 days) in a buffered solution of activated sludge as measured by dissolved organic carbon (DOC). Ionone, methyl- achieved 70.5% of its theoretical biochemical oxygen demand using an acclimated sewage sludge and the modified MITI test (OECD 301C) over a 28-day incubation period. In addition, a test substance known commercially as Isoraldein 70 was found inherently biodegradable (63.4% biodegradation in 28 days) in 1:1 mixture of city and chemical sewage using a modified MITI test OECD 302C as measured by biochemical oxygen demand (BOD). The rate of hydrolysis is expected to be negligible under environmental pH and temperature. The rate of volatilization is considered moderate based on the Henry's Law constants. The members of the ionone derivatives category are expected to have low persistence (P1) and moderate bioaccumulation potential (B2).

Conclusion: The ionone derivatives category is composed of two substances that are grouped together due to their core structure being based on the parent terpene ionone, which occurs in nature as three olefinic isomers (alpha, beta, and gamma). The two members of the ionone derivatives category differ in that one is a methyl ionone and the other is an isomethylionone; however, both are commercially sold as mixtures of several isomers. The members of the ionone derivatives category are liquid mixtures at room temperature with moderate water solubilities and moderate vapor pressures. These substances are expected to have moderate mobility in soil. Volatilization of the members of the ionone derivatives category is considered moderate based on estimated Henry's Law constants. The rate of hydrolysis is considered negligible. The rate of atmospheric photooxidation is considered rapid. The members of the ionone derivatives category are expected to have low persistence (P1) and moderate bioaccumulation potential (B2).

Property	Ionone, methyl-			3-Buten-2-one, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-
	Value			Value
CASRN	1335-46-2			127-51-5
Photodegradation Half-life	0.9 hours (alpha, estimated) ² ; 0.5 hours (beta, estimated) ² ; 1.1 hours (gamma, estimated) ²			0.8 hours (estimated) ²
Hydrolysis Half-life	Stable			Stable
Biodegradation	99.1% biodegradation in 31 days, (readily biodegradable) ^{1,3} ; 70.5% biodegradation in 28 days (readily biodegradable) ^{1,4}			61.8% biodegradation in 28 days (readily biodegradable) ^{1,5} ; 63.4% biodegradation in 56 days (inherently biodegradable) ^{1,6}
Bioaccumulation Factor	BAF = 963 (alpha, estimated) ² ; BAF = 1,057 (beta, estimated) ² ; BAF = 937 (gamma, estimated) ²			BAF = 1,000 (estimated) ²
Log K _{oc}	3.1 (alpha, beta, and gamma, estimated) ²			3.0 (estimated) ²
Fugacity (Level III Model) ²	alpha	beta	gamma	
	<0.1	<0.1	0.15	<0.1
Air (%)	12.5	12.6	12.8	14.3
Water (%)	86.6	86.5	86.2	84.9
Soil (%)	0.89	0.89	0.90	0.90
Sediment (%)				
Persistence ⁷	P1 (low)			P1 (low)
Bioaccumulation ⁷	B2(moderate)			B2(moderate)

¹ The Flavor and Fragrance High Production Volume Chemical Consortia, The Terpene Consortium. August 18, 2004. Revised Robust Summary and Test Plan for Ionone Derivatives. Available online from: <http://www.epa.gov/chemrtk/pubs/summaries/ionederv/c13660tc.htm> as of June 3, 2010.

² U.S. EPA. 2010. Estimation Programs Interface Suite™ for Microsoft® Windows, v4.00. U.S. Environmental Protection Agency, Washington, DC, USA. Available online from: <http://www.epa.gov/opptintr/exposure/pubs/episuitedi.htm> as of June 3, 2010.

³ The test substance used in this biodegradation study was indicated by the sponsor as a mixture of isomers composed of 55–65% alpha-isomethylionone, 22–32% alpha-methylionone, 1–5% beta-methylionone, and 4–8% beta-isomethylionone, and 1–5% of beta or gamma-methylionone.

⁴ The test substance used in this biodegradation study was indicated by the sponsor as a mixture of isomers composed of 60–70% alpha-isomethylionone, 17–30% alpha-methylionone, 0–8% beta-methylionone, and 0–8% beta-isomethylionone.

⁵ The test substance used in this biodegradation study was indicated by the sponsor as a mixture of 55% alpha-isomethylionone and 28% alpha-methylionone.

