Imidacloprid (CAS# 138261-41-3) GreenScreen® for Safer Chemicals (GreenScreen®) Assessment

Prepared for:

Natural Resource Defense Council

January 7, 2016

TOXSERVICES
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TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

GreenScreen® Executive Summary for Imidacloprid (CAS #138261-41-3)

Imidacloprid is a chemical that functions as a neonicotinoid insecticide.

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

- Benchmark **1c**
  - Very High Persistence-P + Very High Group II Human Toxicity (systemic toxicity (single dose)-STs and neurotoxicity (single dose)-Ns)
  - Very High Persistence-P + High Group II* Human Toxicity (neurotoxicity (repeated dose)-Nr*)
  - Very High Persistence-P + Very High Ecotoxicity (acute aquatic toxicity-AA, chronic aquatic toxicity-CA, and acute foliar invertebrates toxicity-AFI[1])

A data gap (DG) exists for respiratory sensitization-SnR*. As outlined in CPA (2013) Section 12.2 (Step 8 – Conduct a Data Gap Analysis to assign a final Benchmark score), imidacloprid meets requirements for a GreenScreen® Benchmark Score of 1 despite the hazard data gap. In a worst-case scenario, if imidacloprid were assigned a High score for the data gap SnR*, it would still be categorized as a Benchmark 1 Chemical.

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 Imidacloprid

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	ATV	AFI	P	B	Rx	F
						single	repeated*	single	repeated*												
M	*L*	*M*	**M**	*M*	*H*	**vH**	**M**	**vH**	*H*	**L**	DG	**L**	**L**	**vH**	**vH**	**H**	**H**	*vH*	**vL**	*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.

[1] Because the maximum score for acute foliar invertebrates and pollinators toxicity per DfE criteria is a High, a score of High was considered equivalent to a Very High for this endpoint for benchmarking purposes

GreenScreen® Assessment for Imidacloprid (CAS #138261-41-3)

Method Version: GreenScreen® Version 1.2[2]
Assessment Type[3]: Certified

Chemical Name: Imidacloprid

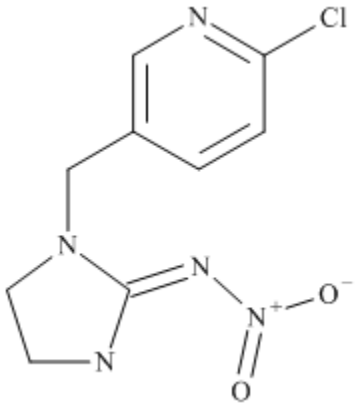
CAS Number: 138261-41-3

GreenScreen® Assessment Prepared By:
Name: Jennifer Rutkiewicz, Ph.D.
Title: Toxicologist
Organization: ToxServices LLC
Date: September 15, 2015 (updated December 10, 2015, January 6, 2016)
Assessor Type: Licensed GreenScreen® Profiler

Quality Control Performed By:
Name: Bingxuan Wang, Ph.D.
Title: Toxicologist
Organization: ToxServices LLC
Date: October 13, 2015 (updated December 11, 2015, January 7, 2016)

Confirm application of the *de minimus* rule[4]: N/A

Chemical Structure(s):



Also called: 1-((6-Chloro-3-pyridinyl)methyl)-N-nitro-2-imidazolidinimine, 1-((6-Chloro-3-pyridyl)methyl)-N-nitro-2-imidazolidinimine, Admire, Admire 2F, Admire Pro, Advantage Flea Adulticide, Advise, AE-F 106464-00GR01B0, AEF 106464, AGST 03001, Alias, Alias 2F, Baimieshi, BAY-NTN 33893, Bayer Advanced Season-Long Grub control, CCRIS 9318, Comodor, Confidate, Confidor, Confidor 200 O-TEQ, Confidor 200 SL, Confidor 200SL, Confidor 240 O-TEQ, Confidor 70WG, Confidor SL, CoreTect, Couraze, Couraze Max, CP 1, EPA Pesticide Chemical Code 129099, Gaucho, Gaucho 600FS, Gaucho Grande, Genesis, Grubex, Hachikusan, HSDB 7373, Imicide, Imidacloprid, Kohinor, Macho Max, Mallet 2F, Marathon, Marathon II, Merit,

[2] Use GreenScreen® Assessment Procedure (Guidance) V1.2
[3] 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)
[4] 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

Merit (insecticide), Meritgreen, NTN 33893, NTN 33893-240FS, Premis, Premise. Premise (insecticide). Premise 75, Preventol, Preventol TM, ProAgro, Provado, Senator (neonicotinoid), Trimax Pro, UNII-3BN7M937V8. (ChemIDplus 2016)

Chemical Structure(s) of Chemical Surrogates Used in the GreenScreen®:
No surrogates were used in the assessment. ToxServices conducted a ChemIDplus structural similarity search to identify a surrogate for the respiratory sensitization data gap, but no suitable surrogates with data were identified.

Identify Applications/Functional Uses: (NPIC 2010)
1. Insecticide
2. Flea protection for pets

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

- Benchmark **1c**
  - Very High Persistence-P + Very High Group II Human Toxicity (systemic toxicity (single dose)-STs and neurotoxicity (single dose)-Ns)
  - Very High Persistence-P + High Group II* Human Toxicity (neurotoxicity (repeated dose)-Nr*)
  - Very High Persistence-P + Very High Ecotoxicity (acute aquatic toxicity-AA, chronic aquatic toxicity-CA, and acute foliar invertebrates toxicity-AFI[6])

A data gap (DG) exists for respiratory sensitization-SnR*. As outlined in CPA (2013) Section 12.2 (Step 8 – Conduct a Data Gap Analysis to assign a final Benchmark score), imidacloprid meets requirements for a GreenScreen® Benchmark Score of 1 despite the hazard data gap. In a worst-case scenario, if imidacloprid were assigned a High score for the data gap SnR*, it would still be categorized as a Benchmark 1 Chemical.

Figure 1: GreenScreen® Hazard Ratings for Imidacloprid

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	ATV	AFI	P	B	Rx	F
						single	repeated*	single	repeated*												
M	*L*	*M*	**M**	*M*	*H*	**vH**	**M**	**vH**	*H*	**L**	DG	**L**	**L**	**vH**	**vH**	**H**	**H**	*vH*	**vL**	*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.

[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.
[6] Because the maximum score for acute foliar invertebrates and pollinators toxicity per DfE criteria is a High, a score of High was considered equivalent to a Very High for this endpoint for benchmarking purposes

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**[7].

Imidacloprid is not expected to be readily biodegradable. However, it is expected to be at least partially degradable in the environment, producing transformation products in soil. It is expected to photodegrade in water as well as undergo slow hydrolysis. The resulting organic moieties are 1-[(6-chloro-3-pyridinyl)methyl]-2-imidazolidinone (imidacloprid urea), 6-chloronicotinic acid, 6-hydroxynicotinic acid, 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-1H-imidazol-2-amine (imidacloprid-guanidine), 6-chloro-nicotinaldehyde, 6-chloro-N-methylnicotinacidamide and 6-chloro-3-pyridyl-methylethylendiamine (Bacey 2000, Fossen 2006). None of the transformation products are present in the Pharos database; however, because imidacloprid is a Benchmark 1 chemical its score is not impacted by the transformation products.

Table 1: Transformation Product Summary Table

Functional Use	Life Cycle Stage	Transformation Pathway	Transformation Products	CAS #	Feasible and Relevant?	GreenScreen® List Translator Score or Benchmark Score[8,9]
Insecticide	In use and disposal	Soil degradation, hydrolysis, photolysis in water	1-[(6-chloro-3-pyridinyl)methyl]-2-imidazolidinone	120868-66-8	Y	Not in the Pharos database
Insecticide	In use and disposal	Soil degradation	6-chloronicotinic acid	5326-23-8	Y	Not in the Pharos database
Insecticide	In use and disposal	Soil degradation, photolysis in water	6-hydroxynicotinic acid	5006-66-6	Y	Not in the Pharos database
Insecticide	In use and disposal	Soil degradation, hydrolysis	1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-1H-imidazol-2-amine	Unknown CAS	Y	Not in the Pharos database
Insecticide	In use and disposal	Photolysis in water	6-chloro-nicotinaldehyde	23100-12-1	Y	Not in the Pharos database
Insecticide	In use and disposal	Photolysis in water	6-chloro-N-methylnicotinacidamide	Unknown CAS	Y	Not in the Pharos database
Insecticide	In use and disposal	Photolysis in water	6-chloro-3-pyridyl-methylethylendiamine	101990-44-7	Y	Not in the Pharos database

Introduction

Imidacloprid is an organic compound that is used as a pesticide. It is an agonist of the nicotinic acetylcholine receptor and functions as a pesticide through interference with neurotransmission in the nicotinic cholinergic system by causing prolonged activation and desensitization of the nicotinic cholinergic receptor. It is more selective towards insects rather than mammals (Cal EPA 2006). It is used to control sucking insects and some chewing insects. It can be topically applied to pets to control fleas, as well as to structures, crops, soil, and as seed treatment (NPIC 2010). Imidacloprid

[7] 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.
[8] The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to screen for GreenScreen® benchmark 1 chemicals (CPA 2012a). Pharos (Pharos 2016) is an online list-searching tool that is used to screen chemicals against the lists in the List Translator electronically.
[9] The way you conduct assessments for transformation products depends on the Benchmark Score of the parent chemical (See Guidance).

is used to protect seedlings from early-season root and leaf-feeding pests, as well as via later season foliar treatments (Morrissey 2015).

ToxServices assessed imidacloprid 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 2016) is an online list-searching tool that is used to screen chemicals against the List Translator electronically. It checks all of the lists in the List Translator with the exception of the U.S. Department of Transportation (U.S. DOT) lists (U.S. DOT 2008a,b) and these should be checked separately in conjunction with running the Pharos query. The output indicates benchmark or possible benchmark scores for each human health and environmental endpoint. The output for Imidacloprid can be found in Appendix B and a summary of the results can be found below:

Mammalian Toxicity

EC - Risk Phrases - R22: Harmful if swallowed
EC - CLP/GHS Hazard Statements - H302 Harmful if swallowed
New Zealand HSNO/GHS - 6.1C (oral) - Acutely toxic (Category 3)
New Zealand HSNO/GHS - 6.9B (oral) - Harmful to human target organs or systems (Category 2)

Neurotoxicity

Lancet - Grandjean & Landrigan Neurotoxic Chemicals - Known to be neurotoxic in man (Grandjean & Landrigan 2014)

Acute aquatic toxicity

EC - CLP/GHS Hazard Statements - H400 - Aquatic Acute 1 - Very toxic to aquatic life
EC - Risk Phrases – R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
New Zealand HSNO/GHS - 9.1A (crustacean) - Very ecotoxic in the aquatic environment (Category 1)

Chronic aquatic toxicity

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.
New Zealand HSNO/GHS - 9.1C (fish) - Harmful in the aquatic environment (Category 3)

Terrestrial toxicity

New Zealand HSNO/GHS - 9.2A - Very ecotoxic in the soil environment
New Zealand HSNO/GHS - 9.3A - Very ecotoxic to terrestrial vertebrates
New Zealand HSNO/GHS - 9.4A - Very ecotoxic to terrestrial invertebrates

When appropriate, HSNO hazard classifications in New Zealand were translated to the corresponding GHS hazard classification (NZ EPA 2012).

Physicochemical Properties of Imidacloprid

Imidacloprid is light yellow powder at room temperature and is moderately soluble in water. Its vapor pressure indicates that it will exist mostly in the solid phase and its low partition coefficient indicates it has low potential to bioaccumulate.

Table 2: Physical and Chemical Properties of Imidacloprid (CAS #138261-41-3)

Property	Value	Reference
Molecular formula	C9-H10-Cl-N5-O2	ChemIDplus 2016
SMILES Notation	c1nc(Cl)ccc1CN1\C(=N\[N+]([O-])=O)NCC1	ChemIDplus 2016
Molecular weight	255.64	ChemIDplus 2016
Physical state	Solid	Cal EPA 2006
Appearance	Light yellow powder	Cal EPA 2006
Melting point	144°C (experimental)	ChemIDplus 2016
Vapor pressure	$1.5x10^{-9}$ mmHg at 20ºC	Cal EPA 2006
Water solubility	610 mg/L at 20°C (experimental)	ChemIDplus 2016
Dissociation constant	pKa1 = 1.56 pKa2 = 11.12	PubChem 2016
Density/specific gravity	1.54 g/cm^3 at 23°C	Cal EPA 2006
Partition coefficient	log K_{ow} = 0.57 (experimental); log K_{ow} = 3.7	ChemIDplus 2016; Bacey 2000

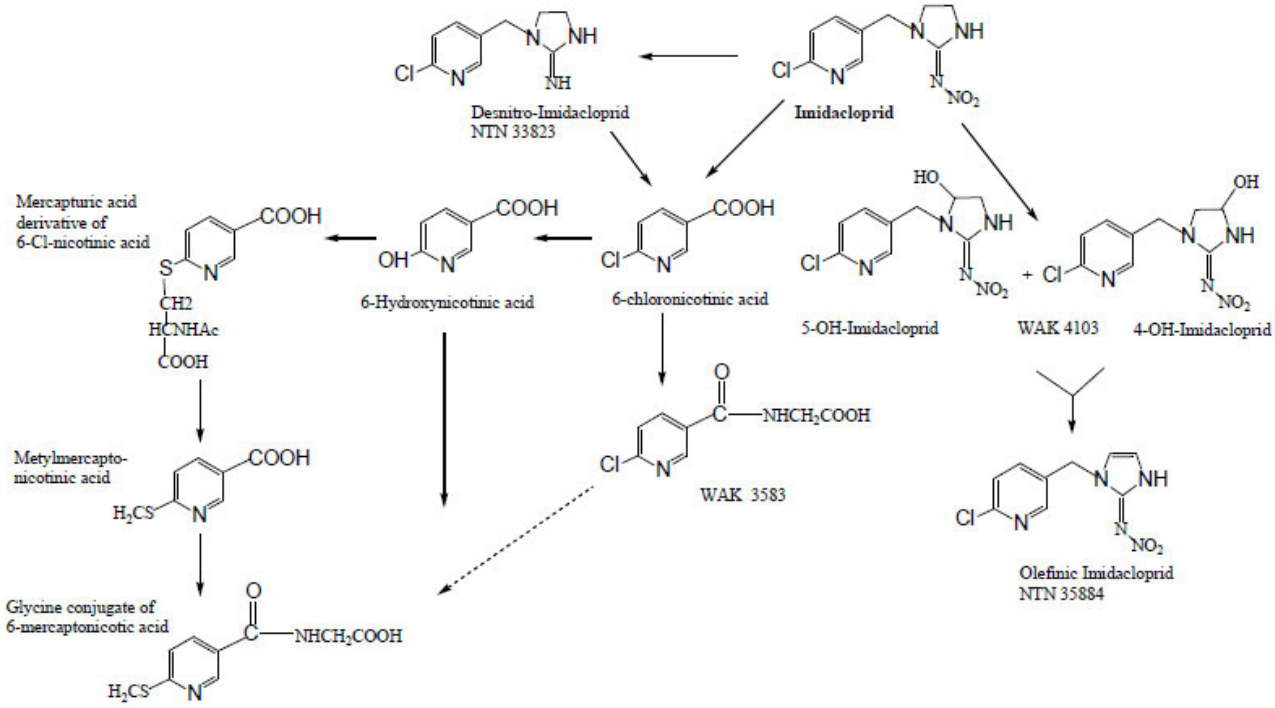
Toxicokinetics

Oral administration studies in rats indicate that radiolabeled imidacloprid is well absorbed, distributed, and eliminated following oral exposure. When rats were orally administered radiolabeled imidacloprid, 95% of the administered dose was absorbed with an estimated half-life of 35 minutes. Plasma concentrations peaked within 2.5 hours. The substance was primarily distributed in the kidney and liver, and was least distributed in the brain (SRC 2005). Similar results were seen in another oral study, which demonstrated more than 90% absorption, based on renal excretion, following oral administration; peak plasma concentrations occurred after 1 hour. Again, the highest concentrations were detected in the liver at 48 hours post administration. Another study in rats that was designed primary to evaluate the distribution of imidacloprid following intravenous or oral administration found the substance distributed rapidly to all tissues, including the skin, aorta, thyroid, and adrenal glands, regardless of the route of administration. Limited amounts were detected in fatty tissues or the central nervous system. Human suicide cases also indicate that imidacloprid is well absorbed following oral exposure, with distribution to the blood, kidneys, liver, and lung (SRC 2005).

The two primary proposed metabolic pathways for imidacloprid are oxidative cleavage to imidazolidine, which is directly excreted in the urine, and 6-chloronicotinic acid. This metabolite is further metabolized via glutathione conjugation and glycine conjugation. The second proposed metabolic pathway involves hydroxylation followed by elimination of water to form an unsaturated olefinic imidacloprid metabolite (Cal EPA 2006). The proposed metabolic pathway is presented in Figure 2.

Excretion of imidacloprid is primarily through the urine, with smaller amounts of the administered dose eliminated through the feces. Of an intravenous administration of 1 mg/kg radiolabeled imidacloprid to rats, 92% was excreted in urine and feces in a 4:1 ratio (WHO 2001) within 48 hours. In a previously described oral study, 90% was found to be excreted renally within 48 hours (SRC 2005). Overall half-lives from oral studies in rats report excretion half-lives ranging from 26-116 hours (Cal EPA 2006).

Figure 2: Proposed Metabolic Pathway for Imidacloprid



(from Cal EPA 2006)

Hazard Classification Summary Section:

<u>Group I Human Health Effects (Group I Human)</u>

Carcinogenicity (C) Score (H, M, or L): *M*
Imidacloprid was assigned a score of Moderate for carcinogenicity based on an increased incidence in a rare tumor in a chronic oral study in rats. GreenScreen® criteria classify chemicals as a Moderate hazard for carcinogenicity when there is limited or marginal evidence of carcinogenicity (CPA 2012a). Confidence in the score is reduced because the increased incidence of the rare tumor in rats was not statistically significant.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Cal EPA 2006, SRC 2005
  - *Oral*: In a 2-year chronic toxicity and carcinogenicity study, Imidacloprid (94.3% active ingredient) was administered to Wistar rats (50/sex/group) in the diet at concentrations of 0, 100, 300, and 900 ppm (0, 5.7, 17, or 51 mg/kg/day in males and 0, 7.6, 25, or 73

mg/kg/day in females). Interim examinations were conducted on an additional 10 rats/sex/dose after 1 year after treatment. A supplemental study to determine the maximum tolerated dose (MTD) was conducted on 50 rats/sex/dose that were either used as controls or administered imidacloprid at a concentration of 1,800 ppm (103 mg/kg/day in males and 144 mg/kg/day in females) for two years. Neoplasms were reported in the liver and thyroid gland, though no dose-related increase in neoplasms was reported in the thyroid gland. Tumors in the liver were found only in males at 1,800 ppm. Authors considered the incidences of hepatic adenomas and carcinomas to be within the historical range for the tumors. Two rats had cholangiocellular carcinomas, which are rarely seen in aging rats. Authors cite another study in which 0 out of 1,270 control male rats developed the tumor. In a previous study, 827 spontaneous tumors occurred in 375 Wistar rats but none of them were the rare cholangiocellular carcinomas. Based on these reports, study authors concluded that the incidence for cholangiocellular carcinoma in this study (2/50 males) was outside the historical range. Nonetheless, the incidence was not statistically significantly different from concurrent controls. Study authors concluded that there was not sufficient evidence to conclude that imidacloprid was carcinogenic in rats.

  - *Oral*: In a 24-month chronic toxicity and carcinogenicity studies, imidacloprid (95.3% active ingredient) was administered to B6C3F1 mice (50/sex/group) in the diet at concentrations of 0, 100, 330, or 1,000 ppm in one study and 0 and 2,000 ppm in the other. An interim evaluation was conducted on an additional 10 mice/sex/dose at 12 months. Reported average daily doses were 0, 20, 66, 208, or 414 mg/kg/day in males and 0, 30, 104, 274, and 424 mg/kg/day in females. Incidences of tumors in all dose-groups were similar to that for control animals. Authors concluded that there was no evidence for carcinogenic potential of imidacloprid from this study.

