1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran (HHCB) (CAS# 1222-05-5) GreenScreen® for Safer Chemicals (GreenScreen®) Assessment

Prepared for:

Women's Voices for the Earth

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HHCB is a chemical that functions as a fragrance ingredient. The product name Galaxolide or Galaxolide 50 (containing 50% main isomer) is the most common form of HHCB; it refers to diluted HHCB with diethyl phthalate (DEP). HHCB has a relatively complete toxicological dataset. A number of the studies were conducted on Galaxolide which contains HHCB (most commonly 65%, as listed with each relevant study) and the diluent DEP or isopropyl myristate. Data on Galaxolide were also included in this GreenScreen® assessment. These data were also considered by the EU (2008) and U.S. EPA (2014) in their assessments of HHCB.

HHCB was assigned a GreenScreen Benchmark™ Score of 1 ("Avoid – Chemical of High Concern"). This score is based on the following hazard score:

- Benchmark 1a
    - PBT = High Persistence (P) + High Bioaccumulation (B) + Very High Ecotoxicity (acute aquatic toxicity (AA) and chronic aquatic toxicity (CA))

Data gaps (DG) exist for reproductive toxicity (R), repeated dose neurotoxicity (Nr*) and respiratory sensitization (SnR*). As outlined in CPA (2013) Section 12.2 (Step 8 – Conduct a Data Gap Analysis to assign a final Benchmark score), HHCB meets requirements for a GreenScreen® Benchmark Score of 1 despite the hazard data gaps. In a worst-case scenario, if HHCB were assigned a High score for the data gaps R, Nr* or SnR*, it would still be categorized as a Benchmark 1 Chemical.

HHCB is the major component (50%) of Galaxolide. Therefore, Galaxolide also has High scores for P and B based on the scores of HHCB. Although the acute and chronic aquatic toxicity values of HHCB are doubled to account for the reduced aquatic toxicity for Galaxolide due to dilution, these values still warrant Very High Scores for both endpoints. Therefore, Galaxolide is also a Benchmark 1.

GreenScreen® Benchmark Score for Relevant Route of Exposure:

As a standard approach for GreenScreen® evaluations, all exposure routes (oral, dermal and inhalation) were evaluated together, so the GreenScreen® Benchmark Score of 1 ("Avoid – Chemical of High Concern") is applicable for all routes of exposure.

GreenScreen® Hazard Ratings for HHCB

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS*	SnR*	IrS	IrE	AA	CA	P	B	Rx	F
						single	repeated*	single	repeated*										
L	L	DG	*L*	*M*	L	*L*	L	L	DG	L	DG	*M*	*L*	vH	vH	*H*	H	*L*	L

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect estimated (modeled) values, authoritative B lists, screening lists, weak analogues, and lower confidence. Hazard levels in BOLD font are used with good quality data, authoritative A lists, or strong analogues. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L) instead of three (i.e., H, M, and L), and are based on single exposures instead of repeated exposures. Please see Appendix A for a glossary of hazard acronyms.

GreenScreen® Assessment for 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran (HHCB) (CAS #1222-05-5)

Method Version: GreenScreen® Version 1.2[1]
Assessment Type[2]: Certified

Chemical Name: 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran (HHCB)

CAS Number: 1222-05-5

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Date: April 16, 2015

Confirm application of the *de minimus* rule[3]: Undiluted HHCB is too viscous to handle as a fragrance ingredient. Therefore, it is diluted in various solvents at the ratio of approximately 65 (HHCB):35 (diluent). The product name Galaxolide or Galaxolide 50 (containing 50% main isomer) is the most common form of HHCB; it refers to diluted HHCB with diethyl phthalate (DEP). Other diluents are benzyl benzoate and isopropyl myristate.

The manufactured HHCB is a mixture of four isomers composing > 95% of the total product weight. HHCB (CAS #1222-05-5) is the major isomer. The other three minor isomers are:

- 1,3,4,6,7,8-Hexahydro-4,6,6,8-tetramethyl-(6 or 8)-ethylcyclopenta- γ -2-benzopyran (CAS #78448-48-3 and 78448-49-4), 6-10% in Galaxolide;
- 1,3,4,7,8,9-Hexahydro-4,7,7,8,9,9-hexamethylcyclopenta[H]-2-benzopyran (CAS #114109-63-6), 5-8% in Galaxolide
- 1,2,4,7,8,9-Hexahydro-1,7,7,8,9,9-hexamethylcyclopenta[F]-2-benzopyran (CAS #114109-62-5), 6-8% in Galaxolide

Known impurities/by-products of Galaxolide are 1,1,2,3,3-pentamethyl-5-t-pentylindan (no CAS #), 1,1,2,3-tetramethyl-5-t-butyl-3-ethylindane (no CAS#), β1,1,2,3,3-hexamethylindan-5-ethanol (CAS #1217-08-9), 5-t-butyl-1,1,2,3,3-pentamethylindan (CAS #66553-13-7) and 1,1,2,3,3-pentamethylindan (CAS #1203-17-4). Each of the impurities is present at < 1% in the commercial preparation.

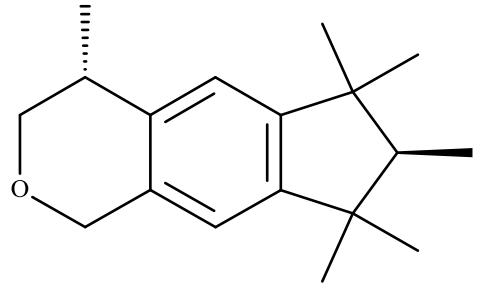
ToxServices screened the solvents, impurities and isomers with the GreenScreen® List Translator Tool (Pharos 2015) as summarized in Table 1 below. As toxicological studies were performed on commercially available preparations that contain all isomers and impurities, and little is known on

[1] Use GreenScreen® Assessment Procedure (Guidance) V1.2
[2] GreenScreen® reports are either "UNACCREDITED" (by unaccredited person), "AUTHORIZED" (by Authorized GreenScreen® Practitioner), "CERTIFIED" (by Licensed GreenScreen® Profiler or equivalent) or "CERTIFIED WITH VERIFICATION" (Certified or Authorized assessment that has passed GreenScreen® Verification Program)
[3] Every chemical in a material or formulation should be assessed if it is:
1. intentionally added and/or
2. present at greater than or equal to 100 ppm

the toxicities of these chemicals alone, no separate GreenScreens® were performed. However, assessing the toxicity of the diluents is out of the scope of this evaluation.

Table 1: List Translator Screening Summary of Non-Active Components of Galaxolide		
Chemical Name	CAS #	GreenScreen® List Translator Score or Benchmark Score[4]
Diethyl phthalate	84-66-2	LT-P1
Benzyl benzoate	120-51-4	LT-P1
Isopropyl myristate	110-27-0	LT-U
1,1,2,3,3-Pentamethyl-5-t-pentylindan	None	N/A
1,1,2,3-Tetramethyl-5-t-butyl-3-ethylindane	None	N/A
β1,1,2,3,3-Hexamethylindan-5-ethanol	1217-08-9	LT-U
5-t-Butyl-1,1,2,3,3-pentamethylindan	66553-13-7	Not in Pharos database, LT-U
1,1,2,3,3-Pentamethylindan	1203-17-4	LT-U
1,3,4,6,7,8-Hexahydro-4,6,6,8-tetramethyl-(6 or 8)-ethylcyclopenta- γ -2-benzopyran	78448-48-3 and 78448-49-4	Not in Pharos database LT-U
1,3,4,7,8,9-Hexahydro-4,7,7,8,9,9-hexamethylcyclopenta[H]-2-benzopyran	114109-63-6	Not in Pharos database LT-U
1,2,4,7,8,9-Hexahydro-1,7,7,8,9,9-hexamethylcyclopenta[F]-2-benzopyran	114109-62-5	Not in Pharos database LT-U

Chemical Structure(s):



1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-γ-2-benzopyran (HHCB) (CAS #1222-05-5), Main isomer (74-76%) in Galaxolide;

Also called: Galaxolide; Hexahydrohexamethyl cyclopentabenzopyran; 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-benzopyran; Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran; Cyclopenta(g)-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-; Galaxolide White; Abbalide; Pearlide, HHCB, (ChemIDplus 2015).

Chemical Structure(s) of Chemical Surrogates Used in the GreenScreen®:
HHCB has a relatively complete toxicological dataset. A number of the studies were conducted on Galaxolide which contains HHCB (most commonly 65%, as listed with each relevant study) and the diluent DEP or isopropyl myristate. Data on Galaxolide were also included in this report. These data were also considered by the EU (2008) and U.S. EPA (2014) in their assessments of HHCB.

[4] The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to screen for GreenScreen® benchmark 1 chemicals (CPA 2012b). Pharos (Pharos 2015) is an online list-searching tool that is used to screen chemicals against the lists in the List Translator electronically.

ToxServices used U.S. EPA's Analog Identification Methodology (AIM) software to search for surrogates of HHCB to fill data gaps. However, no suitable surrogates were identified.

Identify Applications/Functional Uses:
Fragrance ingredient in personal care products (13%), fine fragrances (5%), detergents (25%), fabric softeners (14%), soaps (9%), bath and shower products (10%), hair care products (10%), industrial and household cleaners (8%) and others (6%) (EU 2008)

GreenScreen® Summary Rating for HHCB[5]: HHCB was assigned a GreenScreen Benchmark™ Score of 1 ("Avoid – Chemical of High Concern") (CPA 2014). This score is based on the following hazard score:

- Benchmark 1a
  - PBT = High Persistence (P) + High Bioaccumulation (B) + Very High Ecotoxicity (acute aquatic toxicity (AA) and chronic aquatic toxicity (CA))

Data gaps (DG) exist for reproductive toxicity (R), repeated dose neurotoxicity (Nr*) and respiratory sensitization (SnR*). As outlined in CPA (2013) Section 12.2 (Step 8 – Conduct a Data Gap Analysis to assign a final Benchmark score), HHCB meets requirements for a GreenScreen® Benchmark Score of 1 despite the hazard data gaps. In a worst-case scenario, if HHCB were assigned a High score for the data gaps R, Nr* or SnR*, it would still be categorized as a Benchmark 1 Chemical.

HHCB is the major component (50%) of Galaxolide. Therefore, Galaxolide also has High scores for P and B based on the scores of HHCB. Although the acute and chronic aquatic toxicity values of HHCB are doubled to account for the reduced aquatic toxicity for Galaxolide due to dilution, these values still warrant Very High Scores for both endpoints. Therefore, Galaxolide is also a Benchmark 1.

Figure 1: GreenScreen® Hazard Ratings for HHCB

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS*	SnR*	IrS	IrE	AA	CA	P	B	Rx	F
						single	repeated*	single	repeated*										
L	L	DG	*L*	*M*	L	*L*	L	L	DG	L	DG	*M*	*L*	vH	vH	*H*	H	*L*	L

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect estimated (modeled) values, authoritative B lists, screening lists, weak analogues, and lower confidence. Hazard levels in BOLD font are used with good quality data, authoritative A lists, or strong analogues. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L) instead of three (i.e., H, M, and L), and are based on single exposures instead of repeated exposures. Please see Appendix A for a glossary of hazard acronyms.

Transformation Products and Ratings:
Identify feasible and relevant fate and transformation products (i.e., dissociation products, transformation products, valence states) and/or moieties of concern[6]

[5] For inorganic chemicals with low human and ecotoxicity across all hazard endpoints and low bioaccumulation potential, persistence alone will not be deemed problematic. Inorganic chemicals that are only persistent will be evaluated under the criteria for Benchmark 4.

