d-Limonene (CAS #5989-27-5) GreenScreen™ Assessment

Prepared for:

The Renewable Citrus Products Association

October 22, 2012

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GreenScreen™ Assessment for d-Limonene (CAS #5989-27-5)

GreenScreen™ Version 1.2 Draft Assessment
Note: Validation Has Not Been Performed on this GreenScreen™ Assessment

Chemical Name: d-Limonene

CAS Number: 5989-27-5

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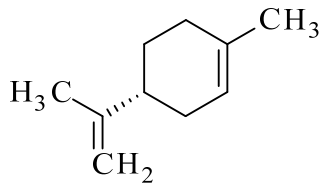
Date: October 22, 2012

Confirm application of the *de minimus* rule[1]: N/A

Chemical Structure(s):



Identify Applications/Functional Uses:
1. Solvent (NICNAS 2002)
2. Fragrance (NICNAS 2002)
3. Flavoring (NICNAS 2002)

GreenScreen™ Summary Rating for d-Limonene[2]: ToxServices assigned a GreenScreen™ Benchmark score of 2 for d-limonene based upon a Very High (vH) Acute Aquatic Toxicity (AA) hazard classification, in addition to a High (H) Skin Sensitization (SnS) hazard classification for d-limonene's oxidation products. On the GreenScreen™ 4-point scoring scale (1 is the worst, 4 is the best), d-limonene's GreenScreen™ score of 2 corresponds to "Use but Search for Safer Substitutes" (CPA 2011a). Data gaps (dg) exist for Reproductive Toxicity (R), Neurotoxicity (N), and Respiratory Sensitization (Snr).

Despite these data gaps, a GreenScreen™ score of 2 is justified for d-limonene as outlined in Section III(1) of Benchmarking Chemicals with Data Gaps (CPA 2011b). In a worst-case scenario, if d-limonene were assigned a High score for Reproductive toxicity (R) or Endocrine Activity (E), it would receive a GreenScreen™ score of 1.

[1] 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

[2] 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.

Figure 1: GreenScreen™ Hazard Ratings for d-Limonene

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	dg	L	dg	L	dg	dg	H	dg	H	*H*	vH	L	vL	L	L	M

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect estimated values and lower confidence. Hazard levels in **BOLD** font reflect values based on test data (See Guidance).
Note: Please see Appendix A for a glossary of hazard acronyms.

Transformation Products and Ratings:
Identify relevant fate and transformation products (i.e., dissociation products, transformation products, valence states) **and/or moieties of concern**[3]

Oxidation products are the primary transformation product of concern for d-limonene. Oxidation of d-limonene with air produces potent skin sensitizers such as cis- and trans- isomers of limonene oxide (CAS #1195-92-2) and carvone (99-49-0) (Karlberg et al. 1992). Hydrolysis of d-limonene is not expected in the aquatic environment (NICNAS 2002). Several transformation products are expected from photochemical reactions in the atmosphere; however, these are not expected to have an impact on human health or aquatic toxicity endpoints (NICNAS 2002). The sensitization potential of air oxidized d-limonene is discussed in greater detail below.

Introduction

d-Limonene belongs to a class of chemicals known as terpenes (NICNAS 2002). d-Limonene is naturally occurring and is the major constituent of the oil from citrus fruits. Its isomer, l-limonene, is also naturally occurring and found predominantly in pine-needle oils, as well as in spearmint and peppermint. Dipentene is the racemic mixture of d- and l-limonene. d-Limonene is a colorless liquid with a citrus odor at room temperature. d-Limonene, like most terpene chemicals, has moderate vapor pressure and low water solubility, which may result in a high rate of vaporization. d-Limonene and terpenes are a naturally-occurring constituent of oils from citrus rinds as well as certain trees and bushes. It is commonly used in fragrances and flavoring and as a solvent in cleaning products. Concentrations in all uses of d-limonene can vary from very low percentages (< 1%) to up to 95% in industrial products. In consumer products, concentrations of d-limonene typically range from < 1% up to 75% (NICNAS 2002). d-Limonene is considered Generally Recognized As Safe (GRAS) by the U.S FDA for addition to food (21CFR§182.60). It is registered as a pesticide (active ingredient) in the United States (U.S. EPA 1994) and also as an inactive ingredient for pesticide formulations.