- Greenop et al. 2013
  - *Epidemiological*: An Australian case-control study investigated the risk of developing childhood brain tumors associated with pesticide exposure before pregnancy, during pregnancy and during childhood. Exposure data were collected by written questionnaires and telephone interviews. Authors found the odds ratios for professional pest control treatments in the home in the year before the index pregnancy, during pregnancy and after the child's birth to be 1.54, 1.52 and 1.04, respectively. The odds ratios for treatment exclusively before pregnancy and during pregnancy were 1.90 and 1.02, respectively. The odd ratios for prenatal home pesticide exposure were increased for low and high grade gliomas. Authors concluded that results suggest that preconception pesticide exposure, and possibly exposure during pregnancy, is associated with an increased childhood brain tumor risk.

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of Moderate was assigned. Although imidacloprid is classified as a "Group E" carcinogen (no evidence of carcinogenicity in humans") (U.S. EPA 1999), ToxServices notes that classifications determined by the U.S. EPA Office of Pesticide Programs are not considered to be authoritative under GreenScreen criteria. The epidemiological study suggesting that preconception pesticide exposure is associated with childhood brain tumors was not weighed heavily in the assessment, as the study considered only overall pesticide treatment and did not differentiate between exposures to different pesticides. There were no effects on tumor incidences in the chronic study in mice. However, an increased incidence

(although not statistically significant) of a rare tumor was found in the rat study. The significance of this observation was unclear, but authors noted that this rare cancer had not been observed in historical controls. Study authors concluded that there was insufficient evidence to conclude that imidacloprid is carcinogenic based on this study. However, because GreenScreen criteria specify score of Moderate when there is limited or marginal evidence of carcinogenicity, a score of Moderate was assigned. ToxServices reduced the confidence level as the increase in tumor incidence was not statistically significant.

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

Imidacloprid was assigned a score of Low for mutagenicity/genotoxicity based on negative results in several bacterial and mammalian cell mutagenicity studies and well conducted *in vivo* clastogenicity assays. GreenScreen® criteria classify chemicals as a Low hazard for mutagenicity/genotoxicity when adequate data are available and are negative for both gene mutations and chromosome aberrations, and the chemical is not present on authoritative or screening lists (CPA 2012a). Confidence in the score is reduced due to conflicting results in some less well reported studies.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Cal EPA 2006
  - *In vivo*: Imidacloprid was negative in a mouse germ cell cytogenetic assay that was conducted in male mice (5/sex/dose) that were administered a single dose of 80 mg/kg imidacloprid (94.1% purity) via gavage and were sacrificed after 6, 24, or 48 hours for the evaluation of spermatogonia. There were no increases in chromosome aberrations at any dose, and positive controls produced the expected results.
  - *In vivo*: Imidacloprid was negative in a micronucleus test that was conducted in male mice (5/sex/dose) that were administered a single dose of 80 mg/kg via gavage and were sacrificed after 24, 48, or 72 hours. No additional details were provided.
- CCRIS 2011
  - *In vitro:* In an Ames bacterial reverse mutation assay, imidacloprid produced mixed results for mutagenicity in *Salmonella typhimurium* strains TA 98 and TA100. Cells were exposed to imidacloprid at doses of 25 – 100 µL/plate with and without metabolic activation in the form of Aroclor 1254 induced rat liver S9 mix. Imidacloprid was negative in strain TA100; imidacloprid was positive in strain TA98. No additional details were provided.
  - *In vitro:* In a micronucleus assay, imidacloprid was found negative for clastogenicity in human lymphocytes. Cells were exposed to imidacloprid at doses of 0.1, 1, 5, 10, 50 and 100 µg/mL without metabolic activation for 41 hours. No assays were conducted with metabolic activation. No additional details were provided.
  - *In vitro:* In a micronucleus assay, imidacloprid produced mixed results for clastogenicity in human peripheral lymphocytes. Cells were exposed to imidacloprid at concentrations of 0.2, 2 and 20 µM with and without metabolic activation in the form of rat liver S9 mix for 24 hours. Imidacloprid was positive for micronucleus formation with metabolic activation at dose 20 µM; imidacloprid was negative for micronucleus formation at all doses without metabolic activation and at the two low doses (0.2 and 2 µM) with metabolic activation. No additional details were provided.
  - *In vivo:* In a chromosome aberration and micronucleus assay conducted in Wistar rats, imidacloprid was administered at doses of 0, 50 and 100 mg/kg orally in the diet for 15

weeks. Imidacloprid was positive for structural changes and micronuclei in bone marrow polychromatic erythrocytes. No additional details were provided.

- *In vivo:* Imidacloprid was evaluated in a micronucleus assay in male Wistar rats. Animals were exposed to imidacloprid at concentrations 100, 200 and 300 mg/kg. Animals were sacrificed 24 hours after treatment and bone marrow polychromatic erythrocytes were evaluated for micronucleus formation. Imidacloprid was positive for clastogenicity at the highest dose tested (300 mg/kg), lower doses were negative for clastogenicity. No additional details were provided.

- Bagri et al. 2015
  - *In vivo:* In a sperm head abnormality (SHA) assay male Swiss albino mice (6/dose) were orally administered 5.5, 11 or 22 mg/kg/day imidacloprid (>98% purity) daily for a period of 7, 14 or 28 days. The control group received 3% aqueous gum acacia at a dose of 1 mL/100 g. Animals were sacrificed on the 8^{th}, 15^{th}, and 29^{th} days of the experiment and the caudate epididymides were dissected out for evaluation. For each animal, 200 sperm were assessed for morphological abnormalities of the sperm head. Among normal head sperm, multiple sperm presented morphological abnormalities, namely banana-shaped, prism-shaped head, head with blunt hook, curved hook head, wrong-angled hook, apical-hooked, hookless head, amorphous-shaped head and pin-headed sperm. In the 7 day experiment, there was no statistically significant dose-dependent increase in SHAs. In the 14 day experiment, there were statistically significant increases in SHAs in all dose groups compared to controls. In the 28 day experiment, dose-dependent increases in SHAs were observed in all dose groups compared to controls and between each other. Thus, authors concluded the increase in SHAs was dose and exposure-duration dependent. Authors concluded that imidacloprid was positive for a mutagenic effect under the conditions of this assay.
  - *In vivo:* In a dominant lethal test (DLT) male Swiss albino mice (6/dose) were orally administered 5.5, 11 or 22 mg/kg/day imidacloprid (>98% purity) daily for 28 days. The control group received 3% aqueous gum acacia at a dose rate of 1 mL/100 g. Males from all dose groups were caged with two sexually mature virgin females for one week 1, 3 and 6 weeks after the end of the treatment. Females were sacrificed on the 17^{th} day after the introduction of the male and uteri were dissected out for evaluation of presence of implementations (total number of large-sized normal fetus implants and reduced sized mole implants in each pregnancy). A decrease in the number of fetus (live implant) and an increase in number of moles (dead implant) were observed in all dose groups compared to controls. The only statistically significant increase in number of moles was recorded in females that were mated with males 6 weeks after treatment with the highest dose (22 mg/kg/day). Authors concluded imidacloprid demonstrated a mutagenic effect on spermatogonial stage of gametogenesis process and that imidacloprid was positive for dominant lethal mutations under the conditions of this study.

- U.S. EPA 1993

  Note: studies that were considered by U.S. EPA to be unreliable were not evaluated.
  - *In vitro:* An Ames bacterial reverse mutation assay (GLP-compliant) was conducted in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA157 and *Escherichia coli* strain WP2uvrA. Cells were exposed to imidacloprid (93.7% purity; DMSO solvent) in concentrations up to 5,000 µg/plate with and without metabolic activation in the form of phenobarbital/5,6-benzoflavone induced microsomal fraction rat liver with NADP(H) generating cofactors. Test cultures were exposed for 48 hours and performed in

replicates of three. No increases in revertants were observed in any strain at any dose. Authors concluded imidacloprid to be non-mutagenic under the conditions of the assay.

- *In vitro:* Imidacloprid was evaluated in a GLP-compliant bacterial reverse mutation assay (Ames test) conducted in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537. Cells were exposed to imidacloprid (95% purity; DMSO solvent) at concentrations up to 12,500 µg/plate with and without metabolic activation in the form of Aroclor 1254 induced microsomal rat liver S9 fraction. No increases in revertant counts were observed in any strain at any dose. Authors concluded imidacloprid to be negative for mutagenicity under the conditions of the assay.
- *In vitro:* In a GLP-compliant mammalian cell gene mutation assay Chinese hamster ovary (CHO) cells were exposed to imidacloprid (95.2% purity; DMSO solvent) at concentrations of 1.25 – 125 µg/mL without metabolic activation and concentrations of 90 – 1,222 µg/mL with metabolic activation in the form of Aroclor 1254 induced rat liver S9 fraction for five hours. Authors concluded imidacloprid to be negative for inducing forward mutation at the hypoxanthine-guanine phosphoribosyl transferase locus of CHO cells exposed *in vitro* under the conditions of the assay.
- *In vitro:* Imidacloprid was evaluated in a GLP-compliant chromosome aberration test conducted in primary human lymphocytes. Cells were exposed to imidacloprid (95.2% purity; DMSO solvent) at doses up to 500 µg/mL without metabolic activation and doses up to 1,300 µg/mL with metabolic activation in the form of Aroclor 1254 induced microsomal rat liver S9 fraction. Increases in aberrations were observed both with and without metabolic activation. Authors concluded imidacloprid to be positive for clastogenicity without metabolic activation and weakly positive for clastogenicity with metabolic activation.
- *In vitro:* Imidacloprid was evaluated in a GLP-compliant sister chromatid exchange assay in Chinese hamster ovary cells. Cells were exposed to imidacloprid (95.2% purity; DMSO solvent) at doses of 25 – 5,000 µg/mL with and without metabolic activation in the form of Aroclor 1254 induced microsomal rat liver S9 fraction. Cytotoxicity was evident at 5,000 µg/mL with and without metabolic activation. A significant increase in SCE frequencies was observed at doses of 500 µg/mL (first experiment) and 250 µg/mL (second experiment) and above without activation and at doses 2,000 µg/mL and above with metabolic activation. Authors concluded that imidacloprid was positive for mutagenicity at cytotoxic concentrations.
- *In vitro:* Imidacloprid was evaluated in a GLP-compliant sister chromatid exchange assay in Chinese hamster ovary cells. Cells were exposed to imidacloprid (95.2% purity; DMSO solvent) at doses of 25, 50, 100, 200, and 400 µg/mL without metabolic activation and 157, 313, 625, and 1,250 µg/mL with metabolic activation. Cytotoxicity was observed at 400 µg/mL. In one assay, there was a significant increase in SCE at 200 µg/mL without metabolic activation, but the increase was within the normal background range. There were no additional increases in SCE at any doses and authors concluded that imidacloprid was negative under the conditions of the assay.
- *In vitro:* Imidacloprid was evaluated in a GLP-compliant unscheduled DNA synthesis assay conducted in male F-344 rat primary hepatocytes. Cells were exposed to imidacloprid (95.2% purity; DMSO solvent) at concentrations of 5 – 1,000 µg/mL. Cytotoxicity was evident at 1,000 µg/mL. No increase in UDS was observed at any dose. Authors concluded imidacloprid to be negative for inducing UDS in rat hepatocytes under the conditions of the assay.

- *In vitro*: Imidacloprid (95.3% purity) was negative for mitotic recombination in a GLP-compliant assay in *Saccharomyces cerevisiae* yeast cells when tested at concentrations of 5,000-10,000 µg/mL (precipitating doses).
- *In vitro*: Imidacloprid (95.3% purity) was negative for DNA-damaging effects in a GLP-compliant assay in *Bacillus subtilis* when tested at doses up to 5,000 µg/disc (solubility limit) with and without metabolic activation.
- *In vivo:* Imidacloprid was evaluated in a GLP-compliant chromosome aberration test conducted in Chinese hamsters. Animals (5/sex) were orally administered imidacloprid (94.6% purity; 0.5% aqueous cremophor emulsion) at single doses of 2,000 mg/kg and sacrificed 6, 24 and 48 hours later. Femoral bone marrow was evaluated for chromosome aberrations. There was no increase in chromosome damage in exposed animals. Authors concluded imidacloprid to be not clastogenic under the conditions of the assay.
- *In vivo:* Imidacloprid was evaluated in a GLP-compliant sister chromatid exchange (SCE) assay conducted in male and female Chinese hamsters. Animals (5/sex/dose) were exposed to imidacloprid (95% purity; 0.5% aqueous cremophor) by oral gavage at doses of 0, 500, 1,000 and 2,000 mg/kg. Cytotoxicity was evident at 1,000 and 2,000 mg/kg. No increase in SCE was observed in the femoral bone marrow of any animal at any dose. Authors concluded imidacloprid to be negative for genotoxicity under the conditions of the assay.

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of Low was assigned. Imidacloprid was primarily negative in *in vitro* gene mutation assays in bacterial and mammalian cells. Positive results were seen in some *in vitro* chromosome aberration and sister chromatid exchanges assays, mostly at cytotoxic doses. With the exception of studies that were poorly reported on CCRIS, *in vivo* chromosome aberration and micronucleus assays were negative. Imidacloprid was positive in a sperm head abnormality assay in mice, but this assay is not a standard genotoxicity assay and available evidence suggests that induction of sperm shape abnormalities indicates physiological damage rather than mutational damage to germ cells (NRC 1989). Positive results were reported in the concurrent dominant lethal assay conducted by the same authors, but this study is of limited reliability due to numerous deviations from guideline procedures (no positive control was used, males were not mated a minimum of 8 times, number of females mated was not sufficient to provide at least 400 total implants, pre- and post-implantation loss were not determined separately to calculate the dominant lethal factor, statistical procedures are not well described and it is not clear that they followed recommendations to consider the male as the experimental unit (OECD 2015)). Therefore this study was not weighed heavily in the assessment. An *in vivo* assay in mice saw no relationship between oral imidacloprid exposure and chromosome aberrations in the spermatogonia. Mixed results were seen in *in vitro* SCE assays (positive at cytotoxic doses), but an *in vivo* assay was negative. A score of Low was assigned based on negative results in gene mutation studies and the most well conducted and reported *in vivo* SCE, micronucleus, and chromosome aberration studies. This is consistent with Cal EPA's conclusion that imidacloprid does not show a clear genotoxic potential (Cal EPA 2006). Confidence in the score is reduced due to conflicting results in some less well reported studies.

Reproductive Toxicity (R) Score (H, M, or L): *M*
Imidacloprid was assigned a score of Moderate for reproductive toxicity based on evidence of testicular degeneration in oral studies of rats, dogs, and rabbits. GreenScreen® criteria classify

chemicals as a Moderate hazard for reproductive toxicity when there is limited or marginal evidence of reproductive toxicity in animals (CPA 2012a). Confidence in the score is reduced because a mating study has only been conducted in rats.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists
    - *Screening:* Not present on any screening lists
- Kapoor et al. 2011
    - *Oral*: In a reproductive toxicity study, female Wistar rats (10/dose) received 0, 5, 10 or 20 mg/kg/day imidacloprid (96% purity) daily, by oral gavage (suspended in corn oil) for 90 days. The control group received 0.4 mL/rat corn oil by oral gavage. Dams were monitored for clinical signs of toxicity for the period of the study. Dam initial and final body weight were recorded. Ovaries were subject to histopathological examination and hormone and antioxidant enzyme levels were evaluated. Treatment did not cause mortality. No adverse clinical signs were reported at doses of 5 and 10 mg/kg/day; however mild to significant toxic signs with a significant decrease in weight gain were observed in rats exposed to the highest dose group (20 mg/kg/day). A significant decrease in relative ovary weight was observed at the highest dose level (20 mg/kg/day). Ovaries of rats administered 20 mg/kg/day showed presence of cytoplasmic clumping and abundant lipofuscin elements in granulosa cells of follicles. Ovaries of rats administered 5 and 10 mg/kg/day showed about normal architecture. Serum FSH levels were significantly increased and LH levels were decreased in rats administered 20 mg/kg/day, rats administered 5 and 10 mg/kg/day did not have any significant alterations in hormone levels. A significant increase in LPO level and decrease in GSH content, SOD, CAT and GPX activity were observed in the ovary of rats exposed to 20 mg/kg/day of imidacloprid. Rats administered 5 and 10 mg/kg/day imidacloprid did not have any significant changes in antioxidant enzyme levels. Authors concluded imidacloprid is capable of altering significant reproductive functions at doses of 20 mg/kg/day and higher based on effects on the ovary, endocrine system, and generation of oxidative stress.
- Memon et al. 2014
    - *Oral*: In a reproductive toxicity study, male rabbits (*Oryctolagus cuniculus*) (10/dose/duration) received 0 or 45 mg/kg imidacloprid (purity not reported) daily by disposable syringe in 10 mL distilled water for 10 or 20 consecutive days. Control groups received distilled water for the same time period. Animals were monitored daily for changes in clinical signs and mortality. Testes were removed after sacrifice and subject to histopathological examination. Animals exposed to imidacloprid had behavioral changes and other health problems, such as decreased movement, trembling, fatigue, convulsion, dizziness, tremors and diarrhea. Both test groups showed highly significant decreases in body weight ($p<0.01$) and testicular weight ($p<0.01$) compared to controls. Histological evaluation indicated that imidacloprid caused testicular damage in test group animals. Animals treated for 10 days showed empty seminiferous tubules in the lumen and the interstitial space became widened. Pyknosis in spermatocytes and vacuolation in the seminiferous tubules were also observed. Animals treated for 20 days showed conspicuous changes in testicular structure with severe congestions in interstitial space. Only a few normal sized Leydig cells were present. Control animals showed normal histological structure of the testes. Authors concluded imidacloprid produces testicular damage that may cause reproductive disorders.

- Cal EPA 2006
    - *Oral*: In a 98-day oral toxicity study in rats, imidacloprid (purity 92.8%) was administered to Wistar rats (10/sex/dose) daily in the diet at concentrations of 0, 120, 600, and 3,000 ppm (0, 11, 57, and 409 mg/kg/day in males and 14, 78, and 513 mg/kg/day in females). Histological examination revealed degenerative changes in the testicular tubuli in 5/10 male rats at 3,000 ppm.
    - *Oral*: In a 4-week oral toxicity study, imidacloprid (purity 92.8%) was administered to Beagle dogs (2/sex/group in 4-week study in the diet at concentrations of 0, 200, 1,000, and 5,000 ppm (0, 7.3, 31, and 49 mg/kg/day). All animals at 5,000 ppm died or were sacrificed prior to the completion of the study. Testicular tubule degeneration was observed at the high dose.
- Clement International 1993
    - *Oral*: In a two-generation reproduction study, male and female Wistar/Han rats (30/sex/dose unknown) received 0, 100, 250 or 700 ppm (estimated 7.3, 18.3 and 52 mg/kg/day for males and 8.0, 20.5 and 57.4 mg/kg/day for females) imidacloprid (95.3% purity) in the diet. F_0 males and females were treated for 84 days, then females mated with males from the same group for a maximum of 22 days; the resulting offspring from this mating were the F_{1A} generation. The F_0 females were rested for two weeks and then mated again with alternative partners for a maximum of 22 days; if no mating occurred females were paired a second time with alternative partners for a maximum of 22 days, and the resulting offspring from this mating were the F_{1B} generation. The F_1 animals (26/sex/dose) were exposed to 0, 100, 250 or 700 ppm imidacloprid (95.3% purity) in the diet for 105 days (presumably after weaning). The F_1 females were paired with one male for mating for a maximum of 21 days; if no mating occurred, females were paired a second time with alternative partners for a maximum of 4 days. Animals were monitored twice daily for changes in clinical signs and mortality. Body weight data were recorded weekly during premating, but not during mating periods. Females were weighed weekly during gestation, male body weights were recorded weekly for the remainder of the study. Food consumption data were recorded weekly. For each litter, the number of live and dead pups, sex, and pup weight at birth and days 1, 4, 7, 14 and 21 as well as gross and behavioral abnormalities were recorded. Uteri of non-pregnant females were evaluated to detect early embryonic loss. Parental animals of both generations and one pup/sex/generation/group were sacrificed and necropsied after weaning. Uterus, cervix, pituitary gland, prostate gland, thyroid gland, liver, ovaries, seminal vesicles with coagulation gland, testes with epididymides, vagina and gross lesions were harvested for histopathological evaluation. No compound related mortalities or adverse clinical signs were observed in either sex or generation. The F_0 males showed a significant reduction in body weight at the highest dose level (700 ppm) during the premating period. The F_0 females showed a significant reduction in body weight at the highest dose level (700 ppm) during the premating period, F_{1A} gestation and lactation periods, and F_{1B} gestation period. The F_1 males in the highest dose level (700 ppm) showed significantly lower body weights on days 1, 8, 15 and 22 of the premating period. The F_1 females in the highest dose group (700 ppm) showed significantly lower body weights through the entire premating period and F_2 gestation and lactation periods. No compound related gross findings were observed in either sex or generation. Relative testes weight and absolute ovarian weight of the F_1 generation were significantly lower than those of controls at the 100 ppm and 700 ppm doses, respectively. Since both these findings were not seen in the previous generation and there were no related histopathologic findings,

authors concluded these observations as incidental and not compound related. There were no statistically significant effects on any reproductive parameters (mating, fertility, or gestation indices, number of live pups, number of live pups/litter, or live birth index). Authors concluded a parental toxicity NOAEL of 700 ppm (52 kg/mg/day for males and 57.4 kg/mg/day for females) due to the absence of parental toxicity in the study. Authors concluded a reproductive toxicity NOAEL of 100 ppm (7.3 mg/kg/day for males and 8.0 mg/kg/day for females) and LOAEL of 250 ppm (18.3 mg/kg/day for males and 20.5 mg/kg/day for females) based on decreased pup body weight. These effects are considered under developmental toxicity, below. ToxServices identified a NOAEL of 700 ppm (52 mg/kg/day for males and 57.4 mg/kg/day for females) based on a lack of effects on any of the fertility parameters at the highest dose tested.