HHCB can undergo photodegradation in the atmosphere by reaction with OH-radicals to the ring and by H-atom abstraction from the C-H bonds of the -CH_2 groups adjacent to the ether O atom. Experimental data suggest that direct photolysis is the primary degradation pathway of photodegradation while indirect photochemical degradation by reactive oxygen species is a minor pathway. Another study identified the formation of a five-ring with aldehyde group or the removal of the oxygen moiety at the pyran side of the molecule during photo-induced degradation. Under atmospheric conditions, the half-life of HHCB is 3.7 hours. The exact structures, identities and persistence of these degradation products were not reported (EU 2008).

HHCB is not readily biodegradable in the aquatic environment, with no mineralization in standard ready biodegradability tests. Primary degradation was measured to be relatively fast, with a half-life of 10 – 15 hours in a sludge test and 100 hours in a river die-away test. Various metabolites were identified at different time points with increasing polarity in time. Galaxolidone (oxidation on the (benzene)pyran ring and hydroxy acid were identified likely transformation products. No other degradation products were reported (EU 2008).

In soil, HHCB completely disappeared within one year with a half-life of 140 – 145 days in sludge amended soil studies. No specified degradation products were identified (EU 2008).

As shown in Table 2 below, neither of the identified degradation products is an LT-1 chemical. Although not all environmental transformation products were identified, there is no indication that HHCB is transformed to chemicals that are known to be more toxic than the parent chemical. Therefore, the Benchmark score of HHCB is not affected by the presence of transformation products.

Table 2: HHCB Transformation Product Summary Table

Functional Use	Life Cycle Stage	Transformation Pathway	Transformation Products	CAS #	Feasible and Relevant?	GreenScreen® List Translator Score or Benchmark Score[7,8]
Fragrance	Disposal	Aquatic degradation	Galaxolidone	507442-49-1	Yes	Not in Pharos
Fragrance	Disposal	Aquatic degradation	Hydroxy acid (or 6-(2-hydroxy-1-methyl-ethyl)-1,1,2,3,3-pentamethyl-indane-5-carboxylic acid)	N/A	Yes	N/A

Introduction

HHCB is a high production volume fragrance ingredient. It is one of the most common synthetic polycyclic musks, and is used in both consumer and commercial products. Technical grade

[6] A moiety is a discrete chemical entity that is a constituent part or component of a substance. A moiety of concern is often the parent substance itself for organic compounds. For inorganic compounds, the moiety of concern is typically a dissociated component of the substance or a transformation product.
[7] The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to screen for GreenScreen® benchmark 1 chemicals (CPA 2012b). Pharos (Pharos 2015) is an online list-searching tool that is used to screen chemicals against the lists in the List Translator electronically.
[8] The way you conduct assessments for transformation products depends on the Benchmark Score of the parent chemical (See Guidance).

Galaxolide consists of four diastereoisomers (i.e., isomers that differ in the spatial arrangement of atoms rather than being mirror images of each other), with HHCB as the major isomer (U.S. EPA 2014). Galaxolide is manufactured by the reaction of α-methyl styrene and tertiary amylene with the catalyst sulphuric acid. The resulting pentamethyl indane is reacted with propylene oxide to form HHCB-alcohol. HHCB (undiluted) is formed by cyclization with paraformaldehyde. HHCB is then diluted to a pourable liquid. The name Galaxolide refers to HHCB diluted with diethyl phthalate (EU 2008).

ToxServices assessed HHCB against GreenScreen® Version 1.2 (CPA 2013) following procedures outlined in ToxServices' SOP 1.37 (GreenScreen® Hazard Assessment) (ToxServices 2013).

GreenScreen® List Translator Screening Results

The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to identify GreenScreen® benchmark 1 chemicals (CPA 2012b). Pharos (Pharos 2015) is an online list-searching tool that is used to screen chemicals against the List Translator electronically. The output indicates benchmark or possible benchmark scores for each human health and environmental endpoint. The output for HHCB can be found in Appendix B and a summary of the results can be found below:

PBT

Oregon DEQ – Priority Persistent Pollutants – Tier 1

Endocrine

ChemSec – Substitute List – Endocrine Disruption
TEDX – Potential Endocrine Disruptors
EC Priority Disrupters – Category 3B (Substances with no or insufficient data gathered)

Aquatic

EC – CLP/GHS Hazard Statements – H400 – Aquatic Acute 1 – Very toxic to aquatic life
EC – CLP/GHS Hazard Statements – H410 – Aquatic Chronic 1 – Very toxic to aquatic life with long lasting effects
EC – Risk Phrases – R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

Restricted List

German FEA – Substances Hazardous to Waters (VwVwS) – Class 2 Hazard to Waters
Environment Canada – Domestic Substances List – Inherently Toxic in the Environment

HHCB is not listed by DOT (U.S. DOT 2008a, b)

PhysioChemical Properties of HHCB

HHCB is a viscous colorless liquid at normal temperature and pressure. It is slightly soluble in water (solubility between 0.1 and 100 mg/L), and the vapor pressure of 5.47×10^{-4} mmHg suggests that it can form a vapor. The partition coefficient of 5.9 indicates that it has a high potential to bioaccumulate (U.S. EPA 2013).

Table 3: Physical and Chemical Properties of HHCB (CAS #1222-05-5)		
Property	Value	Reference
Molecular formula	$C_{18}H_{26}O$	U.S. EPA 2014
SMILES Notation	O1C[C@@H](c2c(cc3c(C([C@@H](C)C3(C)C)(C)C)c2)C1)C	ChemIDplus 2015
Molecular weight	258.44	U.S. EPA 2014
Physical state	Liquid	U.S. EPA 2014
Appearance	Colorless, highly viscous at 20ºC and 1,013 hPa	U.S. EPA 2014
Melting point	-10 to 0ºC	U.S. EPA 2014
Vapor pressure	5.47 x 10^{-4} mmHg at 25ºC (OECD Test Guideline 104)	U.S. EPA 2014
Water solubility	1.65 mg/L at 25ºC 2.3 mg/L at 20ºC (OECD Test Guideline 105)	U.S. EPA 2014
Dissociation constant	Not applicable. HHCB is non-ionizable	U.S. EPA 2014
Density/specific gravity	0.99 – 1.015 g/cm^3 at 20ºC (OECD Test Guideline 109)	EU 2008
Partition coefficient	5.9 (OECD Test Guideline 117)	EU 2008

Hazard Classification Summary Section:

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M, or L): *L*

HHCB was assigned a score of Low for carcinogenicity based on expert judgment by EU and U.S. EPA and the lack of structural alerts. GreenScreen® criteria classify chemicals as a Low hazard for carcinogenicity when adequate data are available and negative, there are no structural alerts, and they are not classified under GHS (CPA 2012a). The confidence level was reduced due to the lack of experimental data.

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists
- EU 2008
  - No data were available on the carcinogenicity of HHCB. HHCB was judged to be of low carcinogenicity potential based on the lack of genotoxicity and lack of indications from repeated dose toxicity studies.
- U.S. EPA 2014
  - No standard 2-year carcinogenicity assays were identified for HHCB. EPA/OPPT concluded that data are not needed for this endpoint based on the weight of evidence from mutagenicity, subchronic toxicity studies and endocrine studies.
- Toxtree 2014
  - HHCB has no structural alerts for genotoxic carcinogenicity or nongenotoxic carcinogenicity (Appendix D).
- Based on the weight of evidence, a score of Low was assigned. No experimental data were identified for this endpoint. HHCB does not have structural alerts for carcinogenicity through the genotoxic or nongenotoxic mechanisms of pathways. OncoLogic could not be used to assess HHCB's carcinogenicity, because its structure does not fit into any of the existing categories in

the program. Both U.S. EPA/OPPT and the EU concluded that HHCB has a low concern for carcinogenicity based on weight of evidence.

Mutagenicity/Genotoxicity (M) Score (H, M, or L): L

HHCB was assigned a score of Low for mutagenicity/genotoxicity based on negative results in two bacterial reverse mutation assays, three *in vitro* chromosomal aberration tests, one UDS assay, one SOS assay, one sister chromatid exchange assay and one *in vivo* micronucleus test. GreenScreen® criteria classify chemicals as a Low hazard for mutagenicity/genotoxicity when adequate data are available and negative, there are no structural alerts, and they are not classified under GHS (CPA 2012a).

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists
- ECHA 2015
  - *In vitro*: HHCB (> 99% pure) was negative in a GLP-compliant chromosomal aberration assay conducted according to OECD TG 473 in Chinese hamster ovary (CHO) cells. Cells were treated with 5 – 20 µg/mL HHCB without metabolic activation and 8.7 – 30 µg/mL with metabolic activation. Cytotoxicity was observed at 30 – 34.5 µg/mL and 20 µg/mL with and without metabolic activation, respectively. There were no significant increases in the structural or numerical chromosomal aberrations at any dose. Positive and negative controls produced the desired responses.
  - *In vitro*: HHCB (> 99% pure) was negative in a GLP-compliant bacterial reverse mutation assay conducted according to OECD TG 471 in *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537 and TA1538 and *Escherichia coli* WP2 uvrA strain at concentrations of 10 – 5,000 µg/plate with and without metabolic activation. Positive and negative controls produced the desired responses. No cytotoxicity was observed.
  - *In vitro:* HHCB (> 99% pure) was negative in a GLP-compliant unscheduled DNA synthesis (UDS) assay conducted according to OECD TG 482 in rat hepatocytes at concentrations of 0.15 – 5,000 µg/mL with and without metabolic activation. There was no increase in net nuclear grain count at concentrations of up to 15 µg/mL, and cytotoxicity was observed at concentrations of 15 µg/mL and higher. Positive and negative controls produced desired responses.
  - *In vitro*: HHCB (Galaxolide containing 65% HHCB in DEP) was negative in a non-GLP micronucleus assay with a protocol similar to OECD TG 487 in human peripheral lymphocytes at concentrations of 0.05 – 194 µM with and without metabolic activation. There was no increase in net nuclear grain count at concentrations of up to 15 µg/mL, and cytotoxicity was observed at 194 µM. Positive controls produced desired responses.
  - *In vitro*: HHCB (Galaxolide containing 65% HHCB in DEP) was negative in a non-GLP micronucleus assay with a protocol similar to OECD TG 487 in human hepatoma cells (HepG2) at concentrations of 0.1 – 387 µM with and without metabolic activation. There was no increase in net nuclear grain count at concentrations of up to 194 µg/mL, and cytotoxicity was observed at 387 µM. Positive controls produced desired responses.
  - *In vitro*: HHCB (Galaxolide containing 65% HHCB in DEP) was negative in a non-GLP sister chromatid exchange assay in human lymphocytes obtained from healthy non-smoking donors at concentrations of 0.025 to 97 µM with and without metabolic activation. Cytotoxicity was observed at concentrations of 48.5 µM and higher. Positive controls produced the desired responses.

  - *In vitro:* HHCB (Galaxolide containing 65% HHCB in DEP) was negative in a non-GLP, non-guideline SOS chromotest for DNA damage and/or repair in *E. coli* PQ37 at concentrations of up to 50 µg, which is the limit of the solubility in this assay, with and without metabolic activation. No cytotoxicity was observed. HHCB did not induce SOS in this study. Positive controls produced the desired responses.
  - *In vivo*: HHCB (purity unspecified) was negative in a GLP-compliant micronucleus assay conducted according to OECD TG 474 in ICR mice. Animals (5/sex/dose) received a single intraperitoneal injection of 376, 750 or 1,500 mg/kg HHCB and bone marrow was harvested at 24, 48 or 72 hours post exposure. The high dose was 70% of the estimated i.p. LD_{50}. All animals at the high dose, 4/15[9] males and 4/15 females at the mid dose and 1/15 females at the low dose had lethargy. No mortality was observed. HHCB did not induce micronucleated polychromatic erythrocytes (PCE). The positive control produced the desired responses.
- EU 2008 (only the study that was not included in ECHA was summarized below)
  - *In vitro*: HHCB (Galaxolide containing 65% HHCB in DEP) was negative in a non-GLP Ames assay with a protocol similar to OECD TG 471 in *S. typhimurium* tester strains TA97, TA98, TA100 and TA102 at concentrations of 5 – 200 µg/plate (limit of solubility) with and without metabolic activation. Cytotoxicity was not reported. Positive controls produced the desired responses.