⁶ The test substance used in this biodegradation study was indicated by the sponsor as a mixture of isomers composed of 60–70% alpha-isomethylionone, 17–30% beta-isomethylionone, alpha- and beta-methylionone, which is known as Isoraldein 70.

⁷ Federal Register. 1999. Category for Persistent, Bioaccumulative, and Toxic New Chemical Substances. *Federal Register* 64, Number 213 (November 4, 1999) pp. 60194–60204.

3. Human Health Hazard

A summary of health effects data submitted for SIDS endpoints is provided in Table 4. The table also indicates where data for tested category members are read-across (RA) to untested members of the category.

Acute Oral Toxicity

Methyl ionone (mixture of isomers) (CASRN 1335-46-2)

(1) Ten rats (information on strain and sex not provided) were administered CASRN 1335-46-2 (mixture of isomers: methyl-*alpha* ionone, *alpha-iso*-ionone, methyl-*beta*-ionone, “pseudo product”) via gavage at 5000 mg/kg and observed for an unspecified number of days. No mortalities were observed.

LD₅₀ > 5000 mg/kg

(2) Ten rats (information on strain and sex not provided) were administered CASRN 1335-46-2 (“Gamma type”, not otherwise specified) via gavage at 5000 mg/kg and observed for 14 days. No mortalities were observed.

LD₅₀ > 5000 mg/kg

alpha-iso-Methylionone (CASRN 127-51-5)

(1) Ten rats (information on strain and sex not provided) were administered CASRN 127-51-5 via gavage at a dose level of 5000 mg/kg and observed for an unspecified number of days following dosing. No mortalities were observed.

LD₅₀ > 5000 mg/kg

(2) Ten rats (information on strain and sex not provided) were administered CASRN 127-51-5 “pseudo product” via gavage at a dose level of 5000 mg/kg and observed for an unspecified number of days following dosing. One animal died at an unspecified time.

LD₅₀ > 5000 mg/kg

(3) CF-1 mice (10/sex/dose) were administered CASRN 127-51-5 via gavage at unspecified doses and observed for an unspecified number of days following dosing. Doses at which mortalities occurred were not provided.

LD₅₀ = 8714 mg/kg

Acute Dermal Toxicity

Methyl ionone (mixture of isomers) (CASRN 1335-46-2)

(1) Ten rabbits (information on strain and sex not provided) were administered CASRN 1335-46-2 (mixture of isomers: methyl-*alpha* ionone, *alpha-iso*-ionone, methyl-*beta*-ionone, “pseudo product”) via the dermal route at a dose of 5000 mg/kg under unspecified conditions and observed for an unspecified number of days. No mortalities were observed.

LD₅₀ > 5000 mg/kg

(2) Rabbits (information on strain and sex not provided) were administered CASRN 1335-46-2 (“Gamma type”, not otherwise specified) via the dermal route (abraded skin) at a dose level of 5000 mg/kg and observed for an unspecified number of days. It is unclear whether 8 or 10 rabbits were exposed. No mortalities were observed.

LD₅₀ > 5000 mg/kg

alpha-iso-Methylionone (CASRN 127-51-5)

Rabbits (10/dose, strain and sex not noted) were administered CASRN 127-51-5 (contains open-chain “pseudo” product) via the dermal route at 2500 or 5000 mg/kg under unspecified conditions and observed for an unspecified number of days. Six of 10 animals died in the 5000 mg/kg group, although the dermal LD₅₀ was calculated to be > 5000 mg/kg. **LD₅₀ > 5000 mg/kg**

Repeated-Dose Toxicity

alpha-iso-Methylionone (CASRN 127-51-5)

FDRL rats (15/sex) were fed a diet containing 2% CASRN 127-51-5 diluted in cottonseed oil, a concentration that was reported to provide an average CASRN 127-51-5 intake of 3.55 mg/kg/day for males and 4.10 mg/kg/day for females for 90 days. No exposure-related effects were observed based on evaluations of body weight, food and water intake, hematology, blood chemistry (endpoints not specified), liver and kidney weights and histopathology (21 tissues examined, including ovary and testis).

NOAEL = 3.55 mg/kg/day (males, only dose tested)

NOAEL = 4.10 mg/kg/day (females, only dose tested)

alpha-Ionone (CASRN 127-41-3, supporting chemical)

(1) FDRL rats (15/sex) were fed a diet containing 2% CASRN 127-41-3 diluted in cottonseed oil, a concentration that was reported to provide an average CASRN 127-41-3 intake of 11.8 mg/kg/day for males and 11.1 mg/kg/day for females for 90 days. No exposure-related effects were observed based on evaluations of body weight, food and water intake, hematology, blood chemistry (endpoints not specified), liver and kidney weights and histopathology (21 tissues examined, including ovary and testis).