ToxServices' summary and conclusion:

- Based on the weight of evidence, a conservative score of Moderate was assigned. Although one study reported effects on the ovary, endocrine system, and generation of oxidative stress in female rats, similar effects were not seen in a 2-generation study in rats. In addition, there were no treatment-related effects on fertility parameters in either generation. Studies in rats, dogs, and rabbits demonstrate the potential for testicular degeneration with repeated exposures in males. A lack of effects on fertility parameters in the 2-generation study in rats indicates that these effects are not expected to produce functional changes in this species. Effects in dogs were only seen at a high dose that also produced severe toxicity and mortality. Overt toxicity was also observed in rabbits that showed effects on the testes. However, as evidence of testicular degeneration has been consistently observed across species and GreenScreen® criteria specify a score of Moderate when there is limited or marginal evidence of reproductive toxicity in animals, ToxServices assigned a conservative score of Moderate. Confidence in the score is reduced because a mating study has only been conducted in rats.

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): M
Imidacloprid was assigned a score of Moderate for developmental toxicity based on effects on offspring body weight in the absence of maternal toxicity in rabbits, pathological changes in the brain in a prenatal developmental toxicity study in rats, and decreased learning activities in a 90-day study in infant rats. ToxServices did not assign a High score because the biological significance of the changes in brain pathology is uncertain, and important study details were not reported in some studies. Therefore, ToxServices considered the observed effects as limited evidence of developmental toxicity. GreenScreen® criteria classify chemicals as a Moderate hazard for developmental toxicity when there is limited or marginal evidence of developmental toxicity in animals (CPA 2012a). Confidence in the score is high because it is based on data from well-conducted studies.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Clement International 1993
  - *Oral*: In a previously described two-generation reproduction study, male and female Wistar/Han rats (30/sex/dose unknown) received 0, 100, 250 or 700 ppm (estimated 7.3, 18.3 and 52 mg/kg/day for males and 8.0, 20.5 and 57.4 mg/kg/day for females) imidacloprid (95.3% purity) in the diet. F_0 males and females were treated for 84 days, then females mated with males from the same group for a maximum of 22 days; the

resulting offspring from this mating were the F_{1A} generation. The F_0 females were rested for two weeks then mated again with alternative partners for a maximum of 22 days; if no mating occurred females were paired a second time with alternative partners for a maximum of 22 days, and the resulting offspring from this mating were the F_{1B} generation. The F_1 animals (26/sex/dose) were exposed to 0, 100, 250 or 700 ppm imidacloprid (95.3% purity) in the diet for 105 days (presumably after weaning). The F_1 females were paired with one male for mating for a maximum of 21 days; if no mating occurred, females were paired a second time with alternative partners for a maximum of 4 days. Animals were monitored twice daily for changes in clinical signs and mortality. Body weight data were recorded weekly during premating, but not during mating periods. Females were weighed weekly during gestation, male body weights were recorded weekly for the remainder of the study. Food consumption data were recorded weekly. For each litter, the number of live and dead pups, sex, and pup weight at birth and days 1, 4, 7, 14 and 21 as well as gross and behavioral abnormalities were recorded. Uteri of non-pregnant females were evaluated to detect early embryonic loss. Parental animals of both generations and one pup/sex/generation/group were sacrificed and necropsied after weaning. No treatment-related mortalities or adverse clinical signs were observed in either sex or generation. The F_0 males showed a significant reduction in body weight at the highest dose level (700 ppm) during the premating period. The F_0 females showed a significant reduction in body weight at the highest dose level (700 ppm) during the premating period, F_{1A} gestation and lactation periods, and F_{1B} gestation period. The F_1 males in the highest dose level (700 ppm) showed significantly lower body weights on days 1, 8, 15 and 22 of the premating period. The F_1 females in the highest dose group (700 ppm) showed significantly lower body weights through the entire premating period and F_2 gestation and lactation periods. At doses of 250 and 700 ppm, decreased body weights among all pups in all litters were observed, with occasional weight reductions at 100 ppm. There were no effects on sex ratio. Authors concluded a parental toxicity NOEL of 700 ppm (52 kg/mg for male and 57.4 kg/mg female) due to the absence of parental toxicity in the study. Authors concluded a developmental toxicity NOAEL of 100 ppm (7.3 mg/kg male and 8.0 mg/kg female) and LOEAL of 250 ppm (18.3 mg/kg male and 20.5 mg/kg female) based on decreased pup body weight.

- Cal EPA 2006
    - *Oral*: In a prenatal developmental toxicity study in rats, imidacloprid (94.2% active ingredient) was administered to mated female Wistar rats (25/dose) by gavage at doses of 0, 10, 30, and 100 mg/kg/day on gestation days 6-15. Dams were sacrificed on gestation day 21 and fetuses were examined for developmental abnormalities. Maternal toxicity (reduced body weight gain and food consumption) was observed at 100 mg/kg/day. Offspring at this dose had an increased incidence of wavy ribs (4.7% compared to 1.2% in the control group), but authors report that the difference was not statistically significant and was within the range for historical controls. At 100 mg/kg/day there were disproportionately more male fetuses compared to female fetuses (59% to 41%). This effect was statistically significant and fell outside the historical control range. Maternal toxicity at 10 and 30 mg/kg/day included a reduction in food intake and a dose-dependent decrease in body weight gain. Cal EPA identified a maternal LOEAL of 10 mg/kg/day for maternal toxicity based on reductions in food intake at that dose and a developmental NOAEL of 30 mg/kg/day based on increased incidence of wavy ribs and high numbers of male fetuses at 100 mg/kg/day.

  - *Oral*: In a prenatal developmental toxicity study in rabbits, mated Chinchilla rabbits (16/dose) were administered imidacloprid (purity not specified) by gavage at concentrations of 8, 24, or 72 mg/kg/day on gestation days 6-18. Dams were sacrificed on gestation day 28 and fetuses were evaluated for developmental effects. Severe maternal toxicity was observed at the highest tested dose, at which two dams died. Food consumption for surviving females was significantly reduced (up to 66% reduction) compared to controls, and there was an overall weight loss during the treatment period. Also at this dose, one dam aborted and two dams had complete resorptions. Females at this dose also had significantly higher post-implantation loss relative to controls, and the number of live fetuses per dam was subsequently reduced. Significantly reduced body weight (by 10%) and delayed ossification were observed in offspring. At 24 mg/kg/day, dams displayed reduced food consumption (by 16%) and reduced body weight gain (by 33%), though the latter was not statistically significant. These effects were considered by Cal EPA to be adverse effects on the dams, so a maternal NOAEL of 8 mg/kg/day was identified. The developmental NOAEL, as established by Cal EPA, was 24 mg/kg/day based on decreased body weight in pups.

- Oak Ridge National Laboratory 2002, Cal EPA 2006
  - *Oral*: In a developmental neurotoxicity study (GLP compliant) that was conducted according to OPPTS 870.6300/OECD Guideline 426, imidacloprid (98.2-98.4% purity) was administered to female Wistar rats (30/dose) via corn oil in the diet at concentrations of 0, 100, 250 or 750 ppm from gestation day 0 through postnatal day 21, when pups were weaned. Average daily intake of imidacloprid was 0, 8.0-8.3, 19.4-19.7 and 54.7-58.4 mg/kg/day during gestation and 0, 12.8-19.5, 30.0-45.4 and 80.4-155.0 mg/kg/day during lactation for the 0, 100, 250 and 700 ppm dose levels, respectively. Pups were fed untreated food after weaning. Maternal animals were checked daily for changes in clinical signs and mortality. On postnatal day 4, offspring were culled to yield four females and four males and were observed for clinical observations, assessment of motor activity, auditory startle response, habituation, learning and memory, and ophthalmology. Pup physical development was assessed by bodyweight, surface righting auditory startle, eye opening, pupillary constriction, vaginal patency in females, and balanopreputial separation in males. The FOB tests were conducted between days 4 and 60. Neural tissues were collected from 10/sex/dose of offspring on postnatal day 11 and termination of the study (75 days of age) for evaluation. No maternal deaths or treatment-related clinical signs were observed for the duration of the study. No treatment-related effects on body weight of mothers were observed; food consumption decreased for dams in the high dose group (700 ppm) during the third week of gestation and first week of lactation. There were no treatment-related effects on the number of litters, liver litter size, number of stillborn pups, live birth index, or viability index. Treatment-related effects for offspring were limited to the high dose group (700 ppm). Body weights of males and females were significantly ($p<0.05$) decreased prior to and after weaning, and during lactation. Overall, there were decreases in motor activity in males on postnatal day 17 and females on postnatal day 21, but the decreases were not significant. At study termination (postnatal day 75), high dose (700 ppm) females had a statistically significant ($p<0.03$) decrease in thickness of the corpus callosum and caudate putamen width compared to controls (this endpoint was not evaluated in the low and mid dose groups). Authors concluded a maternal NOAEL of 20 mg/kg/day and a maternal LOAEL of 55-58 mg/kg/day based on decreased food consumption. Authors concluded an offspring NOAEL of 20 mg/kg/day and LOAEL of 55-58 mg/kg/day based on decreased body

weight, decreased motor activity and decreased caudate/putamen width in females. In its review of this study, Cal EPA also identified a LOAEL of 55-58 mg/kg/day but stated that identification of a NOAEL is not possible due to the lack of morphometric brain measurements in the low and mid dose groups. Cal EPA also noted that because the morphometric brain measurements were not conducted prior to postnatal day 11, the timeline of imidacloprid developmental toxicity cannot be determined.

- Kara et al. 2015
    - Kara et al. evaluated the effects or oral imidacloprid exposure on the developing brains of neonatal Wistar albino rats. Newborn male infant rats (6/group) were administered 0.5, 2, or 8 mg/kg imidacloprid (purity not reported) via gavage for three months and their learning activities were evaluated. Measurements of inotropic glutamate receptor GRIN1, synoptophysin, growth-associated protein 43 and the muscarinic receptor M1 expression levels in the hippocampus were also made. The time at which these measurements were made was not described in the publication. Learning activities (Morris water maze test and probe test) were significantly reduced at the high and/or mid doses. Expression of GRIN1, SYP, and GAP-43 were also altered (not statistically significant). The authors concluded that imidacloprid in high doses caused deterioration in cognitive functions, particularly in infant rats, which may be associated with changes in gene expression.
- Abou-Donia et al. 2011
    - *Intraperitoneal*: In a developmental neurotoxicity study, timed pregnant female Sprague-Dawley (5/dose) rats were administered a single i.p. injection of imidacloprid (99.5% purity) at a dose of 337 mg/kg in corn oil on gestation day 9. Control rats were treated with a single i.p. injection of corn oil on gestation day 9. Animals were observed for overt signs of toxicity and seizures. Number and weight of pups was recorded following parturition. Behavioral (10/sex/dose), biochemical (5/sex/dose) and pathological (5/sex/dose) parameters of offspring were evaluated on postnatal day 30. Brains were removed for evaluation. There were no clinical signs of toxicity or mortality among mothers or offspring of any group. There was no significant difference in litter size or weight gain of the offspring between treated and control groups. Offspring of treated mothers demonstrated sensorimotor deficits by declines in beam-walk time, inclined plane test and grip time. Authors recorded an increase in AChE levels in the midbrain and cortex of male offspring from treated mothers, and in the cortex and brainstem of female offspring from treated mothers. A significant gender effect was seen in plasma BChE activity, male offspring from treated mothers had significant increases compared to female and control groups. Male and female rats from treated mothers had a significant increase in ligand binding to m2mAChR in the cortex and midbrain. No neuronal degeneration was seen in the motor cortex, hippocampus or cerebellum of offspring from treated mothers, however, GFAP immunostaining was increased in layers III and V of the motor cortex, as well as in the dentate gyrus, the CA1 subfield and CA3 pyramidal neurons of the hippocampus. Authors concluded *in utero* exposure to imidacloprid produced significant developmental neurobehavioral and neuropsychiatric abnormalities.
- Hussein et al. 2014
    - *Avian egg injection*: In a teratogenicity study on chick embryos, fertile eggs of white leghorn chicken (35/dose) were administered 5, 12.5, 25 and 50 µg of imidacloprid (purity not stated) at a volume of 5, 12.5, 25 and 50 µL by injection on the third day. Controls were treated with the same volume of normal saline. Eggs were broken to collect embryos on the 20th day of incubation for evaluation. Authors noted the number

of live and dead embryos and gross skeletal malformations. Brains were dissected for evaluation of crown rump length, size of head, and hardness of tissue. Authors observed embryos for failure of retraction of yolk sac, growth retardation, limb deformity, head enlargement, beak deformity, scanty feathers, and ectopia viscerale. Imidacloprid caused developmental delays or smaller embryos at all dose levels. A statistically significant effect on growth retardation was observed at doses of 25 and 50 µg. All dose levels showed significant effects on resorption of the yolk sac as well as an increase in the number of dead embryos. Authors also observed head enlargements, limb deformities, short beak, and ectopia viscerale at doses of 25 and 50 µg. Authors concluded imidacloprid is a potential teratogenic compound due to the dose dependent increase in growth retardation, failure of retraction of yolk sac, head enlargement, limb defects and ectopia viscerale in treated embryos compared to controls.

- Kimura-Kuroda et al. 2012
    - *In vitro*: In a developmental neurotoxicity study, authors used primary cultures of cerebellar neurons from neonatal Sprague-Dawley rats to perform an excitatory Ca^{2+} influx assay as an indicator of neural physiological activity. Imidacloprid is reported to be an agonist of nicotinic acetylcholine receptors (nAChRs); expression of these receptors in the perinatal stage is important for brain development. Authors evaluated imidacloprid's effect on the nAChRs of cerebellar neurons. Cells were administered imidacloprid (purity >98%) at 1 – 100 µM. Authors observed a characteristic excitatory pattern of intracellular Ca^{2+} at all doses of imidacloprid. Authors concluded excitation or desensitization or both of nAChRs by exposure to imidacloprid may affect the developing mammalian nervous system.

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of Moderate was assigned. Several studies have demonstrated a potential for effects on developing organisms, although effects are not consistent between studies. No effects on pup body weight were seen at up to maternally toxic doses in one oral prenatal developmental toxicity study in rats. However, effects on body weight were observed in an oral 2-generation study in rats and an oral prenatal developmental toxicity study in rats that were exposed throughout all of gestation as well as lactation. One study in rats showed an increase incidence in wavy ribs and altered sex ratio at doses that produced maternal toxicity. Reduced pup body weight was seen in rabbit offspring at doses lower than those causing maternal toxicity. Abortions and total resorptions were seen at severely maternally toxic doses. These data suggest that imidacloprid has the potential to cause adverse effects in offspring, although it cannot clearly be determined whether the effects may be secondary to maternal toxicity in some studies. In a well conducted developmental neurotoxicity study, imidacloprid caused histopathological changes (decrease in thickness of the corpus callosum and caudate putamen width) to the brains of rats that were exposed to imidacloprid during gestation and through lactation. Effects were apparent on postnatal day 75. The biological significance of these changes is unclear. Decreased motor activity was observed in male offspring on postnatal day 17 and female offspring on postnatal day 21, although these effects were not statistically significant. Neonatal oral exposure also reduced "learning activities" in rats as tested with Morris water maze test and a probe test at 2 and 8 mg/kg/day by gavage, but the study authors did not report the purity of the material tested, or whether it is the pure imidacloprid compound or the whole pesticide formulation. In addition, the time at which the tests were performed was not reported. Another GLP-compliant developmental neurotoxicity study (Oak Ridge National

Laboratory 2002) also tested learning activity with a water maze test, but did not find any effects on this test at dietary doses as high as 155 mg/kg/day. However, it should be noted that the neonatal rats were not exposed to imidacloprid after weaning (approximately 21 days after birth) in the Oak Ridge National Laboratory study, while animals were dosed for 90 days in the Kara et al. (2015) study. Effects on motor activity in offspring of rats that were administered imidacloprid via i.p. injection during gestation support the potential for neurological effects, as does an *in vitro* study demonstrating that imidacloprid can alter the pattern of intracellular Ca^{2+} in neonatal neuron cultures. A score of Moderate was assigned based on effects on offspring body weight in the absence of maternal toxicity in rabbits, pathological changes in the brain in a prenatal developmental toxicity study in rats, and decreased learning activities in a 90-day study in infant rats. Confidence in the score is high as it was based on well-conducted studies.

Endocrine Activity (E) Score (H, M, or L): *M*
Imidacloprid was assigned a score of Moderate for endocrine disruption based on evidence of interaction with thyroid pathways of rats, dogs and birds. GreenScreen® criteria classify chemicals as a Moderate hazard for endocrine disruption there is evidence of endocrine activity (CPA 2012a). Confidence in the score is reduced because it is unclear whether effects represent a direct vs. indirect effect on the thyroid.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- 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.
- U.S. EPA 2015
  - Imidacloprid was included in the U.S. EPA's Endocrine Disruptor Screening Program (EDSP), and underwent the Tier I assay battery to evaluate the potential of the chemical to interact with the estrogen, androgen, and thyroid signaling pathways. The evaluation included agonism and antagonism at the estrogen and androgen receptors, altered steroidogenesis, and the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary thyroid (HPT) axes. U.S. EPA's evaluation included a review of Tier I toxicity test data (battery of 11 assays) designed to test for effects on receptor binding (estrogen and androgen agonist and antagonist), steroidogenesis, and other effects on the HPG and HPT axes, as well as studies conducted as part of pesticide registrations and published literature studies. The evaluation included an assessment of scientific quality and relevance or each study, consistency of responses, and potential confounding effects of overt systemic toxicity. In the Tier I evaluation, the potential for interaction at multiple levels (e.g. molecular and whole organism) is evaluated in order to form a hypothesis regarding potential interaction, and the data are evaluated to determine if the data provide consistent and robust evidence of interaction, whether compensatory responses are exhibited, and whether effects were seen in the presence of overt systemic toxicity. The U.S. EPA concluded that there is no convincing evidence of potential interaction with the estrogen, androgen, or thyroid pathways. A summary of U.S. EPA's weight of evidence discussion for each pathway is below.
    - *Estrogen pathway*: Imidacloprid tested negative in Tier I *in vitro* estrogen receptor, estrogen receptor transactivation assay, and steroidogenesis assays and was equivocal in an *in vitro* aromatase assay. In literature studies, it was negative

for agonist or antagonist ER transactivation. It did not impact spermatozoa motility in an *in vitro* assay that demonstrated effects on *in vitro* fertilization and embryonic development. It was negative in a fish short term reproduction assay as well as for effects on uterine weight, ovary weight and histopathology, and age and weight at vaginal opening in a female prepubertal assay. A decrease in the mean percentage of cycling animals was attributed to prolonged diestrus period. An evaluation of published mammalian toxicity studies showed no evidence of estrogenic or anti-estrogenic effects. Effects on avian reproduction in some studies were observed in conjunction with effects on the liver and intestines. U.S. EPA concluded that there is no convincing evidence of an interaction with the estrogen pathway in mammals or wildlife.