Reproductive Toxicity (R) Score (H, M, or L): DG

HHCB was assigned a score of Data Gap for reproductive toxicity based on the lack of sufficient data.

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists
- U.S. EPA 2014, ECHA 2015
  - A GLP-compliant reproductive/developmental neurotoxicity study conducted according to the ICH Guideline on the Detection of Toxicity to Reproduction for Medicinal Products with slight modifications was performed to examine the adverse effects to neonates following HHCB exposure through nursing. Pregnant Sprague-Dawley rats (28/group) received HHCB (>95% pure) by gavage at a dose of 0, 2, 6, or 20 mg/kg/day from day 14 of pregnancy through day 21 post-partum. The doses were selected to produce levels of HHCB in milk of lactating rats comparable to and orders of magnitude higher than those reported in human milk, based on pharmacokinetic analysis. F1 offspring received normal diets after weaning and were allowed to produce the F2 generation after 84 days of age. Parameters examined include sex, weight and external abnormalities of F1 offspring after parturition, developmental milestones (i.e. surface righting reflex, startle reflex, air righting reflex and pupil reflex) during pre-weaning period of F1 offspring, neurological parameters (i.e. motor coordination and balance, activity and avoidance) in sexually mature F1 offspring, reproductive parameters (i.e. time of pregnancy, estrous cyclicity, pre-coital time, pregnancy rates and duration of gestation) of F1 offspring, and abnormalities of the F2 generation from parturition to postnatal day 21. The NOAEL for maternal, reproductive and developmental toxicity was established at 20 mg/kg/day, the highest dose tested, based on lack of effects observed.

[9] It was not clear why the total number of animals at each dose was 15/sex, while in the beginning of the record it was stated to be 5/sex/dose.

    - In a GLP-compliant subchronic toxicity study conducted according to OECD TG 408 in Crl:CD (SD) Br rats, animals (15/sex/dose) received HHCB (> 95% pure) in the diet at 0, 5, 15, 50 or 150 mg/kg/day for 13 weeks. Weights of reproductive organs (ovaries and testes) were recorded, and histopathology was performed on ovaries, testes, epididymides, mammary gland, prostate, seminal vesicles, uterus and vagina. Uterine lumen distension was found in all dose groups without dose response. An association between the distension and the proestrus stage of the estrus cycle was found upon histopathological examination of ovaries and vaginas. The authors did not consider these effects toxicologically significant, and identified the NOAEL at 150 mg/kg/day for this study.
    - Toxicokinetics studies on HHCB indicate that it is extensively metabolized and excreted without significant bioaccumulation.
- Based on the weight of evidence, a score of Data Gap was assigned. No standard 2-generation reproductive toxicity studies were identified for HHCB. The reproductive/developmental toxicity study in rats only involved exposure through lactation, and it is not clear how much of the HHCB was still in effect when the F1 generation reached sexual maturity, given the complete metabolism and little bioaccumulation reported in toxicokinetics studies. Although no effects on reproductive organs were found in the repeated dose dietary study in rats, reproductive function was not examined. Therefore, neither of the two identified studies is sufficient to rule out the potential adverse effects on reproductive functions when animals are exposed right before and during mating. Considering the concern for endocrine activity (described below), ToxServices assigned a Data Gap in the absence of studies evaluating reproductive performance following exposure during all stages of reproduction.

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): *L*

HHCB was assigned a score of Low for developmental toxicity based on high quality experimental data and expert judgment. The confidence in this score is reduced as some evidence of developmental toxicity was observed at maternally toxic doses, which the EU judged to be unlikely to be indicative of specific developmental toxicity of HHCB. GreenScreen® criteria classify chemicals as a Low hazard for developmental toxicity when adequate data are available and negative, there are no structural alerts, and they are not classified under GHS (CPA 2012a).

- Authoritative and Screening Lists
    - Not present on any authoritative or screening lists
- EU 2008, ECHA 2015
    - In a dose-range finding study for a developmental toxicity study, pregnant Sprague-Dawley rats (8/group in treated groups, 19 in control group) were exposed to HHCB (>95% pure) by gavage at 0, 100, 250, 500 or 1,000 mg/kg/day on gestational days 7 through 17. Mortality was reported at the highest dose (4/8). There were localized alopecia, decreased motor activity and urine stained fur at the two highest doses. Red perioral substances, ungroomed coat and soft or liquid feces were found at the highest dose. Reduced body weight gain (persisting only at the two highest doses during the post treatment period) and reduced absolute and relative food consumption (persisting in all groups during the post treatment period) were reported at all dose levels. The mean fetal body weights were 89.3% of controls at the highest dose. No other treatment-related effects were observed. It was not clear what developmental toxicity parameters were examined. A maternal toxicity LOAEL of 500 mg/kg/day was identified in the REACH dossier.

- A GLP-compliant developmental toxicity study was performed according to OECD TG 414 in Sprague-Dawley rats, following the range-finding study described above. Pregnant animals (25/dose) were exposed to HHCB (> 95% pure) by oral gavage at 0, 50, 150 or 500 mg/kg/day on gestational days 7 through 17. Dams were monitored for signs of toxicity, body weights and food intake. Upon necropsy on gestational day 20, parameters examined in the uterus, ovaries and placenta of the dams include the number of corpora lutea, number of pregnancies, number and distribution of implantations, live and dead fetuses, and early and late resorptions. Fetal parameters examined include weight, sex, gross external abnormalities, soft tissue alterations, and skeletal alterations. Dams at the high dose had excess salivation, urine-stained abdominal fur, red or brown substance on the forepaws and alopecia. Dams at the mid and high doses had a dose-dependent reduction in body weight gain on gestational days 7-18 (78% and 91% compared to controls, respectively, $p<0.05$). The body weight reductions on gestational day 20 were 2.5, 3.3 and 4.6% for the low, mid and high doses, respectively, compared to controls. Significantly decreased fetus weights were observed at the high dose (7% reduction). There was a significant increase in the fetal incidence of skeletal (vertebral/rib) malformations and the incidence of incomplete ossification and/or no ossification of sternal centra, and a significant decrease in the number of ossification sites in the metatarsals at the high dose. These parameters were also increased for litters. According to the EU, a maternal toxicity NOAEL and LOAEL of 50 and 150 mg/kg/day were identified based on reduced weight gain. The developmental toxicity NOAEL and LOAEL of 150 and 500 mg/kg/day were identified based on reduced fetal body weight, increased incidence of fetal skeletal malformations and decreased ossification of sternal centra and metatarsals. The EU also noted that HHCB is not likely to be a selective developmental toxicant because adverse developmental effects were observed only at maternally toxic doses.
- In the previously-described GLP-compliant reproductive/developmental neurotoxicity study conducted according to the ICH Guideline on the Detection of Toxicity to Reproduction for Medicinal Products with slight modifications that was performed to examine the adverse effects to neonates following HHCB exposure through nursing, pregnant Sprague-Dawley rats (28/group) received HHCB (>95% pure) by gavage at a dose of 0, 2, 6, or 20 mg/kg/day from day 14 of pregnancy through day 21 post-partum. The doses were selected to produce levels of HHCB in milk of lactating rats comparable to and orders of magnitude higher than those reported in human milk, based on pharmacokinetic analysis. F1 offspring received normal diets after weaning and were allowed to produce the F2 generation after 84 days of age. Parameters examined include sex, weight and external abnormalities of F1 offspring after parturition, developmental milestones (i.e. surface righting reflex, startle reflex, air righting reflex and pupil reflex) during pre-weaning period of F1 offspring, neurological parameters (i.e. motor coordination and balance, activity and avoidance) in sexually mature F1 offspring, reproductive parameters (i.e. time of pregnancy, estrous cyclicity, pre-coital time, pregnancy rates and duration of gestation) of F1 offspring, and abnormalities of the F2 generation from parturition to postnatal day 21. The NOAEL for maternal, reproductive and developmental toxicity was established at 20 mg/kg/day, the highest dose tested, based on lack of effects observed.

- Based on the weight of evidence, a score of Low was assigned. The GLP-compliant OECD 414 study reported developmental toxicities (reduced fetal body weight, increased incidence of fetal skeletal malformations and decreased ossification of sternal centra and metatarsals) at a

maternally toxic dose only and the EU concluded that HHCB is unlikely to be a specific developmental toxicant based on the overall weight of evidence.

Endocrine Activity (E) Score (H, M, or L): *M*

HHCB was assigned a score of Moderate for endocrine disruption based on *in vitro* evidence of endocrine activity and the presence on screening lists. GreenScreen® criteria classify chemicals as a Moderate hazard for endocrine activity when there is evidence of endocrine activity (CPA 2012a).

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* ChemSec – Substitute It Now (SIN) List – Endocrine Disruption
  - *Screening:* TEDX – Potential Endocrine Disruptors
  - *Screening:* EC Priority Disrupters – Category 3B (Substances with no or insufficient data gathered)
- Not listed as a potential endocrine disruptor on the OSPAR List of Chemicals of Possible Concern.
- EU 2008
  - *In vitro*: Weak transcriptional activities (approximately 6 orders of magnitude less than estradiol) towards human estrogen receptor (ER)-α but not ERβ was found for HHCB (purity not specified) when added in ethanol human embryonal kidney 293 cells transiently transfected with these receptors for 24 hours.
  - *In vitro*: HHCB (>98% pure) exhibited a slight, non-statistically significant effect in a non-GLP E-screen assay. ER-positive human mammary carcinoma cells (MCF-7) were exposed to HHCB dissolved in ethanol at concentrations of up to 10 μM for 6 days. The cell proliferation rates were measured as a parameter of ER activity and compared with those of hormone-free controls and of cells treated with 17β-estradiol.
  - *In vitro*: Marginal ERα estrogenic activity was reported at only the highest HHCB (purity not specified) concentration (10 μM) in human embryonal kidney 293 cells (HEK 293) and human osteoblastic cells (U2-OS) that were stably transfected with human ERα and ERβ, and transiently transfected with an estrogen responsive reporter gene construct. On the other hand, HHCB dose-dependently repressed 17β-estradiol-induced transcription in both cell lines for both ER isoforms at concentrations of 0.01, 0.1 and 10 μM.
  - *In vitro*: HHCB (purity not specified) exhibited a marginal estrogenic activity towards human ERα only at the highest tested concentration of 10 μM, and did not have any activity toward human ERβ and zebra fish ERα, β and γ. HHCB induced a dose-dependent suppression of estradiol induction towards human ERβ and zebra fish ERγ. HHCB had a weak anti-estrogenic activity towards human ERα and zebra fish ERβ only at the highest tested concentration of 10 μM.
  - *In vitro*: HHCB (purity not specified) exhibited a dose-dependent repression towards androgen receptor (AR) at concentrations of 1 and 10 μM (up to 30%), and a dose-dependent repression towards progesterone receptor (PR) at concentrations of 0.01, 0.1, 1, and 10 μM in U2-OS cells stably transfected with either AR or PR, and a sensitive reporter gene construct. HHCB did not show any agonistic effects in this study.
  - *In vitro*: HHCB (purity not specified) exhibited dose-dependent antagonistic activity towards human ERβ with an IC_{50} of 2.4 μM, and dose-dependent antagonistic activity towards human AR with an IC_{50} of 2.9 μM in HEK293 cells stably transfected with either human ERα or β and a sensitive estrogen responsive reporter gene construct, and in U2-OS cells stably transfected with human AR and an androgen responsive reporter gene

construct. HHCB did not show any agonistic or antagonistic effects on the aryl hydrocarbon receptor.