ToxServices assessed d-limonene against GreenScreen™ Version 1.2 (CPA 2011a) following procedures outlined in ToxServices' SOP 1.37 (GreenScreen™ Hazard Assessment) (ToxServices 2012).

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 2012). Pharos (2012) 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 d-limonene can be found in Appendix B and a summary of the results can be found below:

- EC - CLP/GHS Hazard Statements (EU H-Statements): H400 - Aquatic Acute 1 - Very toxic to aquatic life

[3] 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.

- EC/Oslo-Paris Conv - Priority PBTs & EDs & equivalent concern (OSPAR): PBT - (Substance of Possible Concern)
    - (Available data do not agree with this classification as explained below)
- EC - CLP/GHS Hazard Statements (EU H-Statements): H410 - Aquatic Chronic 1 - Very toxic to aquatic life with long lasting effects
    - (Available data do not agree with this classification as explained below)
- EC - CLP/GHS Hazard Statements (EU H-Statements): H226 Flammable liquid and vapor
- AOEC - Asthmagens (AOEC): Suspected asthmagen (R - reviewed by AOEC and does not meet sensitizer or RADS criteria)
- Intnl Agency for Rsrch on Cancer - Cancer Monographs (IARC): Not classifiable as to carcinogenicity (Group 3: Agent is not classifiable as to its carcinogenicity to humans)
- German FEA - Substances Hazardous to Waters (VwVwS): Hazard to Waters (Water Hazard Class 2)

PhysioChemical Properties of d-Limonene:

Table 1: Physical and Chemical Properties of d-Limonene

Property	Value	Reference
Molecular formula	$C_{10}H_{16}$	NICNAS 2002
SMILES Notation	CC1=CC[C@@H](CC1)C(=C)C	
Molecular weight	136.24	NICNAS 2002
Physical state	Liquid	NICNAS 2002
Appearance	Colorless	NICNAS 2002
Melting point	-74.35°C	NICNAS 2002
Vapor pressure	0.19 kPa @ 20°C	NICNAS 2002
Water solubility	13.8 mg/L @ 25°C	NICNAS 2002
Dissociation constant	N/A	
Density/specific gravity	0.84	NICNAS 2002
Partition coefficient	4.23	NICNAS 2002

Hazard Classification Summary Section:

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M or L): L
d-Limonene was assigned a score of Low for carcinogenicity based on not being classifiable as a carcinogenic compound following GHS (2011) criteria. GreenScreen™ criteria classify chemicals as a low hazard for carcinogenicity as follows: Adequate data are available for classification, studies are negative for carcinogenicity, the chemical does not have structural alerts for genotoxicity, and GHS not classified (CPA 2011c).

- IARC 1999 –
    - d-Limonene is listed by IARC as Group 3 "*not classifiable as to its carcinogenicity to humans"*. d-Limonene induced an increase in renal tubular tumors in male rats. However, the IARC considered these tumors to be the result of an α_{2u}-globulin associated response and not relevant to human exposure.
- NTP 1990 –
    - A two year carcinogenicity/chronic toxicity study was conducting using male and female F344 rats and $B6C3F_1$ mice (50/sex/group). Rats received doses of 0, 75, or 150 mg/kg (males) and 0, 300, or 600 mg/kg (females) via oral gavage (vehicle: corn oil) 5 days a week for 103 weeks. Mice received doses of 0, 250, or 500 mg/kg (males) and 0, 500, or 1,000 mg/kg (females) via oral gavage (vehicle: corn oil) 5 days a week for 103 weeks. Tissues examined included: adrenal glands, brain, cecum, colon, costochondral junction, duodenum, epididymis, seminal vesicles, tunica vaginalis, scrotal sac, prostate, testes, ovaries, uterus, esophagus, eyes, femur, sternebrae, gallbladder (mice), lymph nodes, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular,