- *Androgen pathway*: Imidacloprid was negative in Tier I *in vitro* androgen receptor binding and steroidogenesis assays and an *in vitro* androgen receptor transactivation assay. It did not cause effects on sperm motility or DNA fragmentation in an *in vitro* assay. It did not produce androgenic or anti-androgenic effects on accessory sex tissues in a Hershberger assay and was negative in a fish short term reproduction test. The weight of the dorsolateral prostate was decreased at one dose in the male prepubertal assay but there were no additional effects at that dose. The high dose produced decreased weights of accessory organs/tissues and the pituitary gland, and a 3.4 day delay in preputial separation. There were no effects on testosterone levels or histopathological changes in the testes or epididymides, and because effects were only seen at doses producing systemic toxicity U.S. EPA concluded that they were not likely to be due to an interaction with the androgen pathway. In published mammalian toxicity studies, no androgenic or anti-androgenic effects were seen in the absence of overt systemic toxicity.
- *Thyroid pathway*: Imidacloprid increased TSH levels in males and females in prepubertal assays, but there were no associated changes in T4 levels, thyroid weight, or thyroid histopathology. Pituitary weights decreased in both sexes in these assays. U.S. EPA questioned the biological relevance of these changes, as pituitary weight is expected to increase when TSH levels are increased. Delays in developmental stage and growth in an amphibian metamorphosis assay were not attributed to thyroid effects as thyroid histopathology was unaltered. In published mammalian toxicity studies, the only effect reported was in increase in mineralization of the thyroid in one chronic rat study. U.S. EPA concluded that imidacloprid does not show convincing evidence of potential interaction with the thyroid pathway.

In addition to summarizing the U.S. EPA's evaluation of the endocrine activity of imidacloprid, ToxServices also summarized potential endocrine-related effects observed in studies described elsewhere in this assessment, some of which were included in the U.S. EPA's evaluation. For studies that were evaluated by U.S. EPA, this was noted by ToxServices within the summary. In addition, ToxServices summarized additional studies that either were not included in the U.S. EPA's assessment or were conducted after the assessment was completed.

- Cal EPA 2006
    - In a previously described 2-year chronic toxicity and carcinogenicity study, imidacloprid (94.3% active ingredient) was administered to Wistar rats (50/sex/group) in the diet at

concentrations of 0, 100, 300, and 900 ppm (0, 5.7, 17, or 51 mg/kg/day in males and 0, 7.6, 25, or 73 mg/kg/day in females). Interim examinations were conducted on an additional 10 rats/sex/dose after 1 year after treatment. A supplemental study to determine the maximum tolerated dose (MTD) was conducted on 50 rats/sex/dose that were either used as controls or administered imidacloprid at a concentration of 1,800 ppm (103 mg/kg/day in males and 144 mg/kg/day in females) for two years. Study authors reported lesions in the thyroid gland as the principal treatment-related effect. At 1,800 ppm, parafollicular hyperplasia and a reduced number of colloid aggregation sites in the thyroid were observed. A marked, dose-dependent increase in the incidence and severity of mineralized particles in the thyroid follicles was statistically significant in male rats at 300 ppm and female rats at 900 ppm. The levels of thyroid hormones (T3, T4, and TSH) were not altered at any dose. Cal EPA reported that the occurrence of mineralized particles in the thyroid is considered a sign of biological aging and indicates that imidacloprid may cause premature aging of the thyroid follicles. Cal EPA reported a NOAEL of 5.7 mg/kg/day and LOAEL of 17 mg/kg/day, as mineralization of the thyroid at the low dose did not differ from historical controls (Note: this study was included in the U.S. EPA's evaluation of imidacloprid).

- Kapoor et al. 2011
    - In a previously described reproductive toxicity study, female Wistar rats (10/dose) received 0, 5, 10 or 20 mg/kg/day imidacloprid (96% purity) daily, by oral gavage (suspended in corn oil) for 90 days. The control group received 0.4 mL/rat corn oil by oral gavage. Ovaries were subject to histopathological examination and hormone and antioxidant enzyme levels were evaluated. Serum FSH levels were significantly increased and LH levels were decreased in rats administered 20 mg/kg/day. Rats administered 5 and 10 mg/kg/day did not have any significant alterations in hormone levels. Authors concluded imidacloprid is capable of significantly altering reproductive hormone levels at doses of 20 mg/kg/day and higher (Note: this study was included in the U.S. EPA's evaluation of imidacloprid).
- Ibrahim et al. 2015
    - In a 1-week study in neonatal male Sprague-Dawley rats, animals (5/group) were administered imidacloprid via gavage at doses of 0.529, 1.058, or 2.116 mg/kg/day and blood was collected after 7 days. A significant decrease in plasma T3 was observed at the mid and high doses.
- Bhaskar and Mohanty 2014
    - In an endocrine study to assess imidacloprid's thyroid disrupting potential, Swiss albino mice (12/dose) were exposed to 6.55 mg/kg (0.5% of LD_{50}) of Tatamida® (17.8% wt/wt imidacloprid) orally through the diet from postnatal day 1 to postnatal day 28 through lactating mother. The control group received 0.2 mL olive oil. Body weights and food intake of pups were recorded. Half of the offspring (15-17/dose) were sacrificed on postnatal day 28 for assessment of direct exposure, the other half (15-17/dose) were left until postnatal day 63 for evaluation of the persistence of the effects at sexual maturity. Authors recorded the waist hip ratio and BMI and took plasma samples for hormonal assays (prolactin, T_3, T_4, and TSH) and lipid profiles (total cholesterol, HDL, LDL and TAGs). No significant gain in relative body weight or change in waist hip ratio or BMI was observed in treated female lactating dams or in male or female offspring of treated mothers. No significant changes in hormone levels or lipid profiles were observed in treated dams or their offspring. Authors performed an *in silico* molecular docking simulation to predict the binding of imidacloprid to thyroid hormone receptors (TRs).

Their model predicted imidacloprid could compete with T_3 and disrupt TRα, TRβ and PPARγ functioning. Authors concluded exposure to imidacloprid had no effects on metabolism and body weight under the conditions of the study; however, their modeling demonstrated imidacloprid has the potential to disrupt metabolic regulation through several pathways and contribute to body weight gain through TRs function disruption.

- Cal EPA 2006, SRC 2005
    - *Oral*: In a 4-week oral toxicity study in Beagle dogs, imidacloprid (92.8% purity) was administered to 2 dogs/sex/dose at concentrations of 0, 200, 1,000, or 5,000 ppm (0, 7.3, 31, or 49 mg/kg/day). All animals at the high dose died or were sacrificed before completion of the study. Atrophy of the thyroid glands was observed at this dose. At 1,000 ppm authors reported follicular atrophy of the thyroid.
- Pandey and Mohanty 2014
    - In an endocrine study to assess imidacloprid's thyroid disrupting potential on the pituitary-thyroid axis of a seasonally breeding wildlife avian species, adult male red munia birds (8/dose) were exposed to 1.55 mg/kg/day (0.5% of LD_{50}) imidacloprid (purity unknown) through food using soy oil as a vehicle for 30 days during the preparatory and breeding phases. Control birds were given food with the vehicle. Experiments were performed in each phase in replicates of two. Body weights were recorded every other day throughout the study. At the end of exposure, birds were sacrificed and blood was collected for hormone analysis. Thyroids were dissected out for evaluation of volume of the thyroid gland, number of follicles, volume of colloids, epithelial cell height, epithelial cell nucleus size and nucleus-to-cytoplasm ratio in epithelial and stromal cells. No significant alteration in body weight was observed in exposure groups of the preparatory phase; however, body weight was reduced in the exposed groups of the breeding phase ($p<0.01$). Authors observed a decrease in thyroid weight in the preparatory phase and an increase in thyroid weight in the breeding phase. An increase in thyroid volume was observed in both phases. These changes were not statistically significant compared to controls. In both phases, imidacloprid exposed animals had irregular shaped follicles with ruptured epithelium and lesions in stroma. The number of follicles was significantly ($p<0.01$) reduced and damaged colloids devoid of secretary droplets were observed. A significant ($p<0.05$) increase in colloid volume was observed in the preparatory phase, and an insignificant decrease in colloid volume was observed in the breeding phase. Exfoliation of epithelial cells was observed in both phases; however, shrinkage and mineralization of colloids was only observed in the breeding phase. Epithelial cell height, nucleus size and nucleus to cytoplasm ratio were decreased in both phases in exposed groups. A significant decrease in T_4 levels was seen in both phases; however, T_3 levels were significantly increased during the preparatory phase and significantly decreased in the breeding phase. TSH was also decreased in both phases of imidacloprid exposed groups. Authors concluded substantial thyrotoxicity was induced by exposure to imidacloprid based on damage to thyroid follicles and lesions in stroma and impaired plasma levels of thyroid hormones, and that effects were more prominent in the breeding phase.

ToxServices' summary and conclusion:

- Based on the weight of evidence, a low confidence score of Moderate was assigned. U.S. EPA concluded that imidacloprid does not show convincing evidence of interaction with the estrogen, androgen, and thyroid pathways. Although some studies have indicated that imidacloprid

exposure impacts some aspects of the thyroid pathway, effects were not consistent between studies. U.S. EPA conducted an in depth evaluation of all available data, assessing studies first for quality and then to determine if the data support a consistent and plausible effect on the thyroid pathway. U.S. EPA's evaluation also investigated whether effects could be attributed to an endocrine mediated effect or if they are secondary to systemic toxicity, and based on the studies evaluated the U.S. EPA concluded that there was no evidence of thyroid activity. ToxServices did not identify any additional data suggesting potential interactions with the estrogen or androgen pathways. However, a 1-week oral study in mice showed effects on T3 levels, and a 4-week study in dogs showed evidence of thyroid atrophy (although this was not reproduced in studies of longer duration). A study in birds also showed effects on the number of thyroid follicles, thyroid histopathology, and levels of T3 and T4. These studies were not included in the U.S. EPA's evaluation. As these studies provide further support for effects on the thyroid pathway, a score of Moderate was assigned. Confidence in the score is reduced because it is unclear whether effects represent a direct vs. indirect effect on the thyroid.

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): *H*

Imidacloprid was assigned a score of High for acute toxicity based on oral LD_{50} values of 98 (sex not reported), 131 (males), and 168 (females) mg/kg imidacloprid in acute oral studies in mice. GreenScreen® criteria classify chemicals as a High hazard for acute toxicity when the most conservative oral LD_{50} values are between 50 and 300 mg/kg (CPA 2012a). Confidence in the score is reduced due to the wide range of oral LD_{50} values reported, and that the authoritative H302 corresponds to a score of Moderate.

- Authoritative and Screening Lists
  - *Authoritative:* EU Risk Phrase R22 – Harmful if swallowed
  - *Authoritative:* CLP/GHS Hazard Statement H302 – Harmful if swallowed
  - *Screening:* GHS New Zealand Category 6.1C (GHS Category 3) – Acutely toxic
- Cal EPA 2006
  - *Oral*: LD_{50} (male and female Wistar rat) = 424 mg/kg (males), 450-475 mg/kg (females)
  - *Oral*: LD_{50} (male and female Bor:NMRI-SPF mouse) = 131 mg/kg (males), 168 mg/kg (females)
  - *Dermal*: LD_{50} (male and female Wistar rat) > 5,000 mg/kg
  - *Inhalation*: LC_{50} (male and female Wistar rat, dust) > 5,323 mg/m^3 (> 5.323 mg/L)
  - *Inhalation*: LC_{50} (male and female Wistar rat, liquid aerosol of 2.5% solution) > 69 mg/m^3 (> 0.069 mg/L)
- ChemIDplus 2016
  - *Oral:* LD_{50} (rat, sex and strain not reported) = 410 mg/kg imidacloprid
  - *Oral:* LD_{50} (mouse, sex and strain not reported) = 98 mg/kg
- U.S. EPA 1993
  - *Oral:* LD_{50} (male and female Sprague-Dawley rats) = 2,591 mg/kg (males), 1,858 mg/kg (females)

- *Dermal:* LD_{50} > 2,000 mg/kg imidacloprid (76.1% purity) male and female Sprague-Dawley rats (5/sex/dose)
- *Inhalation:* LC_{50} = 2,650 mg/m^3 (male), 2,750 mg/m^3 (female) imidacloprid (76.1% purity) Sprague-Dawley rats (6/sex/dose)

ToxServices' summary and conclusion:

- Based on the weight of evidence, a conservative score of High was assigned. Available data indicate that imidacloprid is not highly acutely toxic via the dermal and inhalation routes of exposure, as shown by LD/C_{50} values of > 2,000 mg/kg and > 5.323 mg/L in rats. It is associated with EU Risk Phrase R22 – Harmful if swallowed and CLP/GHS Hazard Statement H302 – Harmful if swallowed, which correspond to a score of Moderate. This is consistent with the majority of reported LD_{50} values in rats, which range from 410 mg/kg-2,591 mg/kg. However, the reported LD_{50} values in mice are 98 mg/kg, 131 mg/kg (males), 168 mg/kg (females). As noted in the USDA's human health and ecological risk assessment (SRC 2005), technical grade imidacloprid exhibits a higher order of toxicity than formulations, which may account for the wide range in oral LD_{50} values. Because the LD_{50} values of 131 mg/kg in males and 168 mg/kg in females were obtained in a reliable well conducted and well reported study of a high purity technical grade formulation, ToxServices assigned a score of High based on the assumption that other species may be more sensitive than rats to imidacloprid. Confidence in the score is reduced due to the wide range of oral LD_{50} values reported and due to the inconsistencies with the authoritative H phrase.

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST)
***Group II Score (single dose)* (vH, H, M, or L): vH**
Imidacloprid was assigned a score of Very High for systemic toxicity (single dose) based on evidence of liver toxicity at a dose of 151 mg/kg in an acute oral study in rats. GreenScreen® criteria classify chemicals as a Very High hazard for systemic toxicity (single dose) when evidence of systemic toxicity is seen below the guidance values of 300 mg/kg in an acute oral toxicity study (CPA 2012a). Confidence in the score is high because it is based on experimental data from a well-conducted study.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative or screening lists
  - *Screening:* GHS New Zealand Category 6.9B (GHS Category 2) – Harmful to human target organs or systems.
- SRC 2005, Cal EPA 2006
  - *Oral*: In a previously described acute oral toxicity study in rats, imidacloprid (purity 94.2%) was administered by gavage to Wistar rats (5/sex/group) as a single dose of 50, 100, 250, 315, 400, and 475 mg/kg in males and 100, 250, 315, 400, 475, 500, and 1,800 mg/kg in females. The duration of the observation period was not stated. Clinical signs seen at doses of 100 mg/kg and above included apathy, labored breathing, tremors, spasms, gait incoordination, decreased motility, and nasal and urine staining. Mortality was first observed at 400 mg/kg. Deaths occurred within 3-7 hours following treatment. The NOAEL for systemic effects was reported by Cal EPA to be 50 mg/kg based on the clinical signs at 100 mg/kg.
  - *Oral*: In an acute oral toxicity study that was primarily designed to evaluated neurotoxicity, imidacloprid (98.8% purity) was administered to Sprague-Dawley rats (18/sex/dose) by gavage as a single dose of 0, 42, 151, or 307 mg/kg. Authors reported a

decrease in serum triglycerides of male rats at all doses. A 23% reduction at the low dose was not statistically significant, but statistically significant decreases of up to 73% were seen at higher doses. Cal EPA notes that these effects are consistent with those seen on the liver in repeated dose toxicity studies. The USDA Forest Services risk assessment reports a NOAEL of 42 mg/kg based on clinical chemistry changes, and in addition to the aforementioned effects on serum triglycerides, decreases in serum potassium and cholesterol were observed in females. Therefore the LOAEL is 151 mg/kg.

- *Oral*: In a previously described acute oral toxicity study in mice, imidacloprid (94.2%) was administered to mice (5/sex/group) by gavage at doses of 10, 71, 100, 120, 140, 160, and 250 mg/kg in males and 100, 120, 140, 160, and 250 mg/kg in females. The duration of the observation period was not stated. Animals were observed for clinical signs and gross pathology. All dose groups except the 10 mg/kg group displayed mortality and clinical signs, including apathy, labored breathing, and decreased motility (71/mg/kg/day), staggering gait and severe trembling (100-250 mg/kg). Deaths occurred starting at 100 mg/kg and toxicity was evident within 5-10 minutes following administration. Cal EPA reported a NOAEL for systemic toxicity of 10 mg/kg based on clinical signs in males observed at 71 mg/kg.
- *Dermal*: In the previously described acute dermal toxicity study in Wistar rats, a single dose of 5,000 mg/kg imidacloprid paste (94.2% in 0.9% NaCl) was administered to the skin of the shaved backs of 5 animals/sex and application sites were covered (not specifically stated whether coverage was occlusive or semiocclusive) for 24 hours. The duration of the observation period was not stated. There were no deaths or clinical signs of toxicity, and there were no gross pathological changes. ToxServices identified a NOAEL of 5,000 mg/kg based on a lack of effects at the only dose tested.
- *Inhalation*: In a previously described acute inhalation toxicity study in rats, imidacloprid was administered to Wistar rats (5/sex/dose) in the form of dust via head/nose only inhalation for 4 hours. Concentrations were 1,220, 2,577, and 5,323 mg/m^3 (1.22, 2.577 and 5.323 mg/L). One control group received air. No deaths were observed. Within 4-6 hours post-treatment at concentrations higher than 1,220 mg/m^3, the following clinical signs were observed: difficulty breathing, reduced motility, piloerection, and tremors. Cal EPA reported a NOAEL of 1,220 mg/m^3 (1.22 mg/L) based on clinical signs at 2,257 mg/m^3 (2. 577 mg/L)

- U.S. EPA 1993
  - *Oral:* In a previously described acute oral toxicity study in rats, imidacloprid (76.1% purity) was administered as a single dose to male and female Sprague-Dawley rats (5/sex/dose) by gavage in deionized water at doses of 1,063, 2,180 2,750 and 3,170 mg/kg in females and 1,063, 2,180 and 3,170 mg/kg in males. Animals were observed for toxicity and mortality twice daily for 14 days. Terminal body weights were taken for all animals that died in the duration of the study. All surviving animals were sacrificed on day 14 and gross necropsy was performed on all animals of the study. Male and female deaths occurred during the study in a dose-related manner, all occurring between day 0 and day 10 post treatment. Treatment-related signs of toxicity were observed on day of dosing and included tremors, increased reactivity, decreased activity, noisy breathing, diarrhea, red stains and urine stains; all signs resolved in survivors by day 14. Body weight decreased in a dose related manner from days 0 through 7; recovery was evident in survivors by the end of the observation period. Salivation, lacrimation, reddened lungs and nasal stain were observed in animals found dead. No animals

surviving showed gross lesions. Authors concluded a NOAEL < 1,063 mg/kg for males and females.

- *Dermal:* In a previously described acute dermal toxicity study in rats, imidacloprid (76.1% purity) was administered once to male and female Sprague-Dawley rats (5/sex/dose) at a dose of 2,000 mg/kg. Animals were observed for toxicity and mortality twice daily for 14 days. Body weights were taken on days 7 and 14 post treatment, and all animals were subject to gross pathological examination after sacrifice on day 14 post treatment. No deaths occurred. Treatment-related clinical signs included urine stains on one male, and urine stains and alopecia on one female. Authors concluded a NOAEL of < 2,000 mg/kg for both sexes due to the urine stains and alopecia.
- *Inhalation:* In a previously described acute inhalation study in rats, imidacloprid (76.1% purity) was administered to Sprague-Dawley rats (6/sex/dose) at concentrations of 2,110, 2,810, or 2,990 mg/m^3 (2.11, 2.81, or 2.99 mg/L) as a liquid aerosol in water via nose only inhalation for 4 hours. Animals were observed for 14 days. Deaths were observed at all doses. Clinical signs of toxicity included ataxia, convulsions, hypoactivity, moribundity, nasal and urine stain, and tremors at all doses. Clinical signs resolved by observation day 6. Body weight was significantly reduced in both sexes (12.1% in males and 7.6% in females) at the high dose and in males in the mid dose group (8.9%) at 3 days post treatment; effects resolved by observation days 7 and 14. Reddened turbinates and lungs were seen in animals that died. There were no gross pathological changes in surviving animals.