- *In vitro*: HHCB exhibited weak activity towards human ERα but not human ERβ in human cervical epithelioid carcinoma HeLa cell line stably transfected with human ERα and ERβ at concentrations of 10^{-7} to 10^{-5} M.
- *In vivo*: A non-GLP study was conducted in weanling (21-day old) female Balb/c mice. Animals (6/group) were exposed to HHCB (purity unspecified) at doses of 0, 50 or 300 mg/kg diet for 2 weeks. These are equivalent to doses of 0, 6 and 40 mg/kg/day, according to EU. A group of animals received 17β-estradiol on days 1, 5, 9 and 12 as a positive control. At necropsy, weights of uterus, thymus, liver and body weight were documented. Estrogenic activity was evaluated according to the protocol of Thigpen et al. (1987). Animals in the positive control group had significantly higher uterine weighs and lower thymus weights, while HHCB had no effects on body weight, or uterus or thymus weights. Significant increase in relative liver weight was reported at both doses (8% and 22% at low and high doses, respectively). No histopathology was performed.
- *In vivo*: A non-GLP study was conducted in zebra fish containing a reporter gene construct described above in the *in vitro* studies. HHCB (purity unspecified) did not have any estrogenic activity at concentrations of 0.01, 0.1 and 1 μM, and the concentration of 10 μM was toxic. HHCB exhibited a dose-dependent antagonistic effect against E2 at concentrations of 0.1 and 1 μM in juvenile fish.

- ChemSec 2015
  - HHCB was listed on the SIN list because of reported endocrine effects (unspecified), potential persistence and bioaccumulation, and frequency of presence in humans and the environment.
- TEDX 2011
  - HHCB was included as a potential endocrine disruptor based on an *in vitro* study as described below:
    - In an *in vitro* gene reporter assay, Chinese hamster ovary cells were exposed to six polycyclic musk compounds. HHCB was shown to have agonistic effects toward human ERα, and no effects on human AR or human thyroid hormone receptor (TR)β, or antagonistic effects toward human ERα or human TRβ. HHCB exhibited a dose-dependent antagonistic activity for human AR with the IC_{50} of 1.0 x 10^{-7} M (Mori et al. 2007).
- Based on the weight of evidence, a score of Moderate was assigned. HHCB showed weak estrogenic and anti-estrogenic effects *in vitro*, marginal anti-androgen and anti-progesterone effects *in vitro*, and no estrogenic effects *in vivo*. Although the *in vivo* study was negative, it only examined the estrogenic effects, while the positive anti-androgen, anti-progesterone and anti-estrogen effects *in vitro* were not evaluated *in vivo*. HHCB was listed by ChemSec's SIN List and TEDX as a potential endocrine disruptor, both of which correspond to a score of Moderate to High. HHCB was also listed as an EU category 3B potential endocrine disruptor (corresponding to a score of Low to High), but no supportive evidence was provided. The GreenScreen® criteria assign a High score when there is evidence of endocrine activity and a related human health effect, and a Moderate score when there is evidence of endocrine activity. Available data did show evidence of weak endocrine activity *in vitro*, but no evidence *in vivo*, and no indication of endocrine-driven human health effects was seen in genotoxicity, repeated dose toxicity and reproductive and developmental toxicity studies. The weight of evidence supports a score of Moderate. As the positive evidence was *in vitro*, a reduced confidence level was assigned.

Group II and II* Human Health Effects (Group II and II* Human)

Note: Group II and Group II endpoints are distinguished in the v 1.2 Benchmark system. For Systemic Toxicity and Neurotoxicity, Group II and II* are considered sub-endpoints and test data for single or repeated exposures may be used. If data exist for single OR repeated exposures, then the endpoint is not considered a data gap. If data are available for both single and repeated exposures, then the more conservative value is used.*

Acute Mammalian Toxicity (AT) Group II Score (vH, H, M, or L): L

HHCB was assigned a score of Low for acute toxicity based on measured data. GreenScreen® criteria classify chemicals as a Low hazard for acute toxicity when oral and dermal LD_{50} values are greater than 2,000 mg/kg (CPA 2012a).

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists
- EU 2008, ECHA 2015
  - *Oral*: LD_{50} > 3,250 mg HHCB/kg in rats (non-GLP), performed on Galaxolide 50 containing 65% HHCB in DEP
  - *Oral*: LD_{50} > 3,000 mg HHCB /kg in rats (non-GLP), performed on Galaxolide 50 containing 65% HHCB in DEP
  - *Dermal*: LD_{50} > 6,500 mg HHCB/kg in rats (non-GLP), performed on Galaxolide 50 containing 65% HHCB in DEP
  - *Dermal*: LD_{50} > 3,250 mg HHCB/kg in rabbits (non-GLP), performed on Galaxolide 50 containing 65% HHCB in DEP

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST)
Group II Score (single dose) (vH, H, M, or L): *L*

HHCB was assigned a score of Low for systemic toxicity (single dose) based on lack of systemic toxicity in acute oral and dermal toxicity studies. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (single dose) when no systemic effects are observed at doses of greater than 2,000 mg/kg (oral and dermal), and the chemical is not classified under GHS (CPA 2012a).

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists
- EU 2008, ECHA 2015
  - *Oral*: In a previously-described acute toxicity study in rats, Galaxolide 50 (containing 65% HHCB in DEP) was administered to female Sprague-Dawley rats by gavage at the equivalent HHCB doses of 140, 300, 650, 1,400 or 3,000 mg/kg and the animals were observed for mortality and signs of effects for 7 days before necropsy. One death was found at the mid dose due to gavage error. One animal at 1,400 mg/kg appeared distressed shortly after dosing, but returned to normal after 2 hours. No other effects were observed.
  - *Dermal*: In a previously-described acute toxicity in rats, Galaxolide 50 (containing 65% HHCB in DEP) was administered to the shaved skin of female Sprague-Dawley rats at the equivalent HHCB doses of 300, 650, 1,400, 3,000 or 6,500 mg/kg. Animals were then observed for 7 days. No mortalities were observed and high dose animals had urine stained fur. No gross pathologic abnormalities were found at any dose levels.
  - *Dermal*: In a previously-described acute toxicity in rabbits, Galaxolide 50 (containing 65% HHCB in DEP) was administered to the shaved skin of seven female albino rabbits at the equivalent HHCB dose of 3,250 mg/kg. Signs of irritation were observed,

including moderate skin redness in all animals, moderate skin edema (6/7), and slight edema (1/7).

- Based on the weight of evidence, a score of Low was assigned. No significant systemic toxicities were reported in oral and dermal toxicity studies described above that evaluated clinical signs, mortality and gross necropsy at doses exceeding 3,000 mg/kg. However, due to the lack of inhalation toxicity studies, it is impossible to determine if HHCB is capable of causing respiratory irritation, which warrants classification to GHS category 3. Therefore, the confidence level is reduced.

Group II Score (repeated dose)* (H, M, or L): L

HHCB was assigned a score of Low for systemic toxicity (repeated dose) based on measured data from repeated-dose studies showing a lack of effects below the guidance values. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (repeated dose) when no adverse systemic toxicity is observed at doses of greater than 100 mg/kg/day in studies of 90 days and longer (CPA 2012a).

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists
- EU 2008, ECHA 2015
  - *Inhalation*: In a non-GLP subchronic toxicity study in female rats, Sprague-Dawley rats (20 – 24/group) were exposed (whole-body) to an aerosolized fragrance mixture B at a nominal concentration of 5 mg/m^3 (HHCB concentration 5.7 µg/m^3) for 6 weeks or an aerosolized fragrance mixture G at a nominal concentration of 50 mg/m^3 (HHCB concentration 132 µg/m^3) for 13 weeks. Treatment frequency was 4 hours per day and five days per week. Parameters examined include cage side observation, body weight, hematology, clinical chemistry, urinalysis, gross pathology (adrenal glands, brain, esophagus, eye, heart, muscle with sciatic nerve, kidneys, colon, trachea, lungs, hilar lymph node, liver, pancreas, spleen, stomach, skin, duodenum, spinal cord, sternum, thyroid, urinary bladder, uterus, and ovaries), and histopathology (trachea, lungs, adrenals, brain, heart, kidneys, liver, pancreas, spleen, sternum, uterus and bone marrow from femur). No adverse effects were observed. The EU considered this study of limited value for risk assessment as only mixtures that contained very low levels of HHCB were tested.
  - *Oral*: A dose-range finding study was conducted in Crl:CD (SD)Br rats (5/sex/dose). HHCB (> 95% pure) was administered for 14 days in the diet to achieve the dose levels of 0, 341, 598 and 679 mg/kg/day for males and 0, 352, 633 and 980 mg/kg/day for females. A progressive dose-dependent reduction in body weight was found in both sexes at the two highest doses. A significant and dose-dependent increase in absolute and relative liver weight was found in both sexes at all dose levels, with corresponding moderate centrilobular hypertrophy in the liver in some animals at the high dose upon histopathological examination. A LOAEL of ~350 mg/kg/day based on increased liver weight was established in the REACH dossier.
  - *Oral*: In a GLP-compliant subchronic toxicity study following the range-finding study described above, Crl: CD (SD)Br rats (15/sex/dose) were exposed to HHCB (> 95% pure) in the diet at the target doses of 0, 5, 15, 50 or 150 mg/kg/day for 13 weeks. The actual ingested doses were 0, 5.4, 15.7, 51.8 and 155.8 mg/kg/day in males and 0, 5.1, 15.6, 51.9 and 154.6 mg/kg/day in females. Three animals/sex in the control and highest dose groups were allowed a 4-week recovery period. Parameters examined include clinical signs, body weight, food consumption, ophthalmology, urinalysis, hematology,

clinical chemistry, gross pathology, organ weights and histopathology (on all tissues from control and high dose animals, all gross lesions, and lungs, liver, kidneys and reproductive organs from all animals). No significant adverse effects were observed in this study and the EU identified a NOAEL of 150 mg/kg/day.

- *Dermal:* In two subchronic toxicity studies in Crl:COBS CD (SD) BA rats, animals (female only, 20/dose) were exposed to Galaxolide (65% HHCB in DEP) at 0, 1, 10 or 100 mg/kg/day dissolved in ethanol for 13 weeks or at 0, 9, 18 or 36 mg/kg/day for 26 weeks. The test substance was applied to the anterior dorsal shaved skin under un-occluded conditions. Animals were examined for mortality, body weight, clinical signs, behavioral and motor function, hematology, serum chemistry, organ weights, gross pathology and histopathology. Neuropathological examination was also performed on the brain, spinal cord and peripheral nerves on two animals per dose. In the 13-week study, increased absolute and relative liver weights were observed at the highest dose, but the extent of these changes was not reported. In the 26-week study, decreased body weight gain (extent not reported) at the highest dose. No other effects were noted. EU considered these studies of limited value for risk assessment based on the lack of quantitative information reported, the lack of measures used to prevent ingestion of the tested material, and the lack of information on the area of application. These studies were given a Klimisch score of 4 (reliability cannot be assigned) in the REACH dossier.
- *Dermal*: A third non-GLP dermal toxicity study was conducted in Charles River CD rats (15 males, 35 – 38 females/group) that were exposed daily to a 10% solution of Galaxolide 50 (65% HHCB in DEP) in 95% ethanol at doses of 0, 50, 100 or 200 mg/kg/day for 26 weeks. One group was only dose for 13 weeks and then kept untreated for another 13 weeks. Parameters examined include hematology, clinical chemistry, urinalysis, organ weights and histopathology (26 tissues). Crusty white or brown material and scabbed areas on the dorsal surface of a few animals were observed at the two highest doses. A trend of decreased body weight gain and decreased food consumption was observed at the highest dose in males, but this effect was not statistically significant. Increased relative liver weight was observed in females at the two highest doses (11% and 23%, respectively), and increased kidney weight was observed in males at the highest dose (37%). No histopathological abnormalities were found. The EU considered this study of limited value for risk assessment due to the lack of measures taken to prevent ingestion of the material, the lack of information on the area of application, and the absence of adverse effects. A Klimisch score of 3 (unreliable) was assigned to this study in the REACH dossier due to the inability to determine the actual exposure dose.