mesenteric lymph nodes, nasal cavity, oral cavity, pancreas, parathyroids, pituitary gland, gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, urinary bladder, and zymbal gland. The kidney of male rats was the target organ following the two-year carcinogenicity study. Lesion consisted of tubular cell hyperplasia, adenomas, and adenocarcinomas. The NTP concluded that the specific mechanism of carcinogenicity was unable to be determined. However, study results suggest a link between carcinogenicity and an α_{2u}-globulin associated response. No treatment related neoplasms were reported in mice or female rats.

- U.S. EPA 1994 –
  - Renal adenomas/carcinomas in male rats for 2 years were considered to be related to α_{2u}-globulin-induced nephropathy and not relevant to human exposure by the EPA.
- NICNAS 2002 –
  - In order to investigate the carcinogenic mechanism of d-limonene 150 mg/kg was administered to α_{2u}-globulin deficient F344 rats by oral gavage 5 days/week for 30 weeks. No increases in tumors or preneoplastic lesions were observed.
- Based on the weight of evidence, ToxServices agrees with the IARC and EPA conclusions that the observed adenomas and carcinomas are related to α_{2u}-globulin-induced toxicity and not relevant to human exposure. Therefore, d-limonene is not classifiable as a carcinogenic substance following GHS criteria.

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

d-Limonene was assigned a score of Low for mutagenicity based on no evidence mutagenicity or clastogenicity and not being classifiable as a GHS (2011) germ cell mutagen.

- U.S. EPA 2012 –
  - Several bacterial reverse mutation assays were identified utilizing *Salmonella typhimurium* tester strains TA98, TA100, TA102, TA1535, TA1537, and TA1538 at concentrations of up to 150,000 µg/plate (5,000 µg/plate in TA102) with and without metabolic activation. d-Limonene was reported to be negative for mutagenicity under all test conditions.
  - Two mouse lymphoma assays were conducted utilizing mouse lymphoma L5178Y cells with and without metabolic activation at a concentration of up to 100 µg/ml. No evidence of mutagenicity was reported in either study and d-limonene was considered to be non-mutagenic under the tested conditions.
  - A chromosomal aberration assay was conducted utilizing Chinese Hamster Ovary (CHO) cells at concentrations of up to 500 µg/ml with and without metabolic activation. No increases in chromosomal aberrations or polyploidy were observed and d-limonene was reported as negative for clastogenicity under the tested conditions.
  - Two mammalian cell transformation assays was conducted utilizing Syrian Hamster Embryo (SHE) cells at concentrations of up to 100 µg/ml, with and without metabolic activation. No statistically significant cell transformations were observed and d-limonene was reported as negative for genotoxicity under the tested conditions.
  - A sister chromatic exchange assay was conducted utilizing CHO cells at concentrations of up to 162 µg/ml with and without metabolic activation. No genotoxic effects were observed and d-limonene was reported as negative for genotoxicity under the tested conditions.
  - An *in vivo* mouse spot test was conducted (strain/number/sex not reported). Mice were administered a single dose of 215 mg/kg of d-limonene via intraperitoneal injection. Mouse embryos were treated in utero with limonene on days 10 and 11 post conception. No genotoxic effects were observed and no effects on embryos were reported. d-Limonene is reported as negative for genotoxicity under the tested conditions.
- NICNAS 2002 –
  - There is no evidence that d-limonene or its metabolites are genotoxic or mutagenic.
- U.S. EPA 1994 –
  - Limonene is not mutagenic.

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

- No relevant data were identified.

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M or L): L
d-Limonene was assigned a score of Low for developmental toxicity based on no evidence of developmental toxicity and not being classifiable as GHS (2011) reproductive toxicant.