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of Very High was assigned. The majority of acute oral and inhalation studies reported evidence of neurological effects, which will be discussed under neurotoxicity (single dose), below. Breathing difficulties were observed in one inhalation study, and reddened turbinates and lungs were seen in animals that died in a second study. These effects suggest that inhalation of imidacloprid may irritate the respiratory tract, which corresponds to GHS Category 3 classification and a score of Moderate. No consistent evidence of systemic effects on target organs other than the brain were reported in any of the standard acute studies, but these studies evaluated only clinical signs and gross pathology. However, in one study that was designed primarily to evaluate neurotoxicity, decreases in serum triglycerides indicative of possible liver toxicity were seen with a NOAEL of 42 mg/kg/day and LOAEL of 151 mg/kg/day. Because this is consistent with effects on the liver that have been observed in repeated dose toxicity studies (described below), it is plausible that these changes represent liver damage following a single high dose. Therefore a score of Very High was assigned because effects were seen below the guidance value of 300 mg/kg.

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

Imidacloprid was assigned a score of Moderate for systemic toxicity (repeated dose) based on several subchronic and chronic toxicity studies demonstrating effects between 10 and 100 mg/kg/day. GreenScreen® criteria classify chemicals as a Moderate hazard for systemic toxicity (repeated dose) when the weight of evidence indicates that adverse systemic effects occur between doses of 10 and 100 mg/kg/day (CPA 2012a). Confidence in the score is high because the LOAEL values all fall below the GHS cutoff value of 100 mg/kg/day for classification to category 2.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists

- *Screening:* Not present on any screening lists

Chronic toxicity studies

- Cal EPA 2006, SRC 2005
  - *Oral*: In a previously described 2-year chronic toxicity and carcinogenicity study, imidacloprid (94.3% active ingredient) was administered to Wistar rats (50/sex/group) in the diet at concentrations of 0, 100, 300, and 900 ppm (0, 5.7, 17, or 51 mg/kg/day in males and 0, 7.6, 25, or 73 mg/kg/day in females). Interim examinations were conducted on an additional 10 rats/sex/dose after 1 year after treatment. A supplemental study to determine the maximum tolerated dose (MTD) was conducted on 50 rats/sex/dose that were either used as controls or administered imidacloprid at a concentration of 1,800 ppm (103 mg/kg/day in males and 144 mg/kg/day in females) for two years. At 1,800 ppm, there were substantial reductions in body weight in both sexes at all times. As food intake was similar to controls, this effect was attributed to treatment. Alterations in serum chemistry (elevated serum AP activity, creatinine kinase, and AST, and reduced cholesterol) were also seen at 1,800 ppm. Study authors reported lesions in the thyroid gland as the principal treatment-related effect. At 1,800 ppm, parafollicular hyperplasia and a reduced number of colloid aggregation sites in the thyroid were observed. A marked, dose-dependent increase in the incidence and severity of mineralized particles in the thyroid follicles was statistically significant in male rats at 300 ppm and female rats at 900 ppm. The levels of thyroid hormones (T3, T4, and TSH) were not altered at any dose. Cal EPA reported that the occurrence of mineralized particles in the thyroid is considered a sign of biological aging and indicates that imidacloprid may cause premature aging of the thyroid follicles. There were no additional effects on mortality, clinical signs, clinical chemistry, ophthalmology, organ weights, or pathology. Cal EPA reported a NOAEL of 5.7 mg/kg/day and LOAEL of 17 mg/kg/day, as mineralization of the thyroid at the low dose did not differ from historical controls.
  - *Oral*: In the previously described 24-month chronic toxicity and carcinogenicity study in B6C3F1 mice, animals (50/sex/group) were administered imidacloprid (95.3% active ingredient) in the diet at concentrations of 0, 100, 330, or 1,000 ppm in one study and 0 and 2,000 ppm in the other. An interim evaluation was conducted on an additional 10 mice/sex/dose at 12 months. Reported average daily doses were 0, 20, 66, 208, or 414 mg/kg/day in males and 0, 30, 104, 274, and 424 mg/kg/day in females. Mice at 2,000 ppm had significantly reduced body weights compared to controls (up to 29%) from the first week of treatment but this effect was attributed in part to decreased food consumption. Males at 1,000 ppm also had significantly reduced body weight (up to 10%) in the absence of effects on food consumption. Morphological changes at 2,000 ppm included periacinar hypertrophy of hepatocytes in males and mineralization of the thalamus in females. At 2,000 ppm there was an increase in mortality for males that died intercurrently though the total number of males that died or were euthanized did not differ from controls. Authors reported that a large number of males died during manipulations, which study authors considered an indirect effect of treatment. Animals that died exhibited dyspnea, respiratory failure, and spasms immediately after administration of ether. Males at the high dose showed hypersensitivity to the ether anesthesia, and study authors suggest that imidacloprid may reduce the ability of mice to respond to additional challenges with xenobiotics. There were no effects on clinical signs, clinical chemistry, urinalysis, hematology, organ weights, or histopathology. Study authors identified a NOAEL of 330 ppm (47 mg/kg/day) based on reductions in body weight at the LOAEL of 1,000 ppm (143 mg/kg/day).

- *Oral*: In a 52-week oral toxicity study, imidacloprid (94.9% active ingredient) was administered to Beagle dogs (4/sex/dose) in the diet at concentrations of 200, 500, or 1,250 ppm. At week 17, the 1,250 ppm dose was increased to 2,500 ppm until the end of treatment. Daily doses were reported by Cal EPA to be equivalent to 6, 15, and 41/72 mg/kg/day. Food consumption was decreased in females at the highest dose. There were also increases in metabolic activity (elevated plasma cholesterol and liver cytochrome P450 enzymes) in the liver in both sexes which study authors considered to be associated with increased relative liver weight (as liver/brain ratio). Relative liver weight was not statistically significantly increased when expressed as a liver weight/body weight ratio. The chronic oral NOAEL was identified as 500 ppm (15 mg/kg/day) by study authors, based on liver changes at the highest dose of 41/72 mg/kg/day.

Subchronic toxicity studies

- Clement International 1993
  - *Oral*: In a two-generation reproduction study, male and female Wistar/Han rats (30/sex/dose) received 0, 100, 250 or 700 ppm (estimated 7.3, 18.3 and 52 mg/kg/day for males and 8.0, 20.5 and 57.4 mg/kg/day for females) imidacloprid (95.3% purity) in the diet. The F_0 males and females were treated for 84 days, then females mated with males from the same group for a maximum of 22 days; the resulting offspring from this mating were the F_{1A} generation. The F_0 females were rested for two weeks then mated again with alternative partners for a maximum of 22 days; if no mating occurred females were paired a second time with alternative partners for a maximum of 22 days, and the resulting offspring from this mating were the F_{1B} generation. The F_1 animals (26/sex/dose) were exposed to 0, 100, 250 or 700 ppm imidacloprid (95.3% purity) in the diet for 105 days (presumably after weaning). The F_1 females were paired with one male for mating for a maximum of 21 days; if no mating occurred, females were paired a second time with alternative partners for a maximum of 4 days. Animals were monitored twice daily for changes in clinical signs and mortality. Body weight data were recorded weekly during premating, but not during mating periods. Females were weighed weekly during gestation, male body weights were recorded weekly for the remainder of the study. Food consumption data were recorded weekly. Blood samples and liver samples were collected from 10 random animals/group/sex from the F_1 generation for hematology and clinical chemistry. Parental animals of both generations and one pup/sex/generation/group were sacrificed and necropsied after weaning. Uterus, cervix, pituitary gland, prostate gland, thyroid gland, liver, ovaries, seminal vesicles with coagulation gland, testes with epididymides, vagina and gross lesions were harvested for histopathological evaluation. No compound related mortalities or adverse clinical signs were observed in either sex or generation. The F_0 males showed a significant reduction in body weight at the highest dose level (700 ppm) during the premating period. The F_0 females showed a significant reduction in body weight at the highest dose level (700 ppm) during the premating period, F_{1A} gestation and lactation periods, and F_{1B} gestation period. The F_1 males in the highest dose level (700 ppm) showed significantly lower body weights on days 1, 8, 15 and 22 of the premating period. The F_1 females in the highest dose group (700 ppm) showed significantly lower body weights through the entire premating period and F_2 gestation and lactation periods. No compound related effects were observed in any hematological parameter or clinical chemistry in F_1 males or females. No compound related gross findings were observed in either sex or generation. Relative testes weight and absolute ovarian weight of the F_1 generation were significantly lower than those of controls at the 100 ppm and 700 ppm doses, respectively. Since both

these findings were not seen in the previous generation and there were no related histopathologic findings, authors concluded these observations as incidental and not compound related. Authors concluded a parental systemic toxicity NOAEL of 700 ppm (52 mg/kg/day for males and 57.4 mg/kg/day for females) based on a lack of systemic effects at the highest dose tested.

- Cal EPA 2006, SRC 2005
    - *Oral*: In a 107-day repeated-dose oral toxicity study in mice, imidacloprid (92.8% purity) was administered in the diet to B6C3F1 mice (10/sex/dose) at concentrations of 0, 120, 600, and 3,000 ppm (77, 397, and 2,323 mg/kg/day in males and 91, 453, and 3,075 mg/kg/day in females). Animals at the high dose had rough coats and reduced body weights (15% in males and 27% in females) despite higher feed consumption. Reduced body weights were also seen in the males at the mid dose. At the high dose, males had decreased cholesterol and urea and both sexes had higher serum AP activity. A total of 16 mice died at end of the 14th week, including 1 at 120 ppm, 1 at 600 ppm, and 14 at 3,000 ppm. No gross pathological changes were observed. Study authors speculated that deaths were a result of the weight loss seen at the high dose. Authors reported a NOAEL of 600 ppm (396 mg/kg/day) and LOAEL of 3,000 ppm (2,323 mg/kg/day) based on poor appearance, reduced body weight, and mortality. Cal EPA reviewed the study and noted that the food consumption appeared high and that the report did not indicate whether the quantity of food consumed had been corrected for spillage. Cal EPA adjusted the doses based on normal food consumption for a mouse, and reported a revised NOAEL of 86 mg/kg/day and LOAEL of 427 mg/kg/day.
    - *Oral*: In a 98-day oral toxicity study in rats, imidacloprid (purity 92.8%) was administered to Wistar rats (10/sex/dose) daily in the diet at concentrations of 0, 120, 600, and 3,000 ppm (0, 11, 57, and 409 mg/kg/day in males and 14, 78, and 513 mg/kg/day in females). No deaths occurred at any dose. Body weight reduction was observed in females at 600 ppm and in both sexes at 3,000 ppm though feed consumption was increased in these rats. Study authors therefore concluded that the effect on body weight was treatment-related. Other statistically significant effects in animals at 3,000 ppm included elevated alkaline phosphatase activity and creatinine levels and decreased levels of glucose and cholesterol in the blood. Authors speculated that these effects are indicative of changes in metabolism of carbohydrates and fats. At 3,000 ppm, cellular necrosis was observed in the liver of one male and was considered by authors to be treatment-related since it involved multifocal cell necrosis in central, intermediary, and peripheral lobular zones. Further histological examination revealed degenerative changes in the testicular tubuli in 5/10 male rats at 3,000 ppm. Cal EPA reported a NOAEL of 120 ppm (14 mg/kg/day) based on 11% reductions in body weight of female rats at 600 ppm (78 mg/kg/day).
    - *Oral*: In a 13-week oral toxicity study, imidacloprid (purity 95.3%) was administered to Wistar rats (10 rats/sex/group) in the diet at concentrations of 0, 150, 600, or 2,400 ppm (0, 14, 61, or 300 mg/kg/day for males and 0, 20, 83, or 422 mg/kg/day for females). After the 13-week dosing period, animals were maintained without treatment for a 4 week recovery period. No mortality or clinical signs were observed. Body weights were reduced in males at 600 ppm (8% reduction) and in both sexes at 2,400 ppm (14-16% reduction). Food consumption was increased in both sexes at 2,400 ppm. Body weight reductions persisted through the recovery period. At the high dose, elevated activities of AP and ALT, decreased levels of protein, albumin, and cholesterol, and lengthening of blood clotting time were observed. Focal necrosis, single cell necrosis, swollen nuclei,

and cytoplasmic transformation were also reported in the livers of males at the high dose. At the mid dose, round cell infiltration, necrosis of hepatocytes, and cytoplasmic changes were observed in 3 males. Cal EPA reported that these effects are indicative of liver damage, and reported a NOAEL of 150 ppm (14 mg/kg/day) based on liver toxicity and reduced body weight in male rats at 600 ppm (61 mg/kg/day).

- Cal EPA 2006
    - *Oral*: In a 13-week subchronic neurotoxicity study that was primarily designed to evaluate neurotoxicity, imidacloprid (98.8% a.i.) was administered to Fischer-344 rats (12/sex/dose) in the diet at concentrations of 0, 150, 1,000, and 3,000 ppm (9.3, 63, and 196 mg/kg/day for males and 10.5, 69, and 213 mg/kg/day for females). Body weight was significantly decreased in both sexes at the mid and high doses. Decreases in serum triglyceride were seen at the mid and high doses and authors speculated this may indicate changes in metabolism in the liver. Authors identified a NOAEL of 9.3 mg/kg/day and LOAEL of 63 mg/kg/day.
    - *Oral*: In a 13-week oral toxicity study in Beagle dogs, imidacloprid (92.8% purity) was administered to 4 dogs/sex/group in 13-week study in the diet at concentrations of 0, 200, 600, or 1,800 ppm. A drastic reduction in body weight (8-20%) by week 6 was reported at the high dose, and was accompanied by a decrease in food intake (30-54%). Due to the low food intake, the concentration in the diet was then reduced to 1,200 for the remainder of the study. The adjusted doses were reported as 0, 8. 24, and 46 mg/kg/day. Trembling was seen in all animals at 600 and 1,800/1,200 ppm. Severe tremors were also reported for all dogs in the highest dose group. The mean body weights at the high dose remained lower (by 6% in females and 9% in males) at the end of the study. Cal EPA reports that effects were limited to clinical signs with no effects on survival or tissue damage, and identified a NOAEL of 200 ppm (8 mg/kg/day) based on clinical signs (tremors) at the LOAEL of 600 ppm (24 mg/kg/day). These effects will be considered under neurotoxicity, below. ToxServices identified a NOAEL and LOAEL of 600 ppm (24 mg/kg/day) and 1,800 ppm (46 mg/kg/day) based on reduced food consumption and body weight at the high dose.
- Bhardwaj et al. 2012
    - *Oral*: In a 90 day oral toxicity study in adult female Wistar rats, animals (10/dose) were administered imidacloprid (96% purity) by gavage (corn oil vehicle) at doses of 0, 5, 10 and 20 mg/kg/day. The control group received corn oil by gavage. Animals were monitored for clinical signs of toxicity, food consumption and body weight changes throughout the period of the study. Urine was collected at day 0 and day 90 for analysis (qualitative analysis of pH, specific gravity, presence of blood, ketone, urobilinogen, glucose, bilirubin, and protein). Rats were sacrificed after 90 days of dosing and necropsied; liver; brain, kidney, spleen, adrenal, uterus and ovary were removed, weighed, and examined for gross pathology. Blood was collected for hematology and serum chemistry. Significant clinical signs of toxicity (diarrhea, salivation, dyspnea, and piloerection), a decrease in body weight, and a decrease in food consumption were observed in animals at the highest dose (20 mg/kg/day). Overall weight gain at the high dose was reduced from 64% in the control group to 53% in the high dose group. The lower doses (5 and 10 mg/kg/day) did not produce any signs of toxicity. Relative weights of the liver, kidney and adrenals were significantly increased at the high dose (20 mg/kg/day); no other organs at any dose showed any significant change in weight. No significant changes were observed in hematological parameters or urine examination at any dose. A significant increase in serum GOT, GPT, glucose and blood urea nitrogen

was observed in animals exposed to the highest dose (20 mg/kg/day); all other biochemical parameters showed no changes at any dose. Authors concluded a NOAEL of 10 mg/kg/day and a LOAEL of 20 mg/kg/day based on effects on clinical signs of toxicity, organ body weight ratio, hematology, enzymatic changes, and urinalysis.

Subacute toxicity studies

- Mohany et al. 2012
    - *Oral*: In a 28 day oral repeated dose immunotoxicity study, adult male albino rats (20/dose, strain not specified) were administered imidacloprid as Confidor 20% EC[10] (trade name for imidacloprid) at a dose of 0.21 mg/kg (reported to be 1/100 of the LD_{50}) dissolved in distilled water by a blunt-ended syringe needle daily. Rats were sacrificed at the completion of the exposure, and the liver spleen and thymus were dissected and blood samples were collected for evaluation. A significant ($p<0.05$) increase in total leukocyte counts and a significant ($p<0.05$) decrease in leukocyte phagocytic activity, chemokinesis and chemotaxis were observed in the exposed animals compared to controls. Significant ($p<0.05$) increases in total immunoglobulin, IgG levels and hemagglutination of antibodies was observed in exposed animals compared to controls; there was no effect on IgM levels. Significant increases ($p<0.05$) in AST, ALT and ALP liver enzymes as well as MDA levels were observed in exposed animals compared to controls. During histopathologic examination, the spleens of exposed animals had larger sections of white pulp with low lymphocyte densities, more fibroblasts, red pulp congested with red blood cells, more bundles, and some pyknotic lymphocytes. The thymus of exposed animals exhibited lymphocytic depletion, lymphocyte invasion, fibroblasts, occasional eosinophilic cells, pyknotic nuclei, and focal areas of macrophage activity. Livers of exposed animals showed a heavily congested central vein and blood sinusoids, widely distributed pyknotic nuclei and leukocyte infiltration. Authors concluded imidacloprid is an inducer of immunotoxicity. A NOAEL and LOAEL could not be identified as it is unclear whether doses are for the trade name formulation or the active ingredient.
        - *Authors report that the dose of 0.21 mg/kg was equivalent to 1/100 of the LD_{50} for the formulation; however the corresponding LD_{50} of 21 mg/kg (it is unclear whether the dose presented in the paper is for the trade name formulation or for the active ingredient) is much lower than other reported LD_{50} values for imidacloprid. In light of this fact, and because no analytical measurements were conducted, authors reported severe histopathological effects at doses much lower than NOAEL values in other well-conducted studies, and because hematological changes are inconsistent with the lack of effects on hematological parameters in numerous other subchronic and chronic studies, this study was not weighed heavily in the assessment.*
- Badgujar et al. 2013
    - *Oral*: In a 28 day oral repeated dose immunotoxicity study, female BALB/c mice (6-8/dose) were administered imidacloprid (>98% purity) at doses of 2.5, 5, and 10 mg/kg (0.5% w/v aq. carboxymethyl cellulose vehicle) by oral gavage. Positive controls received cyclophosphamide or dexamethasone for five days, negative controls received carboxymethyl cellulose for 28 days. Animals were weighed daily. Seven days before completion, sets of treated/control mice were immunized by intraperitoneal injection of 0.3 mL sheep red blood cell suspension for evaluation of hemagglutinating antibody (HA) titer. On day 18, mice were sensitized by a subcutaneous injection of 50 µL of

[10] Confidor 20% EC is a trade name for imidacloprid. The composition of this trade name formulation could not be identified. It is unclear whether the dose presented in the paper is for the trade name formulation or from the active ingredient.

sheep red blood cell suspension. After 10 days, these mice were challenged to evaluate delayed-type hypersensitivity (DTH) response. Blood samples were collected at the end of the exposure period and mice were sacrificed. Spleen, liver, kidney and lungs were dissected for evaluation. All animals survived the experimental period. The high dose group (10 mg/kg) had decreased total leukocyte count, percent lymphocytes, and platelet count; the two lower dose groups (2.5 and 5 mg/kg) showed no changes. A slight body weight decrease (approximately 8%) was seen in the high dose group (10 mg/kg); no significant body weight changes were seen in any other dose group. All three dose levels had a decreased spleen weight. High and low doses (20 and 2.5 mg/kg, respectively) had no significant effects on serum anti-SRBC agglutinin titer; however, the mid dose (5 mg/kg) caused a significant ($p \leq 0.01$) decrease in titer. Intensity of DTH response was inversely related to the dose of imidacloprid administered; marked suppression was evident in the high dose group (10 mg/kg). The high dose group (10 mg/kg) had a significant decrease in lymphocyte proliferation; no other dose levels impacted proliferation. A seemingly dose-related depletion in lymphocytes in white pulp was observed in the spleen. The high dose group (10 mg/kg) also showed an increased presence of neutrophils and reticuloendothelial cells, along with congestion. Pathological alterations (moderate fatty degeneration) were reported in the liver in high dose animals. No significant changes were observed in the kidneys and lungs at any dose. Authors concluded that imidacloprid had direct immunotoxic effects in BALB/c mice under the conditions of the study. Authors established a NOAEL of 2.5 mg/kg/day for imidacloprid based on effects on immune function at 5 mg/kg/day.