- Based on the weight of evidence, a score of Low was assigned. The available inhalation toxicity studies did not report any adverse effects, but HHCB was tested at very low levels in the mixture, and the concentrations are well below the GHS cutoff values. Therefore, there is insufficient information to assign a score based on inhalation studies. The available dermal studies were of limited values as well, as the test materials were not covered, and the animals could have ingested them during grooming, making it impossible to determine the NOAEL. Therefore, there is insufficient information to assign a score based on dermal studies. The key GLP-compliant subchronic oral toxicity study reported a NOAEL of 150 mg/kg/day, which is sufficient to warrant a Low score.

Neurotoxicity (N)

Group II Score (single dose) (vH, H, M, or L): L

HHCB was assigned a score of Low for neurotoxicity (single dose) based on the absence of effects in acute oral and dermal toxicity studies. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (single dose) when no adverse neurological effects are observed at oral and dermal doses of higher than 2,000 mg/kg/day, and the chemical is not classified under GHS (CPA 2012a).

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists.
- Not classified as a developmental neurotoxicant (Grandjean and Landrigan 2006, 2014).
- EU 2008, ECHA 2015
  - *Oral*: In a previously-described acute toxicity study in rats, Galaxolide 50 (containing 65% HHCB in DEP) was administered to female Sprague-Dawley rats by gavage at the equivalent HHCB doses of 140, 300, 650, 1,400 or 3,000 mg/kg and the animals were observed for mortality and signs of effects for 7 days before necropsy. One death was found at the mid dose due to gavage error. One animal at 1,400 mg/kg appeared distressed shortly after dosing, but returned to normal after 2 hours. No other effects were observed.
  - *Dermal*: In a previously-described acute toxicity in rats, Galaxolide 50 (containing 65% HHCB in DEP) was administered to the shaved skin of female Sprague-Dawley rats at the equivalent HHCB doses of 300, 650, 1,400, 3,000 or 6,500 mg/kg. Animals were then observed for 7 days. No mortalities were observed and high dose animals had urine stained fur. No gross pathologic abnormalities were found at any dose levels.
  - *Dermal*: In a previously-described acute toxicity in rabbits, Galaxolide 50 (containing 65% HHCB in DEP) was administered to the shaved skin of seven female albino rabbits at the equivalent HHCB dose of 3,250 mg/kg. Signs of irritation were observed, including moderate skin redness in all animals, moderate skin edema (6/7), and slight edema (1/7).
- Based on the weight of evidence, a score of Low was assigned. No evidence of neurotoxicity was observed in the available acute oral and dermal toxicity studies at doses higher than 3,000 mg/kg.

Group II Score (repeated dose)* (H, M, or L): DG

HHCB was assigned a score of Data Gap for neurotoxicity (repeated dose) based on lack of sufficient data.

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists.
- Not classified as a developmental neurotoxicant (Grandjean and Landrigan 2006, 2014).
- EU 2008, ECHA 2015
  - *Inhalation*: In a previously-described non-GLP subchronic toxicity study in female rats, Sprague-Dawley rats (20 – 24/group) were exposed (whole-body) to an aerosolized fragrance mixture B at a nominal concentration of 5 mg/m^3 (HHCB concentration 5.7 µg/m^3) for 6 weeks or an aerosolized fragrance mixture G at a nominal concentration of 50 mg/m^3 (HHCB concentration 132 µg/m^3) for 13 weeks. Treatment frequency was 4 hours per day and five days per week. Gross pathology was performed on brain and muscle with sciatic nerve, spinal cord). Histopathology was performed on brain and bone marrow from femur. No adverse effects were observed. The EU considered this study of limited value for risk assessment as only mixtures were tested that contained very low levels of HHCB.

- *Dermal:* In two previously-described subchronic toxicity studies in CrlLCOBS CD (SD) BA rats, animals (female only, 20/dose) were exposed to Galaloxide (65% HHCB in DEP) at 0, 1, 10 or 100 mg/kg/day dissolved in ethanol for 13 weeks or at 0, 9, 18 or 36 mg/kg/day for 26 weeks. The test substance was applied to the anterior dorsal shaved skin under un-occluded conditions. Animals were examined for clinical signs and behavioral and motor function. Neuropathological examination was also performed on the brain, spinal cord and peripheral nerves on two animals per dose. No effects were noted for neurotoxicity, while the positive control produced clear evidence of neurotoxicity. EU considered these studies of limited value for risk assessment based on the lack of quantitative information reported, the lack of measures used to prevent ingestion of the tested material and the lack of information on the area of application. These studies were given a Klimisch score of 4 (reliability cannot be assigned) in the REACH dossier.
- *Dermal*: In a previously-described non-GLP dermal toxicity study that was conducted in Charles River CD rats (15 males, 35 – 38 females/group), animals were exposed daily to a 10% solution of Galaxolide 50 (65% HHCB in DEP) in 95% ethanol at doses of 0, 50, 100 or 200 mg/kg/day for 26 weeks. One group was only dose for 13 weeks and then kept untreated for another 13 weeks. The purpose of this study was to screen for neurotoxic potential. Histopathology was performed on the nervous system tissues (unspecified). No neurotoxicity was found, while a positive control produced clear evidence of neurotoxicity. The EU considered this study of limited value for risk assessment due to the lack of measures taken to prevent ingestion of the material, the lack of information on the area of application, and the absence of adverse effects. A Klimisch score of 3 (unreliable) was assigned to this study in the REACH dossier due to the inability to determine the actual exposure dose.

- Based on the weight of evidence, a score of Data Gap was assigned. Although no neurotoxicity was found in the studies described above, the doses either were much lower than the GHS cutoff, or could not be accurately determined due to study design deficiencies.

Skin Sensitization (SnS) Group II* Score (H, M, or L): L

HHCB was assigned a score of Low for skin sensitization based on negative results in humans and experimental animals regarding contact sensitization and photosensitization. GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization when adequate data are available and negative, there are no structural alerts, and they are not classified under GHS (CPA 2012a).

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists
- EU 2008, ECHA 2015
  - A non-GLP guinea pig maximization test was conducted on Galaxolide (65% HHCB in DEP). Ten albino Dunkin/Hartley guinea pigs were induced with 0.5% intradermally and 100% topically, and challenged with 25% Galaxolide (equivalent to 0.325%, 65% and 16.25% HHCB, respectively). The doses were selected based on a preliminary irritation test. Equivocal response was observed in one animal, and no evidence of sensitization was observed in other animals. The authors concluded that Galaxolide is not dermally sensitizing.
  - A GLP-compliant photosensitization study that predates, but was largely the same as OECD TG 406 was conducted in 12 albino Hartley guinea pigs on Galaxolide (65% HHCB in DEP). Animals received four injections of 0.1 mL Freund's Compete Adjuvant at the four corners of a 9 cm^2 shaved interscapular region. Galaxolide was dermally

applied to the site at 1% in ethanol (HHCB 0.65%). The doses were selected based on a preliminary photoirritation study that determined the maximum non-photoirritating doses. The sites were exposed to UV light after 25 min for about 1 and half hours. The dermally exposure and UV irradiation were repeated 24 hours later. Ten to 14 days afterwards, animals were topically challenged with 1, 0.3 or 0.1% Galaxolide in ethanol (HHCB concentrations of 0.65, 0.2 and 0.065%) to the shaved lumbar region. The animals were irradiated 30 minutes later and the test material was then applied to fresh sites to check for sensitization reactions at 24 and 48 hours. A second challenge was performed 6 or 7 days later. Galaxolide tested negative in this study.

- In a human repeated insult patch test (HRIPT), 3.75% HHCB was tested along with a cream control. There were a total of nine 24-hour inductions with semi-occlusive patches to the upper arms of the subjects 3 times per week for 3 weeks. A 24-hour challenge patch was applied after 2 weeks and reactions were scored at 48 or 72 hours afterwards. HHCB tested negative in this study.
- In a HRIPT test of 43 subjects, 50% Galaxolide 50 was tested on the upper arms for 24 hours, 3 times per week for 3 weeks. After 2 weeks, duplicate challenge patches were applied with one to the original site and the other to a fresh site. The sites were scored 48 or 72 hours after patch removal. No signs of irritation or sensitization were observed. In another study conducted by the same authors, HHCB was tested at 100% without vehicle on 42 subjects. No signs of irritation or sensitization were observed.
- A human maximization test was conducted on Galaxolide 50 (65% HHCB in DEP) probably in petrolatum in 10 volunteers. Galaxolide 50 was tested under occlusive conditions on the forearm on 5 alternate days for 48 hours. The patch sites were pretreated with 5% sodium lauryl sulfate (SLS) under occlusion for 24 hours to enhance the penetration. After 10 – 14 days, challenge patches were applied under occlusion on the back with and without 30-min pretreatment of SLS. The sites were evaluated 48 or 72 hours afterwards. No signs of irritation or sensitization were observed.
- A similar human maximization test was conducted on Galaxolide 50 with 24 Japanese American subjects. No positive reactions were observed.
- To test the potential of Galaxolide to induce allergic reactions in sensitive patients, Galaxolide (25% in petrolatum) was patch tested in 179 patients suspected of cosmetic allergy. Positive responses were found in 3/179 subjects (1.7%), and the authors noted that these may be the results of occasional false-positive reactions due to the Excited Skin Syndrome.
- In a patch test in 28 patients sensitized to perfumes and sweet smelling constituents, Galaxolide (purity not reported) tested negative at 3%.
- In a patch test in 100 patients, Galaxolide 50 (65% HHCB in DEP) was tested as one of the 48 fragrance materials at 1 and 5% in petrolatum. The material was applied to the back for 2 days and evaluated on days 2 and 3 or days 2 and 4. Galaxolide 50 was negative in this study.
- A modified HRIPT study was conducted to examine the photosensitivity of HHCB. HHCB was tested on the back of 27 panelists at 25% in ethanol/DEP under occlusion twice per week for 3 weeks. Patches were removed after 24 hours and the sites were irradiated with UVB. After 2 weeks of rest period, a single application of duplicate patches was tested on naïve sites for 24 hours and the sites were then irradiated and evaluated at 1, 24 and 48 hours afterwards. Slight to strong (1 or 2 individuals) signs of irritation were observed during the induction period. However, the same reaction rates were observed in HHCB-treated and vehicle-treated subjects, and the rate was even higher at blank sites. A decrease was observed in the severity of the irritation over time

during the induction phase. Two subjects had skin responses during the challenge phase to the sample, vehicle control and the blank sites. Therefore, these reactions were considered to be the result of excess UV exposure, indicative of a sunburn effect. It was concluded that HHCB is not a photosensitizer.

Respiratory Sensitization (SnR) Group II* Score (H, M, or L): DG

HHCB was assigned a score of Data Gap for respiratory sensitization based on lack of data.

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists
- No data were identified.

Skin Irritation/Corrosivity (IrS) Group II Score (vH, H, M, or L): *M*

HHCB was assigned a score of Moderate for skin irritation/corrosivity based on being classified to GHS category 3 based on weight of evidence from animal data, expert judgment by EU, and human data. GreenScreen® criteria classify chemicals as a Moderate hazard for skin irritation/corrosivity when they are classified to GHS category 3 (CPA 2012a).