NOAEL = 11.8 mg/kg/day (males, only dose tested)

NOAEL = 11.1 mg/kg/day (females, only dose tested)

(2) Sprague-Dawley rats (15/sex/dose) were fed diet containing CASRN 127-41-3 at unspecified concentrations calculated to result in target doses of 0, 10 or 100 mg/kg/day for 90 days. The actual intakes of CASRN 127-41-3 for the low and high doses, respectively, were determined to be 11 and 106-108 mg/kg/day for both sexes. Endpoints included body weight, food and water intake, hematology, blood chemistry, urinalysis, organ weights (brain, liver, spleen, kidneys, caecum, adrenals and male gonads) and histopathology. The histopathology examinations were performed on 31 tissues, including male and female gonads, from the control and high dose groups, and on liver from the low dose groups. No effects on any endpoint were observed at the low dose. The principal effect at the high dose was an increase in relative and absolute mean liver weights in males (which were statistically significant), and the only histopathological finding was desquamation of the thyroid in females at the high dose. Quantitative information regarding these effects was not provided.

NOAEL = 106-108 mg/kg/day (highest dose tested)

beta-Ionone (CASRN 14901-07-6, supporting chemical)

(1) FDRL rats (15/sex) were fed diet containing 2% CASRN 14901-07-6 diluted in cottonseed oil, a concentration that was reported to provide an average CASRN 14901-07-6 intake of 11.4 mg/kg/day for males and 11.6 mg/kg/day for females for 90 days. No exposure-related effects were observed based on evaluations of body weight, food and water intake, hematology, blood chemistry (endpoints not specified), liver and kidney weights and histopathology (21 tissues examined including male and female gonads).

NOAEL = 11.4 mg/kg/day (males, only dose tested)

NOAEL = 11.6 mg/kg/day (females, only dose tested)

(2) Sprague-Dawley rats (15/sex/dose) were fed diet containing CASRN 14901-07-6 at unspecified concentrations, calculated to result in target doses of 0, 10 or 100 mg/kg/day for 90 days. The actual intakes of CASRN 14901-07-6 were determined to be 11 and 106-108 mg/kg/day for both sexes. Endpoints included body weight, food and water intake, hematology, blood chemistry, urinalysis, organ weights (brain, liver, spleen, kidneys, caecum, adrenals and male gonads) and histopathology (31 tissues), including male and female gonads, in the control and high-dose groups. Histopathology examination of the liver was also performed at the low dose. No effects on any endpoint were observed at the low dose. Relative and absolute mean liver weights were statistically increased in males at the high dose. Relative brain, caecal, liver and kidney weights were statistically increased in females at the high dose. Quantitative information regarding these effects was not provided in the robust summary.

NOAEL = 106-108 mg/kg/day (males and females, highest dose tested)

Reproductive Toxicity

alpha-iso-Methylionone (CASRN 127-51-5)

No specific reproductive toxicity study is available. However, in the 90-day oral repeated-dose toxicity study described previously, no histopathological effects on male or female reproductive organs were observed.

beta-Ionone (CASRN 14901-07-6, supporting chemical)

(1) In a two-generation reproductive study, white rats (48 males and females, number/sex/dose or strain not specified) were administered an unspecified oil solution containing 0 or 2 mg CASRN 14901-07-6 by gavage every other day for 8 months. The 2 mg dose every other day was stated as corresponding to a daily intake of 1 mg/day or approximately 8-10 mg/kg/day. The females were exposed through three reproduction cycles, and mated with treated males (F0) during each cycle. Prior to reproduction, offspring from the first reproduction cycle (F1) were treated in the same manner as the parental rats and were allowed to reach maturity. There were no treatment-related effects in the F0 or F1 dams, F1 offspring from the three reproduction cycles, or F2 offspring as evaluated by maternal weight, number of pregnancies, number of born offspring, number of offspring born alive, pup weight at birth and after 7 and 21 days, or postnatal viability of the offspring.

NOAEL (systemic and reproductive toxicity) = 8-10 mg/kg/day (only dose tested)

(2) In the 90-day oral repeated-dose toxicity study described previously, no histopathological effects on male or female reproductive organs were observed

alpha-Ionone (CASRN 127-41-3, supporting chemical)

No specific reproductive toxicity study is available. However, in the 90-day oral repeated-dose toxicity study described previously, no histopathological effects on male or female reproductive organs were observed.