- *As with the immunotoxicity study in rats, no information was provided regarding preparation of the dosing solution or analytical measurements, effects on body weight and pathology were seen at doses much lower than those producing systemic toxicity in longer studies in mice, and hematological changes are inconsistent with the lack of effects on hematological parameters in numerous other subchronic and chronic studies. Therefore this study was also not weighed heavily in the assessment.*

- Arfat et al. 2014
  - *Oral*: Nephrotoxicity and hepatotoxicity of imidacloprid were evaluated in a 15-exposure oral toxicity study. Male albino mice (12/dose, strain not specified) were administered imidacloprid as Confidor 20% EC (trade name for imidacloprid) at doses of 5, 10 and 15 mg/kg by oral gavage daily for 15 days[11]. Animals were monitored daily for changes in clinical signs and mortality. Urine was collected on days 0, 5, 10 and 15 for analysis; blood samples were collected on day 15 for analysis. Liver and kidneys were collected for evaluation. Repeated oral exposure to 5 and 10 mg/kg/day did not produce any signs of toxicity or mortality; however, at 15 mg/kg/day a significant decrease in body weight (approximately 6%) and clinical signs of toxicity (not specified) were observed. At the high dose (15 mg/kg/day) a significant increase in absolute and relative weight of the liver and kidney was observed. Statistically significant changes in serum creatinine and blood urea nitrogen in the urine showed renal damage in the high dose (15 mg/kg/day) group. Additionally, a significant increase in serum SGOT, SGPT, ALP and TBIL was observed in the high dose group, causing a significant rise in enzyme levels and total bilirubin. No changes were seen in mice administered 5 or 10 mg/kg/day. Hepatocytes of animals from the high dose group (15mg/kg/day) showed mild focal necrosis with swollen cellular nuclei, hypertrophied blood vessels and cytoplasmic lesions.

[11] Confidor 20% EC is a trade name for imidacloprid. The composition of this trade name formulation could not be identified.

Degeneration of tubules, glomeruli and observed hemolysis were evident in the kidney of the high dose group (15 mg/kg/day). The low dose groups (5 and 10 mg/kg/day) showed no pathological changes in the liver or kidney. Authors concluded a NOAEL of 10 mg/kg and an LOAEL of 15 mg/kg based on effects on organ body weight ratio, clinical chemistry, urinalysis, and histopathology at the high dose.

- *ToxServices did not weigh this study heavily in the assessment as extrapolation from the guidance values due to the short duration (6–fold shorter than 90 days) may be overly conservative.*

- Cal EPA 2006, SRC 2005
  - *Oral*: In a 4-week oral toxicity study in Beagle dogs, imidacloprid (92.8% purity) was administered to 2 dogs/sex/dose at concentrations of 0, 200, 1,000, or 5,000 ppm (0, 7.3, 31, or 49 mg/kg/day). All animals at the high dose died or were sacrificed before completion of the study. Clinical signs reported in dogs that died in this group included marked reduction in food intake, weight loss, severe tremor, ataxia, and vomiting. Atrophy of the thyroid glands and bone marrow, advanced involution of the thymus, and testicular tubule degeneration were also observed at this dose. At 1,000 ppm authors reported a decrease in food consumption, hypertrophy of hepatocytes, and follicular atrophy of the thyroid. Cal EPA reported a NOAEL of 200 ppm (7.3 mg/kg/day) based on treatment-related effects at 1,000 ppm (31 mg/kg/day). Due to the 28-day duration of this study, these values are compared to tripled guidance values (i.e. 30-300 mg/kg/day for a Moderate).
- Bayer AG 1993
  - *Inhalation*: In a 28 day repeated dose inhalation study conducted according to OECD Guideline 412, male and female Wistar rats (10/dose) were exposed to imidacloprid (95.2% purity) dust at concentrations of 0.006, 0.031 or 0.191 mg/L via nose only inhalation for 6 hours a day five days a week. Animals were monitored for signs of toxicity and mortality daily and body weights were recorded initially and at the conclusion of the exposure. Rats were sacrificed and evaluated for ophthalmoscopy, clinical chemistry, hematology, urinalysis, gross pathology, and gravimetric analysis and histopathology or organs. No significant treatment-related signs of toxicity were observed. Slight but statistically significant reductions in body weight (5.9-8.6%) were observed in males at the high dose. Mixed function oxidase induction was observed in the liver tissue in females at the two highest doses and in males at the highest dose. Urinary pH was significantly increased in females at the high dose. Changes in organ weights were minimal and did not exhibit a dose response. No additional treatment-related effects were observed. Authors concluded the liver is the most sensitive organ and there is functional hepatic impairment; however, because these signs were not accompanied by overt toxicity a NOAEL of 0.191 mg/L, the highest dose tested, was established by study authors.

Table 3: Oral Toxicity Studies for Imidacloprid

Species	Duration	Route of Exposure	NOAEL mg/kg/day	LOAEL mg/kg.day	Critical Effect(s)	Reference
Chronic toxicity studies						
Wistar rats	2 years	Diet	5.7	17	Mineralization of the thyroid (no effects on hormones) indicative of premature aging	Cal EPA 2006, SRC 2005
B6C3F1 mice	2 years	Diet	47	143	Body weight reduction; histopathological changes in	Cal EPA 2006, SRC 2005

Table 3: Oral Toxicity Studies for Imidacloprid						
Species	**Duration**	**Route of Exposure**	**NOAEL mg/kg/day**	**LOAEL mg/kg.day**	**Critical Effect(s)**	**Reference**
					liver at higher doses	
Beagle dogs	1 year	Diet	15	41/72	Increased liver weight and plasma cholesterol	Cal EPA 2006, SRC 2005
Subchronic toxicity studies						
Wistar/Han rats	Approximately 105 days	Diet	52 mg/kg/day (males); 57.4 mg/kg/day (females)	NA	No effects at highest dose	Clement International 1993
Wistar rats	98 days	Diet	14	78	Reduced body weight in females; Liver toxicity at higher doses	Cal EPA 2006, SRC 2005
Wistar rats	90 days	Diet	14	61	Increased AP and ALT, decreased protein, albumin, and cholesterol, and histopathological changes in the liver	Cal EPA 2006, SRC 2005
Wistar rats	90 days	Gavage	10	20	Clinical toxicity, changes in organ weight, hematology, urinalysis	Bhardwaj et al. 2012
Fischer 344 rats	90 days	Diet	9.3	63	Body weight reduction, decreased serum triglyceride	Cal EPA 2006
B6C3F1 mice	107 days	Diet	86	427	Poor appearance, reduced body weight, mortality; Liver toxicity at higher doses	Cal EPA 2006, SRC 2005
Beagle dogs	90 days	Diet	24	46	Decreased body weight	Cal EPA 2006
Subacute toxicity studies						
Rat (strain not specified)	28 days	Gavage	Not identified due to poor dose reporting	Not identified due to poor dose reporting	Immunosuppression, increased iver enzymes, histopathological changes in liver	Mohany et al. 2012
BALB/c mice	28 days	Gavage	2.5	5	Immunosuppression	Badgujar et al. 2013
Mice (strain not specified)	15 days	Gavage	10	15	Changes in relative organ weights, clinical chemistry, urinalysis, and, histopathology of kidney and liver	Arfat et al. 2014
Beagle dogs	28 days	Diet	7.3	31	Decreased food consumption, hypertrophy of hepatocytes, follicular atrophy of thyroid	Cal EPA 2006, SRC 2005

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of Moderate was assigned. One subacute inhalation study in rats showed slight effects on body weight at a concentration of 0.191 mg/L, which falls within the guidance values of 0.06-0.6 mg/L for a 28-day dust inhalation study (tripled due to < 90 day duration). Rather than extrapolating from short term oral toxicity studies, ToxServices focused primarily on the subchronic (90 day) and chronic toxicity studies to assign the score, due to the availability of well reported studies. The majority of subchronic and chronic studies reported NOAEL values between the guidance values of 10 and 100 mg/kg/day for an oral toxicity study (presented in Table 3). One exception is the NOAEL of 5.7 mg/kg/day for mineralization of the thyroid, likely representative of premature aging, in a chronic study in rats. Because the LOAEL of 17 mg/kg/day is greater than the guidance values, it is not possible to

determine if effects would have been seen at doses corresponding to a score of High. Similar mineralization has been seen in one previously described study in birds, but this effect has not been reported in other studies in standard laboratory species. Two studies reported effects on the immune system of rats and mice, but these studies are of limited reliability, and the hematological changes seen in these studies were not observed in numerous other subchronic and chronic studies. Remaining subchronic and chronic studies report NOAEL values between 10 and 100 mg/kg/day based on general toxicity, effects on body weight, and effects on the liver. Similar effects were seen in subacute studies, but ToxServices based the hazard classification on the longer studies rather than extrapolating from studies of shorter duration. In addition, ToxServices noted that the studies that reported the lowest LOAELs used the gavage route, while the other studies were conducted via the dietary route. The dietary route is more relevant to humans as the exposure pattern is more similar to the ways humans are exposed to the pesticide. Therefore a score of Moderate was assigned. Confidence in the score is high because the lowest LOAELs fall below the GHS cutoff of 100 mg/kg/day, making it at least classified to GHS category 2. It is not possible to conclusively determine if thyroid mineralization in rats would have been seen below the guidance value for GHS category 1 classification, and two subacute studies of limited reliability suggest the potential for effects on the immune system that were not observed in other studies may raise some uncertainties regarding if imidacloprid can be assigned a higher score than Moderate.

Neurotoxicity (N)

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

Imidacloprid was assigned a score of Very High for neurotoxicity (single dose) based on neurological effects consistently seen at oral doses of less than 300 mg/kg in acute toxicity studies. GreenScreen® criteria classify chemicals as a Very High hazard for neurotoxicity (single dose) when neurotoxic effects are seen at doses of 300 mg/kg and below (CPA 2012a). Confidence in the score is high due to the consistency of effects seen in several acute studies.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Grandjean and Landrigan (2014) –Known to be neurotoxic in man
- Classified as a developmental neurotoxicant (Grandjean and Landrigan 2006, 2014).
- Cal EPA 2006, SRC 2005
  - *Oral*: In a previously described acute neurotoxicity study, imidacloprid (98.8% purity) was administered to Sprague-Dawley rats (18/sex/dose) by gavage as a single dose of 0, 42, 151, or 307 mg/kg. Neurobehavioral signs were evaluated in 12 of the 18 rats using a Functional Observational Battery (FOB) and 6 rats/sex/group were subjected to pathological evaluation (brain, spinal cord, eyes, peripheral nerves (sciatic, sural and tibial), gasserian ganglion and gastrocnemius muscles). Clinical observations and the FOB were conducted prior to treatment, and 90 minutes, 7 days, and 14 days after treatment. Motor activity was evaluated at pretest, 2.5 and 4.5 hours after treatment, and 7 and 14 days after treatment. At the highest dose, four males and ten females died within 24 hours post-treatment. All animals that died were alive four hours post-treatment and displayed severe tremors, cool-to-touch bodies, and reduced body temperatures. Central nervous system (CNS) effects at the high dose included markedly decrease activity, reduced response to stimuli, uncoordinated gait, decreased rearing and gait strength, and impaired aerial righting. At the 151 mg/kg dose, a higher incidence of animals sitting or lying in the cage and tremors was observed. Animals in the 151 and 307 mg/kg dose groups showed a statistically significant decrease in motor and locomotor activity in the

figure-8 maze (21-89% decrease). Females in the lowest dose group exhibited a nonsignificant decrease (25-27%) in motor activity. Decreased activity in surviving males in the high dose group persisted through 7 and 14 days after treatment. Cal EPA identified a NOAEL of 42 mg/kg/day based on statistically significant decreases motor and locomotor activity at the LOAEL of 151 mg/kg/day. Cal EPA reports that U.S. EPA considered the LOAEL to be 42 mg/kg/day. The risk assessment report for the USDA Forest Service also reports a LOAEL of 42 mg/kg/day.

- *Oral*: In a previously described acute oral toxicity study in rats, imidacloprid (purity 94.2%) was administered by gavage to Wistar rats (5/sex/group) as a single dose of 50, 100, 250, 315, 400, and 475 mg/kg in males and 100, 250, 315, 400, 475, 500, and 1,800 mg/kg in females. The duration of the observation period was not stated. Clinical signs seen at doses of 100 mg/kg and above included apathy, labored breathing, tremors, spasms, gait incoordination, and decreased motility. The NOAEL for systemic effects was reported by Cal EPA to be 50 mg/kg based on the clinical signs at 100 mg/kg.
- *Oral*: In a previously described acute oral toxicity study in mice, imidacloprid (94.2%) was administered to mice (5/sex/group) by gavage at doses of 10, 71, 100, 120, 140, 160, and 250 mg/kg in males and 100, 120, 140, 160, and 250 mg/kg in females. The duration of the observation period was not stated. Animals were observed for clinical signs and gross pathology. All dose groups except the 10 mg/kg group displayed clinical signs, including apathy, labored breathing, and decreased motility (71/mg/kg/day), staggering gait and severe trembling (100-250 mg/kg). Toxicity was evident within 5-10 minutes following administration. Cal EPA reported a NOAEL of 10 mg/kg based on clinical signs in males observed at 71 mg/kg.
- *Dermal*: In the previously described acute dermal toxicity study in Wistar rats, a single dose of 5,000 mg/kg imidacloprid paste (94.2% in 0.9% NaCl) was administered to the skin of the shaved backs of 5 animals/sex and application sites were covered (not specifically stated whether coverage was occlusive or semiocclusive) for 24 hours. There were no clinical signs of toxicity, and there were no gross pathological changes. ToxServices identified a NOAEL of 5,000 mg/kg based on a lack of effects at the only dose tested.
- *Inhalation*: In a previously described acute inhalation toxicity study in rats, imidacloprid was administered to Wistar rats (5/sex/dose) in the form of dust via head/nose only inhalation for 4 hours. Concentrations were 1,220, 2,577, and 5,323 mg/m^3 (1.22, 2.577 and 5.323 mg/L). One control group received air. Within 4-6 hours post-treatment at concentrations higher than 1,220 mg/m^3, the following clinical signs were observed: difficulty breathing, reduced motility, piloerection, and tremors. Cal EPA reported a NOAEL of 1,220 mg/m^3 (1.22 mg/L) based on clinical signs at 2,577 mg/m^3 (2.577 mg/L).

- U.S. EPA 1993
  - *Oral:* In a previously described acute oral toxicity study in rats, imidacloprid (76.1% purity) was administered as a single dose to male and female Sprague-Dawley rats (5/sex/dose) by gavage in deionized water at doses of 1,063, 2,180 2,750 and 3,170 mg/kg in females and 1,063, 2,180 and 3,170 mg/kg in males. Animals were observed for toxicity twice daily for 14 days. All surviving animals were sacrificed on day 14 and gross necropsy was performed on all animals of the study. Treatment-related signs of toxicity were observed on day of dosing and included tremors, increased reactivity, and decreased activity; all signs resolved in survivors by day 14. No animals surviving

showed gross lesions. Authors concluded a NOAEL < 1,063 mg/kg for males and females.

- *Dermal:* In a previously described acute dermal toxicity study in rats, imidacloprid (76.1% purity) was administered once to male and female Sprague-Dawley rats (5/sex/dose) at a dose of 2,000 mg/kg. Animals were observed for toxicity twice daily for 14 days. Body weights were taken on days 7 and 14 post treatment, and all animals were subject to gross pathological examination after sacrifice on day 14 post treatment. No neurobehavioral changes were reported.
- *Inhalation:* In a previously described acute inhalation study in rats, imidacloprid (76.1% purity) was administered to Sprague-Dawley rats (6/sex/dose) at concentrations of 2,110, 2,810, or 2,990 mg/m^3 (2.11, 2.81, or 2.99 mg/L) as a liquid aerosol in water via nose only inhalation for 4 hours. Animals were observed for 14 days. Clinical signs of toxicity included ataxia, convulsions, hypoactivity, moribundity, and tremors at all doses. Clinical signs resolved by observation day 6. There were no gross pathological changes in surviving animals.

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of Very High was assigned. Acute oral and inhalation toxicity studies consistently report neurological effects. Some effects such as ataxia and effects may be considered as transient narcotic effects as effects resolved during the observation period in most studies. However, some studies reported more severe effects such as tremors and convulsions. In addition, one oral study in rats that was designed specifically to evaluate neurotoxicity reported effects on mobility that persisted up to 14 days after treatment with 307 mg/kg. While this dose exceeds the guidance value of 300 mg/kg, because it is only slightly above the guidance value and the next lowest dose is 151 mg/kg, it is likely that similar persistent effects would have been observed at doses below 300 mg/kg. Therefore a score of Very High was assigned.

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

Imidacloprid was assigned a score of High for neurotoxicity (repeated dose) based on subchronic oral studies in rats demonstrating statistically significant effects on learning activities at a dose of 8 mg/kg/day and non-statistically significant effects on grip strength, righting response, and locomotor activity at doses of 9.3 and 63 mg/kg/day. GreenScreen® criteria classify chemicals as a High hazard for neurotoxicity (repeated dose) when neurological effects are seen between the guidance values of 10 and 100 mg/kg/day (CPA 2012a). Confidence in the score is reduced as the critical study did not report the purity of the material tested or whether it tested a formulated pesticide product or pure imidacloprid.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Grandjean and Landrigan (2014) –Known to be neurotoxic in man
- Classified as a developmental neurotoxicant (Grandjean and Landrigan 2006, 2014).
- Cal EPA 2006, SRC 2005
  - *Oral*: In a previously described 2-year chronic toxicity and carcinogenicity study, imidacloprid (94.3% active ingredient) was administered to Wistar rats (50/sex/group) in the diet at concentrations of 0, 100, 300, and 900 ppm (0, 5.7, 17, or 51 mg/kg/day in males and 0, 7.6, 25, or 73 mg/kg/day in females). Interim examinations were conducted on an additional 10 rats/sex/dose after 1 year after treatment. A supplemental study to

determine the maximum tolerated dose (MTD) was conducted on 50 rats/sex/dose that were either used as controls or administered imidacloprid at a concentration of 1,800 ppm (103 mg/kg/day in males and 144 mg/kg/day in females) for two years. There were no effects on plasma, red cell, or brain cholinesterase at any dose.