- U.S. EPA 2012 –
    - A developmental toxicity study was conducted using female Wistar rats (n=20). Rats were administered (oral administration, no further details on route) doses of 0, 591, or 2,869 mg/kg of d-limonene on days 9 through 15 of gestation. In the top dose group a delayed ossification of fetuses' metacarpal and proximal phalanx, decreased body weights, and decreased thymus, spleen and ovarian weight were observed. Additionally, maternal toxicity in the top dose group included decreased body weights and increased mortality. No effects were reported a 591 mg/kg. The study authors reported a NOAEL and LOAEL of 591 and 2,869 mg/kg for both maternal and fetal toxicity. As fetal/embryonic toxicity only occurred at high doses in the presence of maternal toxicity this chemical is not classified as a GHS Reproductive toxicant.
    - A second developmental toxicity study was conducted using female Japanese White rabbits (number not reported). Rabbits were administered (oral administration, no further details on route) doses of 0, 250, 500, or 1,000 mg/kg of the test substance on days 6 through 18 of gestation. Significant decreases in maternal body weight and food consumption were reported in the top two dose groups. No treatment related effects were reported on the offspring. A NOAEL of 1,000 mg/kg for developmental toxicity was reported by the study authors.
    - A third developmental toxicity study was conducted using female ICR mice (number not reported). Mice were administered (oral administration, no further details on route) doses of 0, 591, or 2,363 mg/kg of the test substance on days 7 through 12 of gestation. Significantly decreased body weight was reported for females in the top dose group. Increased incidence of fused-ribs and delayed ossification occurred in offspring for the top dose group. Delayed ossification returned to normal following birth. A NOAEL and LOAEL of 591 and 2,363 mg/kg were reported for developmental toxicity. As developmental toxicity only occurred in the presence of maternal toxicity at a high dose classification for this chemical is not required under GHS.
- U.S. EPA 1994 –
    - d-Limonene is not a developmental toxicant. Delayed ossification is considered to be a secondary effect to maternal toxicity.
- JECFA 1998 –
    - There is no evidence that d-limonene has teratogenic or embryotoxic effects in the absence of maternal toxicity.
- Based on the weight of evidence, ToxServices agrees with the JECFA and EPA conclusion that the observed toxic effects were secondary in nature to maternal toxicity. Therefore, d-limonene is not classifiable as a reproductive toxicant following GHS criteria.

Endocrine Activity (E) Score (H, M or L): dg
d-Limonene has been assigned a data gap for endocrine activity. Although it is not a known endocrine disruptor, endocrine disruption testing has not performed on the chemical.

- Not listed as a potential endocrine disruptor on the EU Priority List of Suspected Endocrine Disruptors.
- Not listed as a potential endocrine disruptor on the OSPAR List of Chemicals of Possible Concern.
- Not listed as a potential endocrine disruptor on the Red List of Chemicals (CPA 2011d).
- ACD/Labs 2012 –
    - ACD/I-Lab Estrogen Receptor Binding Affinity predicts that d-limonene is not expected to bind to the estrogen receptor. Refer to Appendix C for the ACD/I-Lab prediction report.
- Insufficient data are available to fully address the endocrine activity endpoint. While no evidence from available studies or QSAR modeling suggests that a potential effect on the endocrine system exists, a two-generation reproductive toxicity study has not been performed and sufficient details from developmental toxicity studies and chronic/sub-chronic toxicity studies were not available to assess the potential effects of d-limonene upon the thyroid or male sex glands. Additionally, adequate QSAR modeling has not been identified to address anti-androgenic or anti-thyroid effects. Therefore, a data gap has been assigned for endocrine activity.

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

d-Limonene was assigned a score of Low for acute mammalian toxicity based on oral and dermal LD_{50} values above the 2,000 mg/kg cut-off for low toxicity (CPA 2011c).