Developmental Toxicity

alpha-iso-Methylionone (CASRN 127-51-5)

In a prenatal developmental toxicity study, CASRN 127-51-5 in a corn oil vehicle was administered via gavage to four groups of presumed pregnant Sprague-Dawley rats on gestational days 7-17, at 0, 3, 10, or 30 mg/kg/day. Animals were observed for clinical signs, abortions, and premature delivery. Body weights and feed consumption were measured regularly. Animals were euthanized on gestational day 21. Uteri from pregnant rats were examined for number and distribution of implantations, live and dead fetuses, and early and late resorptions. The number of corpora lutea in each ovary was also recorded. Fetuses were removed from the uterus, weighed, and examined for gender and gross external alterations. Approximately half of the fetuses in each litter were examined for soft tissue alterations. The remaining fetuses in each litter were eviscerated, cleared, stained, and examined for skeletal alterations.

No mortality occurred during the study. No clinical signs were attributed to CASRN 127-51-5, both because the incidence was not dose related and was equivalent in controls and treated groups. Maternal feed consumption and body weight gains were unaffected at all doses. No effects on caesarean section and litter data were noted at any dose, which included corpora lutea/rat, implantations/rat, resorptions/rat, litter size/rat, fetal body weight/litter, and live or dead fetuses/group. All fetal gross external, soft tissue, or skeletal alterations (malformations or variations) were considered unrelated to CASRN 127-51-5 at any dose because (1) neither the fetal nor litter incidences were dosage dependent; (2) the incidences did not significantly differ from the control group values; and/or (3) the incidences were within ranges of historical controls at the testing facility. [Politano VT et al. "Evaluation of the Developmental Toxicity of Alpha-iso-methylionone in Rats", International Journal of Toxicology 26:271-276. 2007.]

NOAEL (maternal and fetal) = 30 mg/kg/day (highest dose tested)

beta-Ionone (CASRN 14901-07-6, supporting chemical)

In the two-generation prenatal reproductive study described above, no developmental toxicity was observed in F1 offspring from the three reproduction cycles, or F2 offspring. This was evaluated by number of born offspring, number of offspring born alive, pup weight (at birth and after 7 and 21 days), or postnatal viability of the offspring.

NOAEL (maternal/developmental toxicity) = 8-10 mg/kg/day (only dose tested)

Genetic Toxicity – Gene Mutations

In vitro

Methyl ionone (mixture of isomers) (CASRN 1335-46-2)

Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 were incubated with CASRN 1335-46-2 (≤ 5000 $\mu\text{g}/\text{plate}$) in the presence and absence of metabolic activation. DMSO served as the vehicle. No precipitate was observed at any dose. Cytotoxicity was observed, but the reporting on this was unclear. Additional information on study performance (including that on vehicle or dose groups) and a quantitative presentation of findings were not provided.

CASRN 1335-46-2 was not mutagenic in this assay.

alpha-iso-Methylionone (CASRN 127-51-5)

Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 were incubated with CASRN 127-51-5 (≤ 3600 $\mu\text{g}/\text{plate}$) or positive controls, with and without metabolic activation, and were concluded as showing no evidence of mutagenicity. Additional information regarding the study performance (including that on vehicle) and a quantitative presentation of findings were not provided.

CASRN 127-51-5 was not mutagenic in this assay.

Genetic Toxicity –Chromosomal Aberrations

In vivo

alpha-Ionone (CASRN 127-41-3, supporting chemical)

NMRI mice (unspecified numbers of males and females per dose) received intraperitoneal injections of CASRN 127-41-3 (825, 1444 or 2063 mg/kg doses), at 0 and 24 hours. Animals were killed at 30 hours and slides were prepared from bone marrow smears and the mean number of micronucleated polychromatic erythrocytes were determined. The mean number of micronucleated polychromatic erythrocytes/1000 polychromatic erythrocytes were 1.7, 1.0, 0.7, and 1.9 at 0, 825, 1444, or 2063 mg/kg/bw, respectively.

CASRN 127-41-3 did not induce micronuclei in this assay.