- Cal EPA 2006
  - *Oral*: In a 13-week subchronic neurotoxicity study, imidacloprid (98.8% a.i.) was administered to Fischer-344 rats (12/sex/dose) in the diet at concentrations of 0. 150, 1,000, and 3,000 ppm (9.3, 63, and 196 mg/kg/day for males and 10.5, 69, and 213 mg/kg/day for females). A FOB and figure-8 maze activity test were conducted prior to treatment and again at weeks 4, 8, and 13. Reduced grip strength was measured in males at the high dose, and the reduction became statistically significant by week 8. At study termination, an increased incidence of uncoordinated righting response was seen in males at all treatment doses, with 2, 3, and 7 males showing uncoordinated response at the low, mid, and high doses (statistically significant at the high dose), respectively. Females in the high dose group had decreased locomotor activity in all tests at weeks 4, 8, and 13. The magnitude of the effect was up to 21%, but this was not statistically significant and did not reach the magnitude of the effect seen in the acute neurotoxicity study. Cal EPA reports that this may reflect some degree of adaptation over time. Study authors established a NOAEL of 9.3 mg/kg/day based on reductions in body weight and food consumption at 63 mg/kg/day, which are described above for systemic toxicity. ToxServices identified a NOAEL of 63 mg/kg/day for neurotoxicity based on statistically significant effects on grip strength and righting response in males and a nonsignificant decrease in locomotor activity in females.
- Oak Ridge National Laboratory 2002
  - In a previously described developmental neurotoxicity study (GLP compliant OPPT 870.6300/OECD 426) imidacloprid (98.2-98.4% purity) was administered to female Wistar rats (30/dose) via corn oil in the diet at concentrations 0, 100, 250 or 750 ppm from gestation day 0 through postnatal day 21. Average daily intake of imidacloprid was 0, 8.0-8.3, 19.4-19.7 and 54.7-58.4 mg/kg/day during gestation and 0, 12.8-19.5, 30.0-45.4 and 80.4-155.0 mg/kg/day during lactation for the 0, 100, 250 and 700 ppm dose levels, respectively. A functional operation battery (FOB) was performed on all dams on gestation days 6, 13 and 20. A FOB was performed on 10 dams/dose on lactation days 4, 11 and 21. There were no treatment-related effects on functional observation. ToxServices identified a NOAEL of 54.7-58.4 mg/kg/day during gestation and 80.4-155 mg/kg/day during lactation.
- Bhardwaj et al. 2012
  - *Oral*: In a previously described 90 day oral toxicity study in adult female Wistar rats, animals (10/dose) were administered imidacloprid (96% purity) by gavage (corn oil vehicle) at doses of 0, 5, 10 and 20 mg/kg/day. The control group received corn oil by gavage. Animals were monitored for clinical signs of toxicity throughout the period of the study. Rats were sacrificed after 90 days of dosing and necropsied; the brain was removed, weighed, and examined for gross pathology. Authors evaluated spontaneous locomotor activity (SLA) by using a SLA task before the initial exposure and at day 90. The brain did not show any significant change in weight. Significant alterations in various aspects of spontaneous locomotor activity were observed. Animals exposed to 20 mg/kg/day showed significant decreases in distance traveled, ambulatory time and stereotypic time; however there was an increase in resting time. Animals exposed to 10 mg/kg/day showed a decrease in ambulatory time; no other alterations in SLA were found

in animals exposed to imidacloprid at 10 or 5 mg/kg/day. A dose dependent inhibition of AChE activity was observed in the brain and serum; however significant inhibition occurred only at the highest dose (20 mg/kg/day). Histopathologically, exposure to the high dose (20 mg/kg/day) produced necrosed Purkinji cells with loss of dendrites and granules in the granular layer of cerebellum. No pathological changes were observed in animals in the 5 and 10 mg/kg/day dose groups. Authors concluded a NOAEL of 10 mg/kg/day and a LOAEL of 20 mg/kg/day based on effects on histopathology and neurobehavioral examination.

- Kara et al. 2015
    - *Oral*: Kara et al. evaluated the effects or oral imidacloprid exposure on the brains of adult Wistar albino rats. Adult male rats (6/group) were administered 0.5, 2, or 8 mg/kg imidacloprid (purity not reported) via gavage and their learning activities were evaluated by Morris water maze test and a probe test. Measurements of inotropic glutamate receptor GRIN1, synoptophysin, growth-associated protein 43 and the muscarinic receptor M1 expression levels in the hippocampus were also made. The time at which these measurements were made was not described in the publication. Learning activities were significantly reduced at the high dose. Expression of M1 was significantly changed in this dose as well. The authors concluded that imidacloprid in high doses caused deterioration in cognitive functions, which may be associated with changes in gene expression. ToxServices assigned a NOAEL and LOAEL of 2 and 8 mg/kg/day, respectively, based on decreased learning activities.
- Memon et al. 2014
    - *Oral*: In a reproductive toxicity study, male rabbits (*Oryctolagus cuniculus*) (10/dose/duration) received 0 or 45 mg/kg imidacloprid (purity not reported) daily by disposable syringe in 10 mL distilled water for 10 or 20 consecutive days. Control groups received distilled water for the same time period. Animals were monitored daily for changes in clinical signs. Animals exposed to imidacloprid had behavioral changes and other health problems, such as decreased movement, trembling, fatigue, convulsion, dizziness, tremors and diarrhea.
- Keil et al. 2014
    - A case control study investigated the association of autism spectrum disorders (ASD) and imidacloprid exposure. The study used Bayesian logistic models to estimate the association and correct for potential differential exposure misclassification due to recall. The odds of prenatal imidacloprid exposure among children with ASD were slightly higher, with an odds ratio of 1.3. A susceptibility window analysis yielded higher odds ratios for exposures during pregnancy than for early life exposures. When limited to frequent users of imidacloprid, the odds ratio increased to 2.0. Authors concluded the association could possibly result from exposure misclassification alone, and the association warrants further investigation.
- Marfo et al. 2015
    - Marfo et al. evaluated the concentration of several neonicotinoid pesticides, including imidacloprid, in asymptomatic individuals (n=50) as well as individuals displaying typical (n=19) or atypical (n=16) neurological symptoms (typical symptoms included headache, general fatigue, palpitation/chest pain, abdominal pain, muscle pain/weakness/spasm, and cough). Imidacloprid was not detected in the urine of any individuals from the asymptomatic, typical symptomatic, or atypical symptomatic groups.

- Li et al. 2011
    - The action of imidacloprid on human neuronal α4β2 nicotinic acetylcholine receptors was investigated in an *in vitro* study, using human embryonic kidney 293 cells. Imidacloprid directly activated the human nicotinic receptors; however, it was only a weak agonist of the receptor. Exposure to imidacloprid also reduced responses to acetylcholine. Authors concluded imidacloprid to be a low-efficacy but high-affinity agonist of the human α4β2 receptor and that imidacloprid may have stronger side effects on humans.

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of High was assigned. No effects were seen in a FOB in Wistar rats that were orally administered up to 58.4 mg/kg/day during gestation and 155 mg/kg/day during lactation. There were also no effects on plasma, red cell, or brain cholinesterase in Wistar rats administered up to 73 mg/kg/day for 2 years. However, behavioral changes and trembling, convulsions, and tremors were seen in rabbits administered 45 mg/kg/day for 10-20 days. One subchronic study in Wistar rats identified a NOAEL of 10 mg/kg/day based on effects on locomotor activity and brain pathology at a dose of 20 mg/kg/day. A second subchronic neurotoxicity study in Wistar rats identified a NOAEL of 63 mg/kg/day based on statistically significant effects on grip strength, righting response, and locomotor activity at a dose of 196 mg/kg/day. These effects were also observed at doses of 9.3 and 63 mg/kg/day but did not reach statistical significance at these doses. One subchronic gavage study reported significantly reduced learning activities (water maze and probe tests) at 8 mg/kg/day in rats. However, the authors of this study did not report the purity of the material tested, or whether pure imidacloprid or a pesticide formulation was tested. Based on this LOAEL of 8 mg/kg/day, a score of High was assigned. This is supported by *in vitro* studies demonstrating that imidacloprid interacts with the cholinergic system and the well-established mechanism for imidacloprid's activity.

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

Imidacloprid was assigned a score of Low for skin sensitization based on negative results in numerous guinea pig maximization tests and a mouse LLNA. GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization when available data indicate that the chemical does not warrant GHS classification for skin sensitization and the chemical is not present on authoritative or screening lists (CPA 2012a). Confidence in the score is high because it is based on consistently negative results in experimental studies.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists
    - *Screening:* Not present on any screening lists
- U.S. EPA 1993
    - In a dermal sensitization study, male DHPW guinea pigs (15/dose) were dermally administered 0.4 mL of a 7.5% (w/v in deionized water) suspension of imidacloprid (76.1% purity) to their shaved backs. Animals received three topical induction applications (6 hour duration) on days 0, 7 and 14 of the study, followed by a topical challenge application (24 hour duration) on day 27. Dermal irritation scores were determined approximately 24 and 48 hours after each induction and challenge treatment. Animals showed no sensitization response to imidacloprid. Authors concluded imidacloprid to be not dermally sensitizing under the conditions of this study.

- SRC 2005
    - Permatek IM 30, a formulation containing 32 g/L imidacloprid, was not sensitizing in a local lymph node assay in 5 female CBA mice when tested at concentrations of 0, 25, 50, or 100%. No additional details were provided.
    - SRC reviewed the results of numerous guinea pig Buehler and maximization tests that were conducted on several different imidacloprid formulations containing up to 94.2% active ingredient. Negative results were obtained in all of the assays.

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of Low was assigned as imidacloprid was negative in numerous guinea pig maximization tests as well as a mouse LLNA. Confidence in the score is high because it is based on consistently negative results in experimental studies.

Respiratory Sensitization (SnR) Group II* Score (H, M, or L): DG
Imidacloprid was assigned a score of Data Gap for respiratory sensitization based on a lack of adequate data for this endpoint.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists
    - *Screening:* Not present on any screening lists
- Hernandez et al. 2008
    - A cross sectional study was conducted to evaluate potential respiratory function abnormalities following long term pesticide exposure by means of complete pulmonary function testing. The study population was comprised of workers from a prominent agriculture area of southern Spain. A questionnaire was used to determine sociodemographic factors, occupational exposure and clinical symptoms. Multiple regression analysis showed a relationship of short term exposures to pesticides with reduced forced expired volume, and a long term exposure relationship with reduced forced expiratory flow rate. A relationship was found between neonicotinoid insecticides and lower pulmonary volumes, suggestive of restrictive lung disease, and with an increased risk of reporting irritative symptoms.
- Hernandez et al. 2011
    - Several clinical and epidemiological studies have reported an association between exposure to pesticides, such as imidacloprid, and bronchial hyper-reactivity and asthma symptoms. Hernandez et al. summarized that pesticide aerosols can lead to asthma by interaction with functional irritant receptors in the airway and promoting neurogenic inflammation. Some pesticides (organophosphorus) can disrupt negative feedback control of cholinergic regulation in the lungs, acting synergistically with allergen sensitization leaving individuals more susceptible for developing asthma. Overall, many pesticides were found to be sensitizers or irritants capable of directly damaging the airway, increasing the risk of developing asthma.

ToxServices' summary and conclusion:

- Based on the weight of evidence, a Data Gap was assigned. There have been reports of effects on respiratory function due to exposure to pesticides, but it is unclear whether effects represent an allergic asthmatic response rather than an irritant response or other respiratory effect. Furthermore, no studies have specifically investigated the relationship between imidacloprid

exposure and respiratory sensitization. In the absence of sufficient data, a Data Gap was assigned.

Skin Irritation/Corrosivity (IrS) Group II Score (vH, H, M, or L): L
Imidacloprid was assigned a score of Low for skin irritation/corrosivity based on negative results in two dermal irritation studies in rabbits. GreenScreen® criteria classify chemicals as a Low hazard for skin irritation/corrosivity when available data indicate that the chemical does not warrant GHS classification for skin irritation and the chemical is not present on authoritative or screening lists (CPA 2012a). Confidence in the score is high because it is based on consistent results in two studies.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- U.S. EPA 1993
  - In a dermal irritation study, imidacloprid (76.1% purity) was administered to 6 male New Zealand White rabbits at a dose of 500 mg for four hours under semiocclusion. Animals were observed for signs of erythema and edema formation 1 hour, 24 hours, 48 hours, 72 hours and 7 days post dosing; findings were recorded according to the Draize method. Erythema (grade 2) was observed in five animals and edema (grade 1) was observed in one animal one hour following exposure. All signs of irritation were resolved within 7 days. A primary irritation index of 1.08 was calculated. Authors concluded imidacloprid to be mildly irritating and classified it in Category IV for dermal irritation. The scores at 24, 48, and 72 hours were not reported.
- Cal EPA 2006
  - In a dermal irritation study, imidacloprid (purity 94.2%) was administered as a paste to three rabbits (strain, sex not specified) at a dose of 500 mg for four hours (not specifically stated whether the test substance was administered under occlusion or semiocclusion). The skin was then examined for erythema and edema for up to 14 days post-treatment. One animal developed erythema (grade 1) one hour post treatment, but effects resolved within 24 hours. Based on these results, study authors determined that imidacloprid was not a skin irritant.

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of Low was assigned. Two studies in rabbits demonstrate the potential for mild skin irritation within an hour of treatment. One study indicated that effects resolved within 7 days, but the scores at 24, 48, and 72 hours were not reported. The second study demonstrated recovery within 24 hours. These data indicate that imidacloprid does not warrant GHS classification for skin irritation, as the scores for erythema and edema at 24, 48, and 72 hours are expected to be 0.

Eye Irritation/Corrosivity (IrE) Group II Score (vH, H, M, or L): L
Imidacloprid was assigned a score of Low for eye irritation/corrosivity based on negative results in two eye irritation studies in rabbits. GreenScreen® criteria classify chemicals as a Low hazard for eye irritation/corrosivity when available data indicate that the chemical does not warrant GHS classification for eye irritation and the chemical is not present on authoritative or screening lists (CPA 2012a). Confidence in the score is high because it is based on consistent results in two studies.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists

  - *Screening:* Not present on any screening lists
- Cal EPA 2006
  - In an eye irritation study in rabbits, imidacloprid (purity 94.2%) was administered to the eyes of three rabbits (sex, strain not specified) at a dose of 60 mg/eye in 0.1 mL physiologic solution (composition not specified). At 1 hour post treatment, conjunctival irritation was graded as a 2 on a scale of 1-3. Effects resolved within 24 hours. No additional details were provided. Study authors concluded that imidacloprid did not possess an eye irritant potential to the eye.
- U.S. EPA 1993
  - In an eye irritation study, imidacloprid (76.1% purity) was administered to the left eye of six male New Zealand White rabbits at a dose 0.1 mL pulverized test material per animal (0.44 – 0.46 mg). The right eye served as control in each animal. Rabbits were observed for signs of toxicity to the cornea, iris and conjunctivae according to the Draize method. Lacrimation was also assessed. Observations were made 1 hour, 24 hours, 48 hours, 72 hours, 7 days and 14 days post dosing. The cornea and iris were not adversely affected in any of the animals. Conjunctival redness (grade 1), chemosis (grade 1) and ocular discharge (grades 2 or 3) was observed in all six animals. The mean scores for conjunctival redness at 24, 48, and 72 hours were 1 for all animals. The mean scores for chemosis at 24, 48, and 72 hours were 1 in two of the animals and 0.67 in four of the animals. All redness resolved by 7 days except for grade 1 slight redness which persisted past 7 days in one animal but resolved by day 14. Authors concluded imidacloprid to be a minimal eye irritant and classified it in Category III for ocular irritation.

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of Low was assigned. Slight irritation (conjunctival redness and chemosis) was observed in two ocular irritation studies in rabbits, but 24/48/72 hour irritation scores did not reach the guidance value of 2 for conjunctival redness and chemosis and therefore the test substance does not warrant GHS classification for eye irritation.

Ecotoxicity (Ecotox)[12]

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

Imidacloprid was assigned a score of vH for acute aquatic toxicity based on acute EC_{50} values as low as 0.034 mg/L in mysid shrimp and association with H400 – Very toxic to aquatic life and EU Risk Phrase R50 – Very toxic to aquatic organisms. GreenScreen® criteria classify chemicals as a Very High hazard for acute aquatic toxicity when the chemical is associated with H400 and R50 and the most conservative L/EC_{50} values are less than 1 mg/L (CPA 2012a). Confidence in the score is high because it is based on authoritative listings supported by data.

- Authoritative and Screening Lists
  - *Authoritative:* CLP/GHS Hazard Statement H400 – Very toxic to aquatic life
  - *Authoritative:* EU Risk Phrase R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment[13]

[12] Per GreenScreen® guidance, terrestrial toxicity was evaluated according to U.S. EPA's Design for the Environment (DfE) Alternatives Assessment Criteria for Hazard Evaluation (DfE 2011).
[13] This is an authoritative EU R-phrase that addresses a combination of hazards including aquatic toxicity, persistence, and/or bioaccumulation

  - *Screening:* H410 - Aquatic Chronic 1 - Very toxic to aquatic life with long lasting effects (does not correspond to GreenScreen® criteria) [14]
  - *Screening:* GHS New Zealand Category 9.1A (crustacean) (GHS Category 1) – Very ecotoxic in the aquatic environment.
- U.S. EPA 2012a
  - Imidacloprid is designated to the Aliphatic Amines, Halopyrdines, Neonicotinoids, and Neutral Organics ECOSAR chemical classes. The most conservative predicted L/EC_{50} values are 5.132 mg/L in fish (96-hr), 2.812 mg/L in daphnia (48-hr), and 50.658 mg/L in green algae (96-hr) (Appendix D).
- Roessink et al. 2013
  - 96h EC_{50} (immobilization) = 119 µg/L (0.119 mg/L) (*Asellus aquaticus*, freshwater crustacean)
  - 96h LC_{50} = 316 µg/L (0.316 mg/L) (*Asellus aquaticus*, freshwater crustacean)
  - 96h EC_{50} (immobilization) = 18.3 µg/L (0.0183 mg/L) (*Gammarus pulex*, freshwater crustacean)
  - 96h LC_{50} = 263 µg/L (0.263 mg/L) (*Gammarus pulex*, arthropod)
  - 96h EC_{50} (immobilization) = 284 µg/L (0.284 mg/L) (*Chaoborus obscuripes*, insect larvae)
  - 96h LC_{50} = 294 µg/L (0.294 mg/L) (*Chaoborus obscuripes*, insect larvae)
  - 96h EC_{50} (immobilization) = 50.6 µg/L (0.0506 mg/L) (*Sialis lutaria*, insect larvae)
  - 96h LC_{50} >10,000 µg/L (*Sialis lutaria*, insect larvae)
  - 96h EC_{50} (immobilization) = 35.9 µg/L (0.0359 mg/L) (*Plea minutissima*, insect larvae)
  - 96h LC_{50} = 37.5 µg/L (*Plea minutissima*, insect larvae)
  - 96h EC_{50} (immobilization) = 18.2 µg/L (0.0182 mg/L) (*Notonecta* spp., insect larvae)
  - 96h LC_{50} >10,000 µg/L (*Notonecta* spp., arthropod)
  - 96h EC_{50} (immobilization) = 10.8 µg/L (0.0108 mg/L) (*Micronecta* spp., insect larvae)
  - 96h LC_{50} = 28.2 µg/L (*Micronecta* spp., arthropod)
  - 96h EC_{50} (immobilization) = 1.79 µg/L (0.00179 mg/L) (*Limnephilidae*, insect larvae)
  - 96h LC_{50} = 25.7 µg/L (0.0257 mg/L) (*Limnephilidae*, arthropod)
  - 96h EC_{50} (immobilization) = 1.77 µg/L (0.00177 mg/L) (*Caenis horaria*, insect larvae)
  - 96h LC_{50} = 6.68 µg/L (0.00668 mg/L) (*Caenis horaria*, arthropod)
  - 96h EC_{50} (immobilization) = 1.02 µg/L (0.00102 mg/L) (*Cleon dipterum*, insect larvae)
  - 96h LC_{50} = 26.3 µg/L (*Cleon dipterum*, insect larvae)
- Goulson 2013
  - 96h LC_{50} = 65 µg/L (0.065 mg/L) (*Hyalella azteca*, freshwater crustacean)
  - 48h LC_{50} = 17.4 mg/L (*Daphnia magna*, freshwater crustacean)
  - 48h LC_{50} = 361 mg/L (*Artemia* sp., freshwater crustacean)
- LeBlanc et al. 2012
  - 96h LC_{50} = 2.65 µg/L (0.00265 mg/L) (*Chironomus dilutes*, insect larvae)
- Grau 1988
  - 96h LC_{50} = 229.1 mg/L (*Salmo gairdneri*, rainbow trout)
- Young and Hicks 1990
  - 48h EC_{50} = 85.2 mg/L (*Daphnia magna*)
- HSDB 2006
  - 48h LC_{50} = 10.44 mg/L (*Daphnia magna*)

[14] This is a screening EU H-Statement that addresses a combination of hazards including aquatic toxicity, persistence, and/or bioaccumulation

- Bacey 2000
  - 48h LC_{50} = 85 mg/L (*Daphnia magna*)
  - 96h LC_{50} = 34 µg/L (0.034 mg/L) (mysid shrimp)
- NPIC 2010
  - 96h LC_{50} = 237 mg/L (*Leuciscus idus*, golden orfe)
  - 96h LC_{50} = 21 mg/L (*Oncorhynchus mykiss*, rainbow trout)
  - 48h LC_{50} = 85 mg/L (*Daphnia*)
  - 72h LC_{50} >100 mg/L (*Pseudokirchneriella subcapitata*, algae)
- SRC 2005
  - 96-hour EC_{50} (*Anabaena flosaquae*, blue-green algae) = 32.8 mg/L
  - 96-hour EC_{50} (*Navicula pelliculosa*, diatom) = 6.69 mg/L
  - 72 and 96-hour EC_{50} (*Scenedesmus subspicatus*, green algae) > 10 mg/L
  - 120-hour EC_{50} (*Scenedesmus subspicatus*, green algae) > 119 mg/L

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of Very High was assigned. The most conservative LC_{50} of 21 mg/L in rainbow trout is indicates that it is a Moderate hazard for aquatic toxicity to fish. Acute toxicity values in green algae are > 100 mg/L, indicating a low hazard, but lower values for blue-green algae and diatoms suggest a Moderate to High hazard. Acute toxicity values in the least sensitive invertebrates fall into the range for a Moderate, but the most sensitive invertebrate species have values as low as 0.034 mg/L (mysid shrimp). This falls into the range for a Very High and is consistent with the authoritative listings H400 – Very toxic to aquatic life and EU Risk Phrase R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. In addition, LC_{50} values for aquatic dwelling insect larvae fell much below 1 mg/L.