- JECFA 1998 –
  - d-Limonene has reported oral LD_{50} values between 4,400 and 5,100 mg/kg in rats.
  - d-Limonene has reported oral LD_{50} values between 5,300 and 6,800 mg/kg in mice.
  - d/l-Limonene has a reported dermal LD_{50} value of greater than 5,000 mg/kg in rats.
- ESIS 2000 –
  - d-Limonene has a reported dermal LD_{50} value of greater than 2,000 mg/kg in rabbits.
- U.S. EPA 2012 –
  - d-Limonene has a reported dermal LD_{50} value of greater than 5,000 mg/kg in New Zealand rabbits.

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST)

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

- No relevant data were identified.

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

d-Limonene was assigned a score of Low for systemic toxicity/organ effects based on repeated exposure as only minimal enzymatic effects with increased liver weights were identified at a LOAEL of 400 mg/kg. Therefore, this chemical is not classifiable as a GHS (2011) systemic toxicant.

- NTP 1990 –
  - A two year carcinogenicity/chronic toxicity study was conducting using male and female F344 rats and $B6C3F_1$ mice (50/sex/group). Rats received doses of 0, 75, or 150 mg/kg (males) and 0, 300, or 600 mg/kg (females) via oral gavage (vehicle: corn oil) 5 days a week for 103 weeks. Mice received doses of 0, 250, or 500 mg/kg (males) and 0, 500, or 1,000 mg/kg (females) via oral gavage (vehicle: corn oil) 5 days a week for 103 weeks. Both male and female rats has significantly reduced weight in the top dose group and increased mortality. Increased α_{2u}-globulin nephropathy was reported for male rats in all dose groups. As this is not relevant to humans a NOAEL and LOAEL of 300 mg/kg and 600 mg/kg were reported for female rats and 75 and 150 mg/kg for male rats. Males in the top dose group exhibited liver cells with abnormal numbers of nuclei and cytomegaly. Therefore, a NOAEL and LOAEL of 250 and 500 mg/kg were established for male mice. Female mice had significantly decreased bodyweights in the top dose. A NOAEL and LOAEL of 500 and 1,000 mg/kg were reported for female mice.
  - A 90-day oral toxicity was conducted using male and female F344 rats and $B6C3F_1$ mice (10/sex/group). Rats received doses of 0, 150, 300, 600, 1,200, or 2,400 mg/kg via oral gavage (vehicle: corn oil) 5 days a week for 13 weeks. Mice received doses of 0, 125, 250, 500, 1,000, or 2,000 mg/kg via oral gavage (vehicle: corn oil) 5 days a week for 13 weeks. Increased mortality was observed in the high dose groups of rats. Body weights of male rats were significantly decreased in the top three dose groups. Rough hair coats, lethargy, and excessive lacrimation were observed for all animals at the two highest dose levels. α_{2u}-Globulin nephropathy was reported for all groups of male rats. A NOAEL and LOAEL of 300 and 600 mg/kg were reported for rats based on body weight decreases. Increased mortality, decreased body weights, and decreased activity were reported in the top two dose groups for mice. As a result a NOAEL and LOAEL of 500 and 1,000 mg/kg were established for mice.
- U.S. EPA Undated –
  - A 13- week oral toxicity study was conducted using male rats (strain/number not reported). Rats were administered doses of 0, 2, 5, 10, 30, or 75 mg/kg via oral gavage. An increase in lesions of the kidney was observed at 30 mg/kg and above. Additionally, an increase in relative liver weight with no