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists
- EU 2008, ECHA 2015
  - In a GLP-compliant dermal irritation study conducted according to EU Directive 79/831EEC in female New Zealand White rabbits, 0.5 mL Galaxolide (65% HHCB in DEP) was tested on an area of about 6 cm^2 for 4 hours under semi-occlusion (held in place with Elastoplast plastic adhesive bandage 10 cm wide) on the shaved and clipped dorsal skin. Another two groups of three rabbits received undiluted DEP or benzyl benzoate (BB) as controls. Application sites were scored at 1, 24, 48, 72 and 168 hours afterwards. The mean scores at 24, 48, and 72 hours for Galaxolide were 1.3 for erythema (all three animals) and 0.4 for edema (highest score 1). Erythema persisted at the 168-hour observation in two animals, and slight desquamation was found in all three rabbits. Scores for DEP and BB were 0 for both erythema and edema in all animals.
  - In a GLP-compliant dermal irritation study conducted according to EU Directive 79/831EEC in female New Zealand White rabbits, 0.5 mL Galaxolide (65% HHCB in DEP), DEP and BB were applied to a surgical lint of 2.5 cm^2 in area, which was then put on the skin of each of 4 rabbits and held by a 10 cm wide Elastoplast adhesive bandage for 4 hours. Application sites were scored at 1, 24, 48, 72 and 168 hours afterwards. The mean scores at 24, 48, and 72 hours for Galaxolide were 2.1 for erythema (all three animals) and 1.5 for edema (highest score 1). Both erythema and edema persisted in 4/4 and 3/4 animals, respectively, at the 168-hour observation. The mean scores for DEP were 0.2 (erythema) and 0 (edema) and for BB were 1.2 (erythema) and 0.4 (edema).
  - In a GLP-compliant dermal irritation study conducted according to EU Directive 79/831EEC in female New Zealand White rabbits, 0.5 mL undiluted or 50% solutions of Galaxolide (65% HHCB in DEP) or Galaxolide 50 BB (65% HHCB in BB) were applied to a surgical lint of 2.5 cm^2 in area, which was then put on the skin of each of 4 rabbits and held by a 10 cm wide Elastoplast adhesive bandage for 4 hours. The mean scores at 24, 48, and 72 hours for erythema/edema were 1.8/1.3 for undiluted Galaxolide (reversible after 168 hours), 1.3/0/3 for 50% Galaxolide (reversible in 3/4 animals after 168 hours), 1.8/0/8 for undiluted Galaxolide 50 BB (reversible in 3/4 animals after 168 hours), and 1.3/0.7 for 50% Galaxolide 50 BB (reversible after 168 hours).

  - In a non-GLP skin irritation study on Galaxolide 50 (65% HHCB in DEP) in three albino rabbits (strain not specified), 0.5 mL undiluted material was applied to an area of 2x2 (unit not specified) of shaved, clipped and/or abraded skin. The application site was covered with a Webril patch and sealed with Blenderm Surgical tape for 24 or 72 hours. Rabbits were immobilized for the first 24 hours. Sites were evaluated according to Draize criteria at 24, 48 and 72 hours. The mean scores at 24 hours and at 72 hours were both 1 for erythema. No edema was observed at any time point. The authors of this study concluded that Galaxolide 50 was a moderate skin irritant, while the authors of the REACH dossier concluded that Galaxolide 50 was not a skin irritant based on the EU criteria.
  - In a non-GLP skin irritation study using an identical protocol as the study described immediately above, Galaxolide 50 was tested at 25% and 50% and 100% (equivalent to 16%, 33% and 65% HHCB, respectively) in alcohol SDA 39C. The exposure time was 24 or 72 hours. No erythema or edema was observed with the lowest concentration. Erythema score of 1 was reported at 24 hours on abraded skin for the 50% dilution, but no other effects were observed at any other sites and any other time points for this material. Erythema score of 1 was reported at 24 hours for the undiluted Galaxolide 50, while no other effects were observed at any other sites and any other time points for this material. The st5udy authors concluded that Galaxolide 50 and the 50% dilution were very mild skin irritants. The authors of the REACH dossier concluded that Galaxolide 50 was not a skin irritant based on the EU criteria.
  - In an HRIPT study, 0.5 mL 100% HHCB was tested on the upper arms of 42 individuals for 24 hours, 3 times per week for 3 weeks as the induction phase. Sites were examined at 24 and 72 hours after patch removal. No irritation was found in any of the subjects.
  - In another HRIPT study in 40 subjects, 3.75% Galaxolide was tested under semi-occlusion on the upper arms 3 times per week for 3 weeks as the induction phase. Little or no primary irritation was found.
  - No photoirritation was observed in human studies and in an *in vitro* phototoxicity test performed according to EU TG B.41.
- Based on the weight of evidence, three GLP-compliant skin irritation studies performed on Galaxolide reported erythema/edema scores of up to 2.1. Some of the effects, including slight desquamation, erythema and edema were not reversible after 7 days. However, none of the studies lasted long enough as specified by the GHS criteria to examine the reversibility of these effects at day 14. In two non-GLP skin irritation studies, Galaxolide induced at most an erythema score of 1 only at 24 hours after a longer exposure duration of 24 – 72 hours compared to OECD test guidelines. No irritation was observed in humans with neat HHCB. According to the GHS criteria, inflammation (particularly alopecia, hyperkeratosis, hyperplasia and scaling) that persists to the end of 14 day observation period may warrant classification to category 2. The EU evaluated the above studies in a meeting and concluded that HHCB is not a skin irritant. This means that HHCB is not a GHS category 1 or 2 skin irritant. According to GHS criteria, a score of between 1.5 and 2.3 for erythema/edema warrants classification to category 3. Therefore, ToxServices classified HHCB to GHS category 3 based on erythema/edema scores of up to 2.1 in GLP-compliant animal studies on Galaxolide. This corresponds to a score of Moderate. The confidence level was reduced as Galaxolide contains only 65% of HHCB, and it is not clear if 100% HHCB is more irritating to animals. Additionally, neat HHCB did not cause any irritation effects in an HRIPT, which is not consistent with animal study results.

Eye Irritation/Corrosivity (IrE) Group II Score (vH, H, M, or L): L

HHCB was assigned a score of Low for eye irritation/corrosivity based on animal data on Galaxolide demonstrating that it is not classifiable under GHS. GreenScreen® criteria classify chemicals as a Low hazard for eye irritation/corrosivity when adequate data are available and negative, there are no structural alerts, and they are not classifiable under GHS (CPA 2012a). Confidence in this score was reduced as the highest concentration of HHCB tested was 65%.

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists
- EU 2008, ECHA 2015
  - A GLP-compliant eye irritation study was carried out according to the OECD TG 405 with the following deviations: no observation was made at 1 hour and the eyes were not washed at 24 hours. A volume of 0.1 mL undiluted Galaxolide (65% HHCB in DEP) was instilled into the right eye of 6 New Zealand rabbits, and the eyes were scored at 24, 48, 72, 96 and 168 hours according to the method of Draize. Small central opacity (score 2) was observed in one animal at 24 hours, which was reversible within 72 hours. The same animal had 24-hour iris/conjunctival redness/conjunctival discharge scores of 1/1/1, which resolved at later time points. Another animal had a conjunctival redness score of 1 at 24 and 48 hours, which resolved at later time points. Primary eye irritation scores were 3.5, 1.17 and 0 at 24, 48, and 72 hours, respectively. The authors concluded that Galaxolide was practically non-irritating to the eye.
  - No irritation was seen in a poorly reported eye irritation study conducted with a protocol similar to that described above with 0.1 mL Galaxolide 50 (65% HHCB in DEP) with an observation time of 168 hours.
  - In another poorly reported eye irritation study conducted in three rabbits on 50% Galaxolide 50 (65% HHCB in DEP) dissolved in ethanol, animals received 0.1 mL test material and observed for up to 7 days. Scores for conjunctival redness, chemosis and discharge were 1 to 2 at 24 hours in all three animals. Effects were reversible in two animals by 48 hours and in the third animal by 7 days. Primary eye irritation indices for three rabbits were 4.7, 2.7 and 2.7. The rabbit with the highest primary irritation index had a corneal opacity score of 2 and an area score of 2 (> 25% < 50%) after 96 hours, which was not reversible after 7 days (study termination). In control animals which were treated with ethanol only, the primary eye irritation index was 7.5, 5.3 and 2.3 at 24, 48, and 72 hours, respectively. EU considered this study of limited value because the solvent ethanol has eye irritation potential.
  - In another poorly reported eye irritation study in rabbits, an unspecified dilution of Galaxolide (65% HHCB in DEP) was tested and the animals were observed for up to 7 days. Scores for conjunctival redness, chemosis and discharge were 1 to 2 at 24 hours in all three animals. Discharge resolved by 48 hours, but redness and chemosis persisted until 96 hours. EU considered this study of limited value because the solvent ethanol has eye irritation potential.

Ecotoxicity (Ecotox)

Acute Aquatic Toxicity (AA) Score (vH, H, M, or L): vH

HHCB was assigned a score of Very High for acute aquatic toxicity based on a 48h EC_{50} of 0.282 mg/L, a 96h EC_{50} of 0.153 – 0.831 mg/L in juvenile freshwater mussel, a 48h LC_{50} of 0.47 mg/L in marine copepod, and a 72h EC_{50} of 0.72 mg/L in green algae and based on being associated with H400. GreenScreen® criteria classify chemicals as a Very High hazard for acute aquatic toxicity

when acute aquatic toxicity values are no greater than 1 mg/L or when they are associated with H400 (CPA 2012a).

- Authoritative and Screening Lists
  - *Authoritative:* EC – CLP/GHS Hazard Statements – H400 – Aquatic Acute 1 – Very toxic to aquatic life
  - *Authoritative:* EC – Risk Phrases – R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment – Based on Ecotoxicity, P and/or B
  - *Screening:* Environment Canada – Domestic Substances List – Inherently Toxic in the Environment
- U.S. EPA 2014, ECHA 2015
  - 96h LC_{50} = 1.36 mg/L (measured) in freshwater bluegill sunfish (*Lepomis macrochirus*) (OECD TG 204) (statistically derived from a 21-day GLP study)
  - 48h EC_{50} (immobilization) = 0.282 mg/L (measured) in daphnia (*Daphnia magna*) (OECD TG 202)
  - 48h LC_{50} (mortality) = 0.999 - > 1.75 mg/L (measured) in freshwater mussel (*Lampsillis cardium*)
  - 96h EC_{50} (growth) = 0.153 - 0.831 mg/L (measured) in juvenile freshwater mussel (*Lampsillis cardium*)
  - 96h LC_{50} (mortality) = 1.9 mg/L (measured) in saltwater invertebrate estuarine copepods (*Nitocra spinipes*) (SIS 1991 Guideline)
  - 48h LC_{50} (mortality) = 0.47 mg/L (nominal) in saltwater invertebrate marine copepod (*Acartia tonsa*) (ISO 1997)
  - 72h EC_{50} (growth) > 0.85 mg/L (measured) in green algae (*Pseudokirchneriella subcapitata*) (OECD TG 201)
  - 72h EC_{50} (biomass) = 0.72 mg/L (measured) in green algae (*Pseudokirchneriella subcapitata*) (OECD TG 201)

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): vH

HHCB was assigned a score of Very High for chronic aquatic toxicity based on chronic aquatic toxicity values as low as 0.068 mg/L in fish and 0.007 mg/L in saltwater invertebrates. GreenScreen® criteria classify chemicals as a Very High hazard for chronic aquatic toxicity when NOECs or EC_{10} values are no greater than 0.1 mg/L (CPA 2012a).