Conclusions: The acute oral and dermal toxicities of the ionone derivatives category to rats and mice, and rabbits, respectively, are low. In single-dose 90-day oral (dietary) repeated-dose studies in rats with CASRN 127-51-5 and the supporting chemical, CASRN 127-41-3, no treatment-related effects were observed. The NOAEL for systemic toxicity for CASRN 127-51-5 is 3.55 - 4.10 (male – female) mg/kg/day (only dose tested) and for CASRN 127-41-3 is 11.1 – 11.8 (female-male) mg/kg/day (only dose tested). Similarly, in a single-dose 90-day dietary study in rats, the supporting chemical, CASRN 14901-07-6, showed no treatment-related effects. The NOAEL for systemic toxicity is 11.4 – 11.6 (male – female) mg/kg/day (only dose tested). In another 90-day repeated-dose toxicity study in rats, the supporting chemical, CASRN 14901-07-6 showed no treatment-effects; the NOAEL for systemic toxicity is 106-108 mg/kg/day (highest dose tested). No treatment-related effects were observed on the reproductive organs of rats in the 90-day repeated-dose studies described above. The supporting chemical, CASRN 14901-07-6, did not cause reproductive effects in a two-generation single dose dietary reproduction study in rats; the NOAEL for reproductive/maternal/developmental toxicity is 8-10 mg/kg/day (only dose tested). In an oral prenatal developmental toxicity study in rats with CASRN 127-51-5, no maternal or fetal toxicity was observed; the NOAEL for maternal and developmental toxicity is 30 mg/kg/day (highest dose tested). CASRNs 1335-46-2 and 127-51-

5, were negative in bacteria *in vitro* and the supporting chemical, CASRN 127-41-3, was negative in a mouse *in vivo* micronucleus test.

Table 4. Summary of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program – Human Health Data

Endpoints	SPONSORED CHEMICAL Methyl ionone (mixture of isomers) ^a (1335-46-2)	SPONSORED CHEMICAL <i>alpha-iso</i> -Methylionone (127-51-5)	SUPPORTING CHEMICAL <i>alpha</i> -Ionone (127-41-3)	SUPPORTING CHEMICAL <i>beta</i> -Ionone (14901-07-6)
Acute Oral Toxicity LD50 (mg/kg)	> 5000	>5000	–	–
Acute Dermal Toxicity LD50 (mg/kg)	> 5000	> 5000	–	–
Repeated-Dose Toxicity NOAEL/LOAEL Oral (mg/kg/day)	No Data NOAEL = 3.55 (only dose tested) (RA)	NOAEL = 3.55 (only dose tested)	NOAEL = 106	NOAEL = 11
Reproductive Toxicity NOAEL/LOAEL Oral (mg/kg/day) Reproductive Toxicity	No Data NOAEL = 3.55 (only dose tested) (RA)	NOAEL = 3.55 (only dose tested) No effects on reproductive organs in a 90-day oral repeated-dose toxicity study in rats .	NOAEL = 11 (only dose tested) No effects on reproductive organs in a 90-day oral repeated-dose toxicity study in rats.	NOAEL = 8-11 (only one dose tested) No effects on reproductive organs in a 90-day oral repeated-dose toxicity study in rats.
Developmental Toxicity NOAEL/LOAEL Oral (mg/kg/day)	No Data NOAEL = 30 (RA)	NOAEL = 30	–	
Genetic Toxicity – Gene Mutation In vitro	Negative	Negative	–	–
Genetic Toxicity – Chromosomal Aberrations or Micronucleus In vivo	No Data Negative (RA)	No Data Negative (RA)	Negative	–

Measured data in bold text; (RA) = Read Across; – indicates that endpoint was not addressed for this substance

^aMixture of *alpha*-, *beta*- and *gamma-n*-methylionone.

4. Hazard to the Environment

A summary of aquatic toxicity data submitted for SIDS endpoints is provided in Table 5. The table also indicates where data for tested category members are read-across (RA) to untested members of the category. Additional results are from an ecological impact test of chemical substances carried out by the Japanese Ministry of the Environment (MOE) in 2007.

Acute Toxicity to Fish

Methyl ionone (mixture of isomers) (CASRN 1335-46-2)

Japanese medaka (*Oryzias latipes*) were exposed to CASRN 1335-46-2 (mixture of isomers composed of 70.9% *alpha* methyl ionone, 11.4% ionone, n-methyl *beta*, 5.0% n-methyl *gamma* ionone, 1.8% *iso*-methyl *alpha* ionone and < 1% *iso*-methyl *beta* ionone and pseudo methyl ionones, 90% sum of isomers) in DMF at nominal concentrations of 0.500, 0.890, 1.60, 2.80, 5.00 mg/L under static-renewal conditions for 96 hours. Mean measured concentrations were 0.432, 0.814, 1.50, 2.53 and 4.62 mg/L. At 96 hours there was 10% (1/10) mortality in the non-solvent control and at 0.814, 1.50 and 2.53 mg/L, and 100% mortality at 4.62 mg/L (MOE, 2007).