histopathological effects was observed at 75 mg/kg and above. Increased liver weight in this study was considered to be due to microsomal induction resulting from physiological adaption by the U.S. EPA (Undated) and the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) assessment report (NICNAS 2002).
    - A 30-day oral toxicity study was conducted using male rats (strain/number not reported). Rats were administered 400 mg/kg of the test substance via oral gavage. Following 30 days of dosing slight increases were observed in relative liver weights, hepatic phospholipid content, and enzyme activity. Additionally, liver and serum cholesterol levels were significantly decreased. No histopathological examinations were conducted.
    - A 26-day oral toxicity was conducted using male rats (strain/number not reported). Rats were administered doses of 0, 75, 150, and 300 mg/kg of the test substance via oral gavage. α_{2u}-Globulin nephropathy was reported for all groups. Increased liver weights were reported in the 300 mg/kg, but no evidence alterations were identified in liver sections in any dose group. As α_{2u}-Globulin nephropathy is not relevant to human being a NOAEL and LOAEL of 150 and 300 mg/kg were established based in increased liver weights. No changes in enzyme activity were reported and no histopathological examinations were conducted.
- The U.S. EPA's Exposure and Risk Assessment on Lower Risk Pesticide Chemicals (Undated) considered the LOAEL of 400 mg/kg from the continuous 30 day study in male rats to be the critical endpoint for risk assessment as various liver effects occurred outside of relative weight changes. Both the 26-day and 13-week oral toxicity studies reported only increased relative liver weights with no histopathological changes. Based on the U.S. EPA evaluation, ToxServices agrees with a NOAEL and LOAEL of 300 and 400 mg/kg for liver effects. Following GHS criteria d-limonene is not classifiable as a systemic toxicant as the LOAEL value is greater than 100 mg/kg.

Neurotoxicity (N)
Group II Score (single dose)(vH, H, M or L): dg
- Not classified as a developmental neurotoxicant (Grandjean and Landrigan 2006).
- Not listed as a potential neurotoxicant on the Red List of Chemicals (CPA 2011d).
- No relevant data were identified.

Group II Score (repeated dose)*(H, M, L): dg
- Not classified as a developmental neurotoxicant (Grandjean and Landrigan 2006).
- Not listed as a potential neurotoxicant on the Red List of Chemicals (CPA 2011d).
- JECFA 1998 –
    - Decreased motor activity was reported in rats following two studies with doses above 1,000 mg/kg. However, it was not clear if this was a result of a general intoxicant effect or a direct effect of d-limonene exposure. Therefore, insufficient data are available to assess this endpoint.

Skin Sensitization (SnS) Group II* Score (H, M or L): H
d-Limonene was assigned a score of High for skin sensitization as some of its oxidation products are known to be potent skin sensitizers.
- JECFA 1998 –
    - d-Limonene is sensitizing when in the oxidized form. d-Limonene was sensitizing to guinea pigs following an open epicutaneous test, maximization test, and Freund's complete adjuvant test.
- ESIS 2000 –
    - Associated with EU Risk Phrase R43 "May cause sensitization by skin contact".
- NICNAS 2002 –
    - Air-oxidized d-limonene is a potent skin sensitizer. The non-oxidized form has been found to be non-sensitizing.
- Karlberg et al. 1992 –
    - It is well known that the air oxidation of d-limonene produces potent skin sensitizers such as the cis- and trans- isomers of limonene oxide (CAS #1195-92-2) and carvone (99-49-0).
- As outlined in CPA (2011b) Section II (3)(Identifying Transformation Products to Assess), d-limonene has been assigned a High score for skin sensitization based on the well-established sensitizing potential of its air-oxidized products.

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

- No relevant data were identified.

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

d-Limonene was assigned a score of High for skin irritation/corrosivity based on being classified as a GHS (2011) category 2 dermal irritant following a primary irritation index rating of 3.5.

- JECFA 1998 –
  - d-Limonene is considered a skin irritant. d-Limonene was ranked 3.5 out of 8 following OECD Guideline 404, which corresponds to GHS (2011) Category 2 for skin irritation as total score is between 2.3 and 4.0. OECD scores chemicals on an 8 point scale, 4 points for severity of erythema and 4 points for severity of oedema. However, no further data were provided to assess the details of this study.
- ESIS 2000 -
  - d-Limonene is irritating to the skin and eyes.
- NICNAS 2002 –
  - d-Limonene is considered a skin irritant. Irritancy was tested following OECD 404 and a primary irritation index of 3.5 out of 8 was reported for rabbits.
- Following GHS (2011) Skin Irritation criteria (Chapter 3.2), a total skin irritation score of 2.3 to 4.0 is classified as a Category 2 skin irritant.