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Authoritative:* EC – Risk Phrases – R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment – Based on Ecotoxicity, P and/or B
  - *Screening:* EC – CLP/GHS Hazard Statements – H410 – Aquatic Chronic 1 – Very toxic to aquatic life with long lasting effects
  - *Screening:* Environment Canada – Domestic Substances List – Inherently Toxic in the Environment
- U.S. EPA 2014
  - 21-day NOEC (clinical signs) = 0.093 mg/L (measured) in bluegill sunfish (*Lepomis macrochirus*) (OECD TG 204)
  - 36-day NOEC (survival, growth and development) = 0.068 mg/L (measured) in fathead minnow (*Pimphales promelas*) (OECD TG 210)
  - 21-day NOEC (reproduction) = 0.111 mg/L (measured) in water flea (*Daphnia magna*) (OECD TG 202)

- 6-day NOEC (development) = 0.038 mg/L (measured) in marine copepods (*Acartia tonsa*) (OECD TG Draft Invert Life Cycle)
- 5-day EC_{10} (development) = 0.037 mg/L (nominal) in marine copepods (*Acartia tonsa*) (OECD TG Draft Invert Life Cycle)
- 22-day NOEC (development) = 0.007 mg/L (measured) in estuarine copepods (*Nitocra spinipes*) (Test guideline not reported)
- 72h NOEC (growth and biomass) = 0.201 mg/L (measured) in green algae (*Pseudokirchneriella subcapitata*) (OECD TG 201)

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): *H*

HHCB was assigned a score of High for persistence based on a reportedly most relevant half-life of 105 days in soil, its predicted major partitioning compartment. GreenScreen® criteria classify chemicals as a High hazard for persistence when half-lives are between 60 and 180 days in soil (CPA 2012a). Confidence level was reduced as modeling was used to predict the predominant compartment of HHCB.

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists
- ECHA 2015
  - In a CO2 evolution test for ready biodegradability conducted according to OECD 301B (GLP status not reported), HHCB (purity not reported) reached 2% degradation in 28 days, and the HHCB was not inhibitory to the degradation of the reference substance under the conditions of the test. It was concluded that HHCB did not show any significant mineralization.
  - In a GLP-compliant CO2 evolution test for ready biodegradability conducted according to OECD 301B, HHCB containing 32.2% isopropyl myristate was tested. The inoculum was pre-exposed to HHCB in a SCAS test. Excluding the degradation of isopropyl myristate, HHCB alone was not degraded in 28 days. It was concluded that no mineralization occurred for HHCB.
- EU 2008, U.S. EPA 2014
  - HHCB is not readily biodegradable as demonstrated by the lack of mineralization in ready biodegradability studies. However, it is considered inherently biodegradable.
  - In water, primary degradation to more polar metabolites was reported. The radio labelled parent HHCB disappeared with half-lives of 10 – 15 hours and 21 hours in studies with activated sludge. In a river die-away test, 0.5 - 5 µg/L HHCB disappeared with half-lives of 43 – 100 hours. In water, volatilization is an important process of HHCB removal.
  - In soil, field measurements reported complete disappearance within one year. Based on unfrozen conditions in sludge-amended soil studies, the half-life was about 140 – 145 days. After 1 year, only 10 – 14% of the initial concentrations remain in soil.
  - An atmospheric half-life of 3.4 hours was calculated for HHCB based on a reported rate constant for hydroxyl radical oxidation.
  - In EU's derivation of potential environmental exposure concentrations (PECs), conservative half-lives of 60 days, 150 days and 150 days were used for surface water, soil and sediment, respectively.
  - U.S.EPA's summarized available data on the half-lives or half-disappearance times for HHCB as 33 – 100 hours in water, 79 days in sediment, 95 – 239 days in soil, 10 – 69 hours in sludge, and 105 – 144 days in biosolids-amended soil.

  - Both EU and the U.S. EPA consider the reported half-life of 105 days in the sludge amended soil test the most relevant half-life for the fate of HHCB in soil.
- U.S. EPA 2012
  - The BIOWIN modeling Ready Biodegradable Predictor indicates that HHCB is not expected to be readily biodegradable. Fugacity modeling predicts 10.9% will partition to water with a half-life of 60 days, 73.2% will partition to soil with a half-life of 120 days, and 15.7% will partition to sediment with a half-life of 542 days (Appendix E).
- Based on the weight of evidence, a score of High was assigned. Fugacity modeling indicates that HHCB predominantly partition to soil. Measured half-lives in soil or biosolids-amended soil were 95, 239, 105 and 141-144 days in four studies. Although the longest half-life of 239 days warrants a score of Very High (> 180 days in soil), both U.S. EPA and the EU considers the half-life of 105 days the most relevant half-life for the fate of HHCB in soil. Therefore, ToxServices used this value as the basis of this endpoint.

Bioaccumulation (B) Score (vH, H, M, L, or vL): H

HHCB was assigned a score of High for bioaccumulation based on measured BCF/BAFs of up to 1,584 in fish and 2,692 in invertebrates. GreenScreen® criteria classify chemicals as a High hazard for bioaccumulation when BCF/BAF values are between 1,000 and 5,000 (CPA 2012a).

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists
- U.S. EPA 2014
  - BAF = 20 in rudd (*SScardinius erythrophthalmus*, aquatic vertebrate)
  - BAF = 510 in tench (*Tinca tinca,* aquatic invertebrate)
  - BAF = 580 in crucian carp (*Carassius carassius*, aquatic vertebrate)
  - BCF = 862 and BAF = 290 in Eel (*Anguilla Anguilla*, aquatic vertebrate)
  - BAF = 620 in zebra mussel (*Dreissena polymorpha*, aquatic vertebrate)
  - BCF = 1,584 in bluegill sunfish (*Lepomis macrochirus*, aquatic vertebrate) (GLP)
  - BCF = 620 in zebrafish (*Danio rerio*, aquatic vertebrate)
  - BAF = 31 – 106 in smallmouth bass (aquatic vertebrate)
  - BAF = 30 – 146 in largemouth bass (aquatic vertebrate)
  - BAF = 21 – 333 in white perch (aquatic vertebrate)
  - BAF = 18 – 371 in catfish (aquatic vertebrate)
  - BCF = 85 – 138 in midge (*Chironomus riparius*, invertebrate)
  - BCF = 2,692 in blackworm (*Lumbriculus variegatus*, invertebrate)
  - BCF = 2,395 (calculated) in earthworm (*Lumbricus terrestris,* invertebrate)
  - U.S. EPA concluded that HHCB has low to moderate concern for bioaccumulation. The BCF of 1,584 for bluegills and 2,692 for *Lumbriculus* indicate a high bioaccumulation concern. However, BAF values in fish are between 20 and 620, indicating low bioaccumulation potential. Based on these data, aquatic food-chain modeling (Arnot-Gobas model) and monitoring data, HHCB is considered not subject to biomagnification.
- EU 2008
  - Log K_{ow} = 5.9 as measured in a test conducted according to OECD TG 117
  - Based on the studies summarized above, the EU concluded that HHCB does not meet the criterion for bioaccumulation (BCF > 2,000). Available data in lower invertebrates indicate that HHCB may accumulate in those species which are not capable of metabolizing it. Measured data in fish (higher up in the food chain) report BCF and BAF values of < 2,000, which indicate that HHCB is metabolized in fish.

- Based on the weight of evidence, a score of High was assigned. Although the measured log K_{ow} of 5.9 corresponds to a score of Very High, measured BCF/BAF values in fish and invertebrates are up to 2,692, and BCF/BAF values in fish are much lower than those in lower invertebrates, indicating that fish, but not invertebrates, are capable of metabolizing HHCB, thereby reducing its bioaccumulation potential. Therefore, measured BCF/BAF values are more appropriate than partition coefficient in determining the bioaccumulation potential of HHCB and the score of High is based on the experimental BCF values of 1,584 in bluegill sunfish and 2,692 in blackworms.

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): *L*

HHCB was assigned a score of Low for reactivity based on being predicted to be non-explosive and based on expert judgment of the structure. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when they are not explosive, and there are no data indicating they are reactive otherwise (CPA 2012a). Confidence level was reduced due to the lack of measured data.

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists
- EU 2008
  - HHCB is not explosive as calculated by CHETAH (v7.0)
  - HHCB does not have oxidizing propertied based on expert judgment on its structure
- UN 2013
  - Based on examination of the structure, ToxServices determined that HHCB is not an organic peroxide, does not contain reactive groups associated with self-reactive properties, and is not an organometallic substance that may produce flammable gases on contact with water.
- Based on the weight of evidence, a score of Low was assigned. HHCB was predicted to be non-explosive, and judged to be non-oxidizing by the EU. ToxServices did not find any other reactivity parameters relevant to HHCB. Confidence level was reduced due to the lack of measured data.

Flammability (F) Score (vH, H, M, or L): L

HHCB was assigned a score of Low for flammability based on experimental flashpoints. GreenScreen® criteria classify chemicals as a Low hazard for flammability when they are not classifiable under GHS (CPA 2012a).

- Authoritative and Screening Lists
  - Not present on any authoritative or screening lists.
- EU 2008
  - HHCB has a flash point of > 100ºC as determined by a closed cup test conducted according to EU Directive 84/449/EEC, A.9 guideline.
  - HHCB is not a flammable liquid. It is combustible (can burn), but it has no pyrophoric properties.
- U.S. EPA 2014
  - HHCB has a flash point of 144ºC
- Based on the weight of evidence, a score of Low was assigned. The flash points of 144ºC and > 100ºC are higher than the GHS cutoff of 93ºC for classification. Therefore, HHCB is not classifiable as a flammable liquid under GHS.

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APPENDIX A: Hazard Benchmark Acronyms
(in alphabetical order)

(AA) Acute Aquatic Toxicity

(AT) Acute Mammalian Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(D) Developmental Toxicity

(E) Endocrine Activity

(F) Flammability

(IrE) Eye Irritation/Corrosivity

(IrS) Skin Irritation/Corrosivity

(M) Mutagenicity and Genotoxicity

(N) Neurotoxicity

(P) Persistence

(R) Reproductive Toxicity

(Rx) Reactivity

(SnS) Sensitization- Skin

(SnR) Sensitization- Respiratory

(ST) Systemic/Organ Toxicity

APPENDIX B: Results of Automated GreenScreen® Score Calculation for HHCB (CAS #1222-05-5)

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

GREEN SCREEN FOR SAFER CHEMICALS

GreenScreen® Score Inspector

Table 1: Hazard Table

			Group I Human					Group II and II* Human								Ecotox		Fate		Physical	
			Carcinogenicity	Mutagenicity/Genotoxicity	Reproductive Toxicity	Developmental Toxicity	Endocrine Activity	Acute Toxicity	Systemic Toxicity		Neurotoxicity		Skin Sensitization*	Respiratory Sensitization*	Skin Irritation	Eye Irritation	Acute Aquatic Toxicity	Chronic Aquatic Toxicity	Persistence	Bioaccumulation	Reactivity	Flammability
Table 2: Chemical Details									S	R *	S	R *	*	*								
Inorganic Chemical?	Chemical Name	CAS#	C	M	R	D	E	AT	STs	STr	Ns	Nr	SNS*	SNR*	IrS	IrE	AA	CA	P	B	Rx	F
No	HHCB	1222-05-5	*L*	L	DG	*L*	*M*	L	*L*	L	L	DG	L	DG	*M*	*L*	vH	vH	*H*	H	*L*	L

Table 3: Hazard Summary Table

Benchmark	a	b	c	d	e	f	g
1	Yes	No	No	No	No		
2	STOP						
3	STOP						
4	STOP						

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
HHCB	1

Note: Chemical has not undergone a data gap assessment. Not a Final GreenScreen™ Score