96-h LC₅₀ = 3.04 mg/L

Acute Toxicity to Aquatic Invertebrates

Methyl ionone (mixture of isomers) (CASRN 1335-46-2)

(1) Water fleas (*Daphnia magna*) were exposed to CASRN 1335-4602 (mixture of isomers composed of 55 – 65% *alpha-iso*-methylionone, 22 – 32% *alpha*-methylionone, 1-5% *beta*-methylionone, 4-8% *beta-iso*-methylionone and 1-5% of *beta*- or *gamma*-methylionone, 98% sum of isomers) at nominal concentrations of 0.78, 1.3, 2.2, 3.6 or 6.0 mg/L under static-renewal conditions for 48 hours. Mean measured concentrations were 0.621, 1.14, 1.82, 2.99 and 5.32 mg/L. The NOEC was 1.14 mg/L and the LC₅₀ was 3.11 mg/L.

48-h EC₅₀ = 2.65 mg/L

(2) Water fleas were exposed to CASRN 1335-46-2 (mixture of isomers composed of 70.9% *alpha* methyl ionone, 11.4% ionone, n-methyl *beta*, 5.0% n-methyl *gamma* ionone, 1.8% *iso*-methyl *alpha* ionone and < 1% *iso*-methyl *beta* ionone and pseudo methyl ionones, 90% sum of isomers) in DMF at nominal concentrations of 0.500, 0.900, 1.60, 2.80 or 5.00 mg/L under static-renewal conditions for 48 hours. Mean measured concentrations were 0.441, 0.802, 1.45, 2.51 and 4.73 mg/L. At 48 hours, 15% (3/20) were immobilized at 2.51 mg/L and 100% were immobilized at 4.73 mg/L (MOE, 2007).

48-h EC₅₀ = 3.14 mg/L

Toxicity to Aquatic Plants

Methyl ionone (mixture of isomers) (CASRN 1335-46-2)

Green algae (*Pseudokirchneriella subcapitata*) were exposed to CASRN 1335-46-2 (mixture of isomers composed of 55 – 65% *alpha-iso*-methylionone, 22 – 32% *alpha*-methylionone, 1 – 5% *beta*-methylionone, 4 – 8% *beta-iso*-methylionone and 1 – 5% of *beta*- or *gamma*-methylionone, 98% sum of isomers) at nominal concentrations of 0, 0.5, 1.0, 2.0, 4.0, 8.0 or 16 mg/L under static conditions for 72 hours. Initial mean measured concentrations were 0.404, 0.954, 1.68, 2.94, 6.74 and 14.0 mg/L; final measured concentrations were 40 – 50% of nominal concentrations. The NOEC was 1.68 mg/L.

72-h EC₅₀ (growth rate) = 7.47 mg/L

72-h EC₅₀ (cell number) = 3.23 mg/L

72-h EC₅₀ (biomass) = 2.89 mg/L

Chronic Toxicity to Aquatic Invertebrates

No data were submitted for this endpoint.

Table 5. Summary of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program – Aquatic Toxicity Data		
Endpoints	SPONSORED CHEMICAL Methyl ionone (mixture of isomers) (1335-46-2)	SPONSORED CHEMICAL <i>alpha-iso</i>-Methylionone (127-51-5)
Fish 96-h LC₅₀ (mg/L)	3.04	No adequate data 3.04 (RA)
Aquatic Invertebrates 48-h LC₅₀ (mg/L)	2.65	No Data 2.65 (RA)
Aquatic Plants 72-h EC₅₀ (mg/L) Biomass Growth	2.89 7.47	No Data 2.89 7.47 (RA)
Chronic Toxicity to Invertebrates 21-day LC₅₀ (mg/L)	No data	No data

bold = measured data (i.e., derived from testing); (RA) = Read Across; – indicates that endpoint was not evaluated for this substance

Conclusions: For CASRN 1335-46-2, the 96-h LC₅₀ for fish is 3.04 mg/L, the 48-h EC₅₀ for aquatic invertebrates is 2.65 mg/L and the 72-h EC₅₀ for aquatic plants is 2.89 mg/L for biomass and 7.47 mg/L for growth rate. There are no data for the chronic toxicity to aquatic invertebrates.