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

d-Limonene was assigned a score of High for eye irritation/corrosivity based on being reported as irritating to the eyes of laboratory animals. A low confidence score was assigned as sufficient study data were not available to assign a GHS categorization.

- JECFA 1998 –
  - d-Limonene caused irritation to the eyes of rabbits.
- ESIS 2000 -
  - d-Limonene is irritating to the skin and eyes.

Ecotoxicity (Ecotox)

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

d-Limonene was assigned a score of Very High for acute aquatic toxicity based on L/EC_{50} values below 1 mg/L and being associated R-Phrase 50/53 (CPA 2011c).

- JECFA 1998; NICNAS 2002 –
  - d-Limonene has a reported LC_{50} value of 0.688 to 38.5 mg/L (fish, 96-hr).
  - d-Limonene has a reported EC_{50} value of 0.73 to 69.6 mg/L (daphnid, 48-hr).
  - d-Limonene has a reported EC_{50} value of 4.08 mg/L (algae, 96-hr).
- ESIS 2000 –
  - Associated with the EU Risk Phrase R50/53 "Very Toxic to Aquatic Organisms, may cause long-term adverse effects in the aquatic environment"

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

d-Limonene was assigned a score of Low for chronic aquatic toxicity based on low water solubility and bioaccumulation and a high volatility.

- Based on the relatively low water solubility (13.8 mg/L) and bioaccumulation potential (BCF = 246 to 262), and high volatility of d-limonene (vapor pressure = 0.19 kPa), chronic aquatic toxicity is not expected from d-limonene, as it will not be present in the water compartment (NICNAS 2002).

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): vL
d-Limonene was assigned a score of Very Low for persistence based on being classified as readily biodegradable following an OECD 301C "Modified MITI Test", as well as having an expected half-life of hours for volatilization from soil, sediment and water.

- U.S. EPA 2012 –
  - d-Limonene was reported as readily biodegradable following sealed vessel biodegradation assay. d-Limonene was depleted beyond detectable limits in 8 days under the tested conditions.
- ESIS 2000 –
  - d-Limonene was reported as inherently biodegradable with 100% degradable in 28 days. No further details were reported.
- NICNAS 2002 –
  - d-Limonene was found to be readily biodegradable following an OECD 301C "Modified MITI Test", and almost completely disappeared following an OECD 303A "Simulation Test – Aerobic Sewage Treatment: Coupled Units test". However, NICNAS authors reported that these tests are not suitable for highly volatile substances such a d-limonene as a large portion of the removal was likely due to volatilization. Limonene is not expected to be persistent in the aquatic environment. The estimated half-life for volatilization to the atmosphere from a model river is 3.4 hours. Additionally, limonene is expected to volatilize from soil and sediments. Once in the atmosphere d-limonene is expected to rapidly undergo gas-phase reactions.

Bioaccumulation (B) Score (vH, H, M, L, or vL): L
d-Limonene was assigned a score of Low for bioaccumulation based on having a BCF between 100 and 500.

- NICNAS 2002 –
  - Limonene has a calculated BCF of 246 to 262. Chemicals with a BCF of < 500 are considered low for bioaccumulation.

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M or L): L
d-Limonene was assigned a score of Low for reactivity based on not being classified as a GHS (2011) explosive or reactive compound.

- ESIS 2000 –
  - d-Limonene is not an oxidizing compound.
- d-Limonene is not classifiable as a GHS explosive or reactive compound.

Flammability (F) Score (vH, H, M or L): M
d-Limonene was assigned a score of Moderate for flammability based on having a flashpoint of 47°C and being classified as a GHS (2011) Category 3 flammable liquid.

- ESIS 2000 –
  - d-Limonene has a flashpoint of 47°C, indicating that it is a moderately flammable compound and is classified as a GHS Category 3 flammable liquid.

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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

(Cr) Corrosion/ Irritation (Skin/ Eye)

(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: Pharos List Translator Results

D-LIMONENE

CAS RN: 5989-27-5

Direct Chemical and Compound Hazard Quickscreen — Detailed Hazard Listings

Very High Hazard of...