Table 6

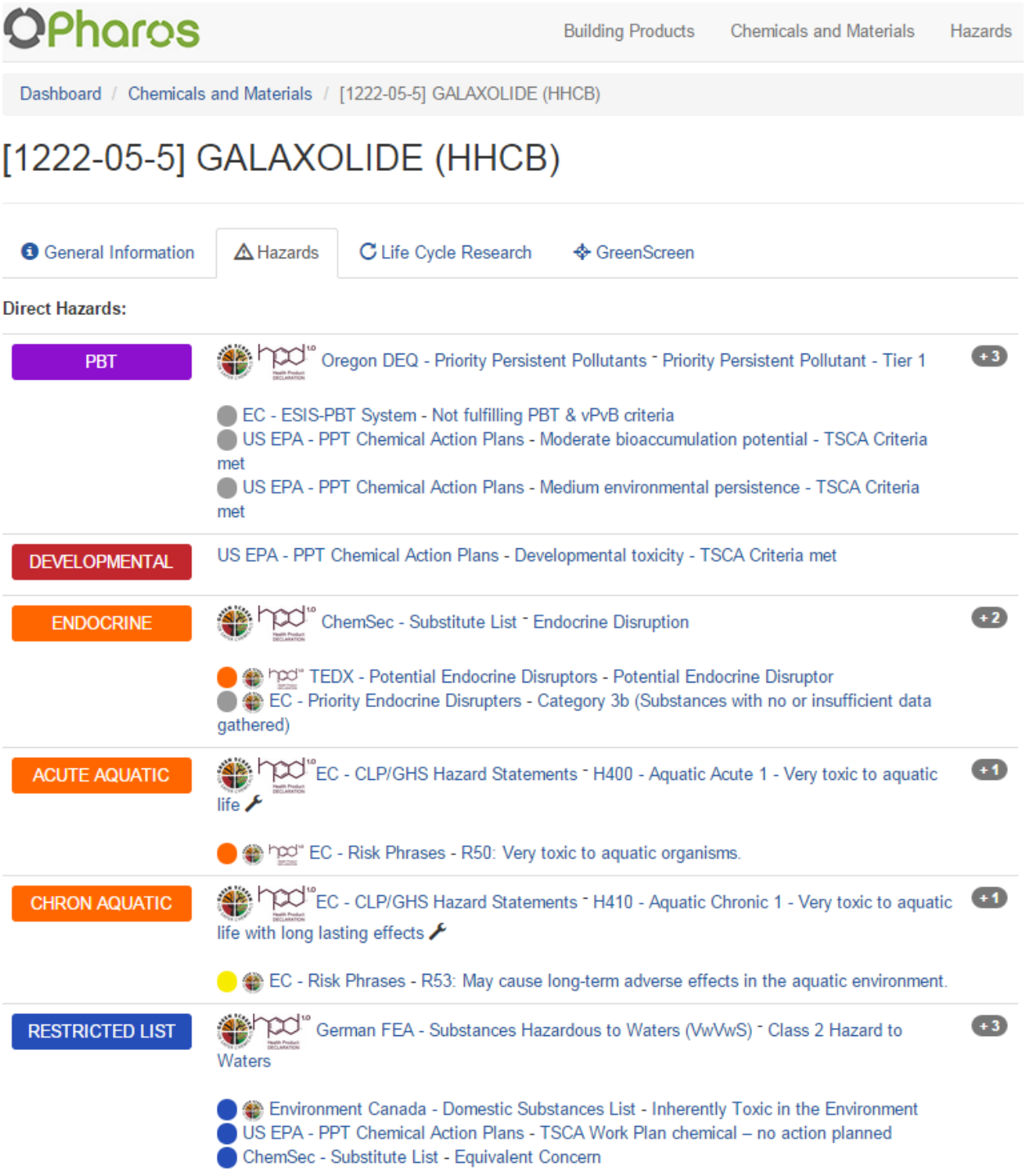
Chemical Name	Final GreenScreen® Benchmark Score
HHCB	1

After Data gap Assessment
Note: No Data gap Assessment Done if Preliminary GS Benchmark Score is 1.

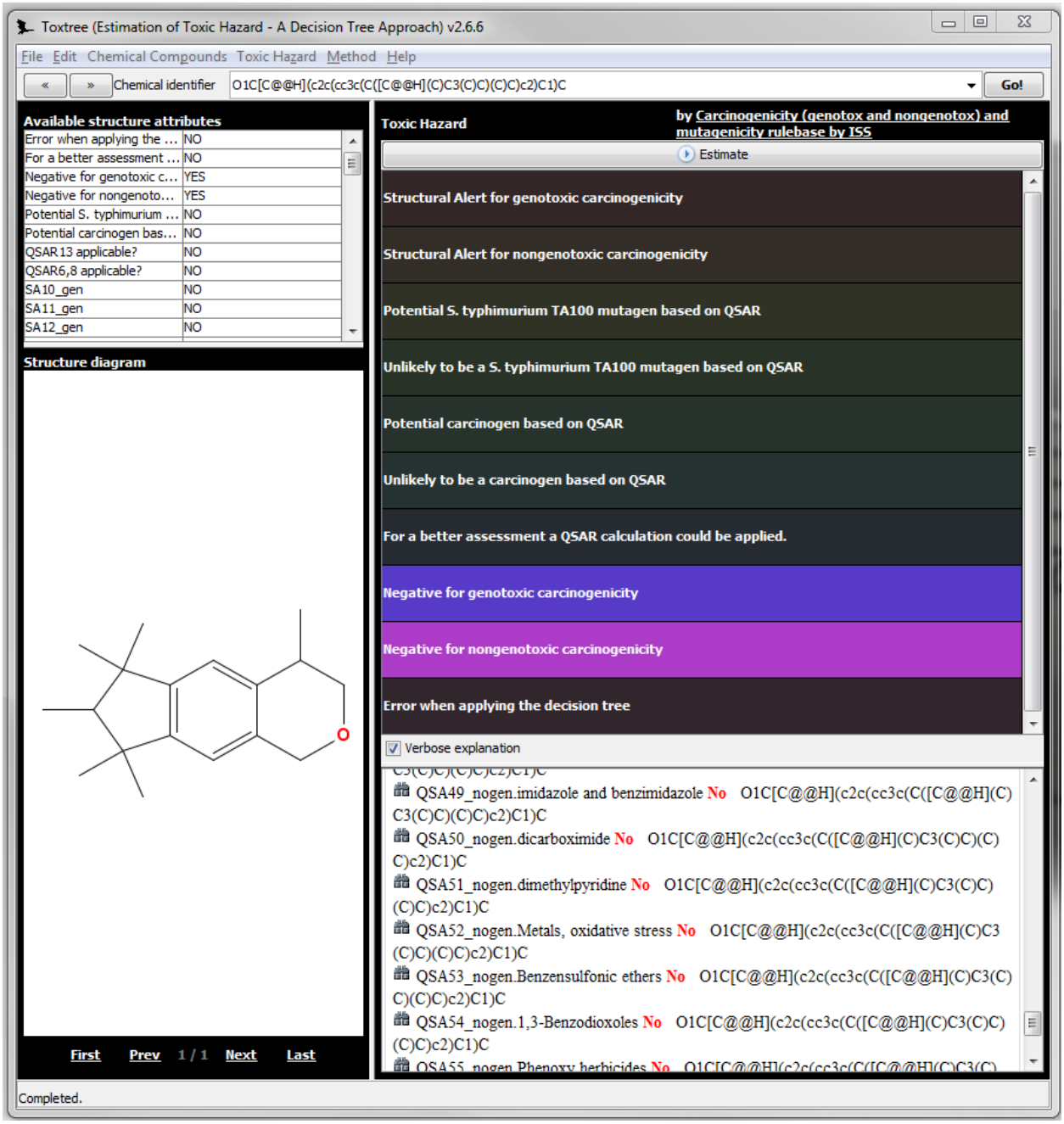
Table 5: Data Gap Assessment Table

Datagap Criteria	a	b	c	d	e	f	g	h	i	j	bm4	End Result
1												1
2												
3												
4												

APPENDIX C: Pharos Output for HHCB (CAS #1222-05-5)



APPENDIX D: Toxtree Carcinogenicity Modeling Results for HHCB (CAS #1222-05-5)



APPENDIX E: EPISuite Modeling Results for HHCB (CAS #1222-05-5)

CAS Number: 1222-05-5
SMILES : O(CC(c(c1cc(c2C(C3C)(C)C)C3(C)C)c2)C)C1
CHEM : Cyclopenta g -2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamet
hyl-
MOL FOR: C18 H26 O1
MOL WT : 258.41

---------- EPI SUMMARY (v4.11) ----------
Henry LC (atm-m3/mole) : ------
Log Kow (octanol-water): ------
Boiling Point (deg C) : ------
Water Solubility (mg/L): ------
Physical Property Inputs:
Vapor Pressure (mm Hg) : ------
Melting Point (deg C) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.68 estimate) = 6.26
Log Kow (Exper. database match) = 5.90
Exper. Ref: US EPA (2004)

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 325.30 (Adapted Stein & Brown method)
Melting Pt (deg C): 102.64 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 0.000512 (Modified Grain method)
VP (Pa, 25 deg C) : 0.0683 (Modified Grain method)
MP (exp database): -5 deg C
BP (exp database): 325 deg C
VP (exp database): 5.45E-04 mm Hg (7.27E-002 Pa) at 25 deg C

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 0.1943
log Kow used: 5.90 (expkow database)
no-melting pt equation used
Water Sol (Exper. database match) = 1.75 mg/L (25 deg C)
Exper. Ref: US EPA (2004)

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 0.20116 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found: Neutral Organics

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 1.32E-004 atm-m3/mole (1.34E+001 Pa-m3/mole)
Group Method: 7.56E-007 atm-m3/mole (7.66E-002 Pa-m3/mole)
For Henry LC Comparison Purposes:

User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 8.960E-004 atm-m3/mole (9.078E+001 Pa-m3/mole)
VP: 0.000512 mm Hg (source: MPBPVP)
WS: 0.194 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 5.90 (exp database)
Log Kaw used: -2.268 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 8.168
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : -0.0360
Biowin2 (Non-Linear Model) : 0.0009
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 2.1204 (months)
Biowin4 (Primary Survey Model) : 3.0898 (weeks)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.0657
Biowin6 (MITI Non-Linear Model): 0.0255
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -0.9272
Ready Biodegradability Prediction: NO

Hydrocarbon Biodegradation (BioHCwin v1.01):
Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 0.0727 Pa (0.000545 mm Hg)
Log Koa (Koawin est): 8.168
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 4.13E-005
Octanol/air (Koa) model: 3.61E-005
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 0.00149
Mackay model : 0.00329
Octanol/air (Koa) model: 0.00288

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 37.7872 E-12 cm3/molecule-sec
Half-Life = 0.283 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 3.397 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):
0.00239 (Junge-Pankow, Mackay avg)
0.00288 (Koa method)

Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 1.969E+004 L/kg (MCI method)
Log Koc: 4.294 (MCI method)
Koc : 1.253E+004 L/kg (Kow method)
Log Koc: 4.098 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Rate constants can NOT be estimated for this structure!

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 3.560 (BCF = 3629 L/kg wet-wt)
Log Biotransformation Half-life (HL) = 0.5542 days (HL = 3.582 days)
Log BCF Arnot-Gobas method (upper trophic) = 3.090 (BCF = 1231)
Log BAF Arnot-Gobas method (upper trophic) = 3.261 (BAF = 1826)
log Kow used: 5.90 (expkow database)

Volatilization from Water:
Henry LC: 7.56E-007 atm-m3/mole (estimated by Group SAR Method)
Half-Life from Model River: 1247 hours (51.94 days)
Half-Life from Model Lake : 1.373E+004 hours (572.2 days)

Removal In Wastewater Treatment:
Total removal: 91.68 percent
Total biodegradation: 0.77 percent
Total sludge adsorption: 90.91 percent
Total to Air: 0.00 percent
(using 10000 hr Bio P,A,S)

Removal In Wastewater Treatment (recommended maximum 95%):
Total removal: 96.60 percent
Total biodegradation: 26.01 percent
Total sludge adsorption: 70.59 percent
Total to Air: 0.00 percent
(using Biowin/EPA draft method)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.149	6.79	1000
Water	10.9	1.44e+003	1000
Soil	73.2	2.88e+003	1000
Sediment	15.7	1.3e+004	0

Persistence Time: 1.94e+003 hr

Licensed GreenScreen® Profilers

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