ACUTE AQUATIC	EC - CLP/GHS Hazard Statements (EU H-Statements): H400 - Aquatic Acute 1 - Very toxic to aquatic life - GreenScreen Benchmark Unspecified - occupational hazard only

High Hazard of...

PBT	EC/Oslo-Paris Conv - Priority PBTs & EDs & equivalent concern (OSPAR): PBT - (Substance of Possible Concern) - GreenScreen Benchmark 1
SKIN IRRITATION	EC - CLP/GHS Hazard Statements (EU H-Statements): H315 Causes skin irritation - GreenScreen Benchmark Unspecified {and 1 other}
CHRON AQUATIC	EC - CLP/GHS Hazard Statements (EU H-Statements): H410 - Aquatic Chronic 1 - Very toxic to aquatic life with long lasting effects - GreenScreen Possible Benchmark 1 - occupational hazard only

Medium Hazard of...

FLAMMABLE	EC - CLP/GHS Hazard Statements (EU H-Statements): H226 Flammable liquid and vapour - GreenScreen Benchmark Unspecified - occupational hazard only

Low Hazard of...

RESPIRATORY	AOEC - Asthmagens (AOEC): Suspected asthmagen (R - reviewed by AEOC and does not meet sensitizer or RADS criteria)

Potential concern...

CANCER	Intnl Agency for Rsrch on Cancer - Cancer Monographs (IARC): Not classifiable as to carcinogenicity (Group 3: Agent is not classifiable as to its carcinogenicity to humans) - GreenScreen Benchmark Unspecified
RESTRICTED LIST	German FEA - Substances Hazardous to Waters (VwVwS): Hazard to Waters (Water Hazard Class 2) - GreenScreen Possible Benchmark 1

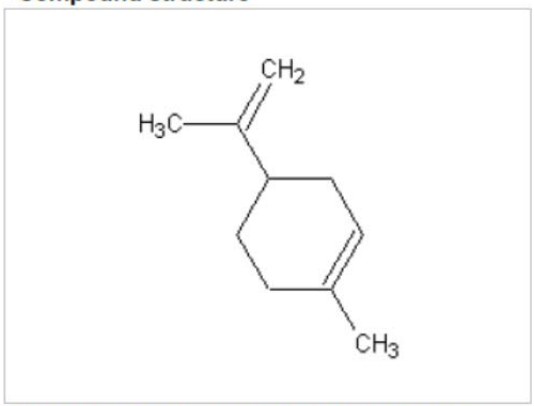
This chemical is NOT present on the hazard lists scanned for the following health and ecotoxicity endpoints...

DEVELOPMENTAL	REPRODUCTIVE	ENDOCRINE	GENE MUTATION	NEUROTOXICITY
MAMMALIAN	EYE IRRITATION	SKIN SENSITIZE	ORGAN TOXICANT	TERRESTRIAL
REACTIVE	GLOBAL WARMING	OZONE DEPLETION		

APPENDIX C: ACD/I-Lab Results

ACD/Labs
I-Lab 2.0 - ilab.acdlabs.com
Wednesday 15th of August 2012 09:07:00 AM. Algorithm Version: v5.0.0.184

Compound structure



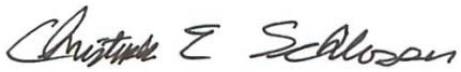
Experimental Values for Similar Structures

No binding to Estrogen Receptor alpha (LogRBA<-3)

Probability of Estrogen Receptor Binding:
LogRBA > -3: 0.09
LogRBA > 0: 0

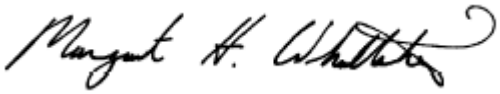
Authorized Reviewers

d-Limonene GreenScreen™ Evaluation Prepared By:



Christopher E. Schlosser, M.F.S.
Associate Toxicologist
ToxServices LLC

d-Limonene GreenScreen™ Evaluation QC'd By:



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