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

Imidacloprid was assigned a score of Very High for chronic aquatic toxicity based on chronic NOEC values as low as 0.000163 mg/L for aquatic invertebrates. GreenScreen® criteria classify chemicals as a Very High hazard for chronic aquatic toxicity when chronic aquatic toxicity values are less than 0.1 mg/L (CPA 2012a). Confidence in the score is high because it is based on experimental data.

- Authoritative and Screening Lists
  - *Authoritative:* EU Risk Phrase R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment[15].
  - *Screening:* H410 - Aquatic Chronic 1 - Very toxic to aquatic life with long lasting effects (does not correspond to GreenScreen® criteria) [16]
- U.S. EPA 2012a
  - Imidacloprid is designated to the Aliphatic Amines, Halopyrdines, Neonicotinoids, and neutral organics ECOSAR chemical classes. The most conservative predicted ChV values are 5.207 mg/L in fish, 0.218 mg/L in daphnia, and 3.856 mg/L in green algae (Appendix D).

[15] This is an authoritative EU R-phrase that addresses a combination of hazards including aquatic toxicity, persistence, and/or bioaccumulation

[16] This is a screening EU H-Statement that addresses a combination of hazards including aquatic toxicity, persistence, and/or bioaccumulation

- Roessink et al. 2013
  - 28d EC_{50} (immobilization) = 11.9 µg/L (0.0119 mg/L) (*Asellus aquaticus*, freshwater crustacean)
  - 28d LC_{50} = 20.3 µg/L imidacloprid (*Asellus aquaticus*, freshwater crustacean)
  - 28d EC_{50} (immobilization) = 15.4 µg/L (0.0165 mg/L) (*Gammarus pulex*, freshwater crustacean)
  - 28d LC_{50} = 33.8 µg/L (0.0338 mg/L) (*Gammarus pulex*, freshwater crustacean)
  - 28d EC_{50} (immobilization) = 11.8 µg/L (0.0118 mg/L) (*Chaoborus obscuripes*, arthropod)
  - 28d LC_{50} = 12.6 µg/L (0.0126 mg/L) (*Chaoborus obscuripes*, freshwater crustacean)
  - 28d EC_{50} (immobilization) = 3.46 µg/L (0.00346 mg/L) (*Sialis lutaria*, insect larvae)
  - 28d LC_{50} = 32.5 µg/L (0.00325 mg/L) (*Sialis lutaria*, insect larvae)
  - 28d EC_{50} (immobilization) = 6.45 µg/L (0.00645 mg/L) (*Plea minutissima*, insect larvae)
  - 28d LC_{50} = 9.80 µg/L (0.0098 mg/L) (*Plea minutissima*, arthropod)
  - 28d EC_{50} (immobilization) = 0.126 µg/L (0.000126 mg/L) (*Caenis horaria*, insect larvae)
  - 28d LC_{50} = 0.316 µg/L (0.000316 mg/L) (*Caenis horaria*, arthropod)
  - 28d EC_{50} (immobilization) = 0.123 µg/L (0.000123 mg/L) (*Cleon dipterum*, insect larvae)
  - 28d LC_{50} = 0.195 µg/L (0.000195 mg/L) (*Cleon dipterum*, insect larvae)
- Goulson 2013
  - 28d LC_{50} = 7.1 ppb imidacloprid (*Hyalella Azteca*, freshwater crustacean)
- SRC 2005
  - SRC reviewed available chronic aquatic toxicity data and reported the following most conservative chronic NOEC values for fish, invertebrates, and algae:
    - 25 mg/L (sensitive fish species)
    - 50 mg/L (tolerant fish species)
    - *Mysidopsis bahia* (sensitive aquatic invertebrate species) = 0.000163 mg/L, duration not specified, growth and reproductive success
    - *Daphnia magna* (tolerant aquatic invertebrate species) = 1.8 mg/L, 21-day immobility
    - 6.69 mg/L (sensitive algae species), 4-day, endpoint not specified
    - 119 mg/L (tolerant algae species), 5-day, biomass and growth

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of Very High was assigned. Association with R53 – May cause long-term adverse effects in the aquatic environment corresponds to a score of Moderate. The most conservative chronic NOEC values in fish and algae correspond to a Low and Moderate, respectively. However, the most conservative NOEC value of 0.000163 mg/L for *Mysidopsis bahia*, as well as several NOEC values for other aquatic invertebrate species and water dwelling insect larvae, fall into the range for a Very High. Therefore a score of Very High was assigned based on the chronic toxicity values for the most sensitive trophic level.

Acute Terrestrial Vertebrates[17] Toxicity (ATV) Score (vH, H, M, or L): H
Imidacloprid was assigned a score of High for acute terrestrial vertebrate toxicity based on LD_{50} values as low as 14 mg/kg in birds. GreenScreen® criteria classify chemicals as a High hazard for

[17] Includes birds and mammals

acute terrestrial vertebrate toxicity when the LD_{50} values in birds are between 10 and 50 mg/kg as per the U.S. EPA DfE classification criteria (CPA 2012a, DfE 2011). Confidence in the score is high because it is based on consistent results in several experimental studies.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* GHS New Zealand Category 9.2A – Very ecotoxic in the soil environment (does not correspond to GreenScreen® criteria)
  - *Screening:* GHS New Zealand Category 9.3A – Very ecotoxic to terrestrial vertebrates (does not correspond to GreenScreen® criteria)
  - *Screening:* GHS New Zealand Category 9.4A – Very ecotoxic to terrestrial invertebrates (does not correspond to GreenScreen® criteria)
- Goulson 2013
  - *Oral:* LD_{50} = 283 mg/kg (mallard duck)
  - *Oral:* LD_{50} = 31 mg/kg (Japanese quail)
  - *Oral:* LD_{50} = 14 mg/kg (grey partridge)
  - *Oral:* LD_{50} = 104 mg/kg (chicken)
  - *Oral:* LD_{50} = 152 mg/kg (bobwhite quail)
  - *Oral:* LD_{50} = 41 mg/kg (sparrow)
  - *Oral:* LD_{50} = 21 – 50 mg/kg (canary)
- ABC 2013
  - *Oral:* LD_{50} = 25 mg/kg (female) 25 – 50 mg/kg (male) imidacloprid (rock dove)

ToxServices' summary and conclusion:

- Per GreenScreen® guidance, terrestrial toxicity was evaluated according to U.S. EPA's Design for the Environment (DfE) Alternatives Assessment Criteria for Hazard Evaluation (DfE 2011). Oral LD_{50} values in birds range from 14-283 mg/kg, with several falling between 10-50 mg/kg. Therefore imidacloprid warrants a score of High based on acute toxicity to birds.

Chronic Terrestrial Vertebrates (CTV) Toxicity Score: N/A

Imidacloprid was not assigned a score for chronic terrestrial vertebrates toxicity as the U.S. EPA DfE (DfE 2011) guidance does not report criteria for chronic toxicity. Therefore the hazard scoring of this endpoint is not applicable due to lack of recognized criteria. Toxicity studies on the chronic exposure of imidacloprid to birds were identified and are described below.

- ABC 2013
  - In a reproductive toxicity assay bobwhite quail were administered 0, 30, 60, 120 and 240 ppm of imidacloprid. Authors identified a NOEL at 120 ppm and LOEL at 240 ppm based on effects to male weight only. Results were difficult to interpret and no true reproductive effects were observed. No additional details were provided.
  - In a reproductive toxicity study mallard ducks were administered 0, 60, 120 and 240 ppm of imidacloprid. Authors identified a NOAEL of 120 ppm and LOAEL at 240 ppm based on effects to hatching and egg laying at higher doses. No additional details were provided.
- Pandey and Mohanty 2014
  - In an endocrine study to assess imidacloprid's thyroid disrupting potential on the pituitary-thyroid axis of a seasonally breeding wildlife avian species, adult male red munia birds (8/dose) were exposed to 1.55 mg/kg/day (0.5% of LD_{50}) imidacloprid (purity unknown) through food using soy oil as a vehicle for 30 days during the

preparatory and breeding phases. Control birds were given food with the vehicle. Experiments were performed in each phase in replicates of two. Body weights were recorded every other day throughout the study. At the end of exposure, birds were sacrificed and blood was collected for hormone analysis. Thyroids were dissected out for evaluation of volume of the thyroid gland, number of follicles, volume of colloids, epithelial cell height, epithelial cell nucleus size and nucleus-to-cytoplasm ratio in epithelial and stromal cells. No significant alteration in body weight was observed in exposure groups of the preparatory phase; however, body weight was reduced in the exposed groups of the breeding phase ($p<0.01$). Authors observed a decrease in thyroid weight in the preparatory phase and an increase in thyroid weight in the breeding phase. An increase in thyroid volume was observed in both phases. These changes were not statistically significant compared to controls. In both phases, imidacloprid exposed animals had irregular shaped follicles with ruptured epithelium and lesions in stroma. The number of follicles was significantly ($p<0.01$) reduced and damaged colloids devoid of secretary droplets were observed. A significant ($p<0.05$) increase in colloid volume was observed in the preparatory phase, and an insignificant decrease in colloid volume was observed in the breeding phase. Exfoliation of epithelial cells was observed in both phases; however, shrinkage and mineralization of colloids was only observed in the breeding phase. Epithelial cell height, nucleus size and nucleus to cytoplasm ratio was decreased in both phases in exposed groups. A significant decrease in T_4 levels was seen in both phases; however, T_3 levels were significantly increased during the preparatory phase and significantly decreased in the breeding phase. TSH was also decreased in both phases of imidacloprid exposed groups. Authors concluded substantial thyrotoxicity was induced by exposure to imidacloprid based on damage to thyroid follicles and lesions in stroma and impaired plasma levels of thyroid hormones, and that effects were more prominent in the breeding phase.

ToxServices' summary and conclusion:

- Available data indicate that imidacloprid may have the potential to produce chronic, sublethal effects on avian reproduction. However, no score was assigned due to the lack of criteria for this endpoint.

Acute Foliar Invertebrates and Pollinators[18] (AFI) Toxicity Score (H, M, or L): H

Imidacloprid was assigned a score of High for acute foliar invertebrates and pollinators toxicity based measured oral and dermal LD_{50} values in bees as low as 1.1 ng/bee. GreenScreen® criteria classify chemicals as a High hazard for acute foliar invertebrates and pollinators toxicity when the oral and dermal LD_{50} values in bees are less than 2 µg/bee (2,000 ng/bee) as per the U.S. EPA DfE criteria. Confidence in the score is high because it is based on consistent results in several experimental studies.

- FERA 2015
    - *Oral:* LD_{50} = 0.069 µg/bee = 69 ng/bee (24-hour), 0.014 µg/bee = 14 ng/bee (48-hour), and 0.006 µg/bee = 6 ng/bee (72-hour) (bumblebee, *Bombus terrestris*)
    - *Contact:* LD_{50} = 0.04 µg/bee = 40 ng/bee (leafcutter bee, *Megachile rotundata*)
    - *Contact:* LD_{50} = 0.04 µg/bee = 40 ng/bee (alkali bee, *Nomia melanderi*)
    - *Contact:* LD_{50} = 3.8 µg/bee = 3,800 ng/bee (hornfaced bee, *Osmia cornifrons*)
    - *Contact:* LD_{50} = 0.0011 µg/bee = 1.1 ng/bee (stingless bee, *Melipona beecheii*)

[18] Includes bees

  - *Contact:* LD_{50} = 0.554 µg/bee = 554 ng/bee (24-hour), 0.014 µg/bee = 14 ng/bee (48-hour), and 0.005 µg/bee = 5 ng/bee (72-hour) (bumblebee, *Bombus terrestris*)
- Marletto et al. 2003
  - *Oral:* LD_{50} = 0.04 µg/bee = 40 ng/bee (24-hour), 0.02 µg/bee = 20 ng/bee (48-hour) (bumblebee, *Bombus terrestris*)
  - *Contact:* LD_{50} = 0.02 µg/bee = 20 ng/bee (48-hour) (bumblebee, *Bombus terrestris*)
- Pisa et al. 2014
  - *Oral:* LD_{50} = 3.1 ng/bee (honey bee, *Apis mellifera*)
- Cresswell 2011
  - *Oral:* LD_{50} = 4.5 ng/bee (honey bee, *Apis mellifera*)
- Fossen 2006
  - *Oral:* LD_{50} = 5 ng/bee (honey bee, *Apis mellifera*)
  - *Contact:* LD_{50} = 14-24 ng/bee (honey bee, *Apis mellifera*)
- Goulson 2013
  - *Oral:* LD_{50} = 5 ng/bee (honey bee, *Apis mellifera*)
  -
- Iwasa et al. 2004
  - *Contact:* LD_{50} = 17.9 ng/bee (>99% purity) honey bee, *Apis mellifera*
- Poquet et al. 2015
  - In an acute toxicity study, honey bee workers (*Apis mellifera*) were exposed to imidacloprid (99% purity) either on the thorax or on the wings at doses of 0, 5, 10, 25, 50, 75, 100, 200, or 400 ng/bee. Eight replicates of 30 bees per contact area were performed. Bees were observed for mortality 24, 48, 72, 96 and 120 hours after exposure. Authors determined 120 hour LD_{50} values of 26.55 ng imidacloprid for wing exposure and 25.10 ng imidacloprid for the thorax exposure.

ToxServices' summary and conclusion:

- The LD_{50} values in several species of bees range from 1.1-3,800 ng/bee (0.0031-0.024 µg/bee), with all but the highest value falling well below 2 µg/bee (2,000 ng/bee). Therefore imidacloprid warrants a score of High based on acute toxicity to bees.

Chronic Foliar Invertebrates and Pollinators (CFI) Toxicity Score: N/A
Imidacloprid was not assigned a score for chronic foliar invertebrates toxicity as the U.S. EPA DfE (DfE 2011) guidance does not report criteria for chronic toxicity. Therefore the hazard scoring of this endpoint is not applicable due to lack of recognized criteria. Toxicity studies on the chronic exposure of imidacloprid to bees were identified and are described below.

- Lu et al. 2013
  - In a terrestrial toxicity study, the role of imidacloprid in colony collapse disorder (CCD) in honey bees (*Apis mellifera*) was investigated *in situ*. Authors used a replicate split-plot design consisting of 4 sites with 5 honey bee hives. Hives (4/dose) were exposed to concentrations of imidacloprid (purity not reported) in high fructose corn syrup at concentrations of 0, 0.1, 1, 5 and 10 µg/kg for four weeks, followed by concentrations of 0, 20, 40, 200 and 400 µg/kg for an additional nine weeks. Hives were monitored for 6 months post dosing. Authors observed brood production bi-weekly. By the end of the experiment, brood rearing was inversely related to imidacloprid dosages. Hive weakness of imidacloprid treated hives was observed post dosing; significant loss of hives was

recorded at 18 weeks post dosing. Authors concluded exposure to imidacloprid causes honey bees to exhibit symptoms of CCD 23 weeks post imidacloprid dosing.

- Derecka et al. 2013
    - In a terrestrial toxicity study the molecular profiles of worker-honey bee larvae were investigated after hives in the field were given access to syrup tainted with imidacloprid. Authors examined genome wide RNA transcriptional responses and lipid profiles of worker-bee larvae of imidacloprid exposed hives. Two 15 day long feeding trials were performed in three hives; 2 µg/L of imidacloprid (purity unknown) was prepared in syrup daily. Authors observed significantly different levels of expression of 15 microRNAs between exposed and control groups, and differential expression of 300 genes between exposed and control groups. A significant enrichment in genes functioning in lipid-carbohydrate-mitochondrial networks was detected. RNA levels for a cluster of genes encoding detoxifying P450 enzymes were elevated and a coordinated downregulation of genes in glycolytic and sugar-metabolizing pathways was observed. Expression of the Hsp90 gene was also reduced in exposed groups. Authors concluded imidacloprid alters the expression of metabolic networks in honey bee larvae and results in altered lipid profiles.
- Eiri and Nieh 2012
    - In a terrestrial toxicity study, the adverse effects of imidacloprid exposure on honey bee foraging activity were investigated. Authors measured the short term effects of imidacloprid on honey bee sucrose response threshold and the longer term effects of imidacloprid metabolites on honey bee foraging preferences and waggle dancing. Bees were exposed to imidacloprid (purity not stated) at concentrations of 0.21 ng or 2.16 ng suspended in unscented pure sucrose solution. Two independent experiments were performed. In the first experiment, a proboscis extension response assay was conducted using 523 foragers from three *Apis mellifera* colonies. Sucrose response was measured 1 hour after exposure. Authors recorded the lowest sucrose concentration that elicited proboscis extension and the total number of proboscis extensions elicited by the sucrose. In the second experiment, responses of 65 free-flying foragers from two *Apis mellifera* colonies visiting and dancing for a sucrose feeder were recorded 24 hours after treatment. Authors recorded the number of visits made by each bee to a series of feeders, the unloading wait time, and the number of dance circuits made by the forager. Foragers exposed to imidacloprid had increased sucrose response thresholds and decreased number of proboscis extension responses compared to controls. Authors observed a significant variation in the sucrose feeder concentrations bees accepted; however, no significant effects of treatment were observed in the number of times a bee fed at a sucrose feeder. Imidacloprid treated bees performed significantly less dance circuits compared to controls. Authors concluded imidacloprid does have significant time dependent neurological effects on honey bee foraging behavior.
- Nahar and Ohtani 2015
    - In a terrestrial toxicity study, the effect of sub-lethal doses of imidacloprid on behavior and homing ability of honey bees (*Apis mellifera*) was investigated. Bees were exposed to imidacloprid (purity not reported; DMSO solvent) by injection at concentrations of 2, 5, and 10 ng/bee. A total of 10 forager bees were used per treatment in three replications. Bees were observed for behavior in transparent plastic boxes for 30 minutes after injection for the highest dose tested (10 ng/bee). Bees were released 50 meters away from the hive and authors recorded their ability to return to the hive. Authors observed abnormalities such as trembling, tumbling, vomiting, abnormal fanning, nausea, restless

running, being stationary, lying on the back, and lack of coordination in bees treated with imidacloprid. Homing of forager bees was disturbed in a dose-dependent manner by exposure to imidacloprid; treated bees exhibited abnormal flying behaviors and a decrease in the number of bees returning to the hive. Authors concluded imidacloprid disrupts the homing ability of forager bees which reduced the number of foragers in hives and can result in collapse of the entire colony.

- Dively et al. 2015
    - In a terrestrial toxicity study, the fate of imidacloprid residues in hive matrices and the effects on honey bee colonies was investigated. To assess the chronic sub-lethal effects of prolonged exposure to imidacloprid, colonies of honey bees (*Apis mellifera*) were exposed to 0, 5, 20 and 100 µg/kg of imidacloprid (purity not stated) by a pollen diet substitute spiked with the test chemical. Colonies were sampled bi weekly to estimate the percentage of the frame area covered with drawn cells, bees, capped brood, cells with older larvae, and cells packed with beebread and honey. Foraging activity was measured biweekly by weight of pollen on the entrance traps and number of foraging bee returning with and without pollen loads. Samples of bees and other hive matrices were analyzed for residue levels of imidacloprid. Imidacloprid exposure of doses up to 100 µg/kg had no significant effects on colony health or foraging activity during or shortly after exposure; however, later imidacloprid exposed colonies at 20 and 100 µg/kg experienced higher rates of queen failure and broodless periods weakening the health of the colony. A significant dose –related effect was observed in colony survival for imidacloprid treated colonies. Authors concluded exposure to imidacloprid exposure at concentrations of 20 µg/kg and higher could cause negative impacts on honey bee colony health; however, the most likely encountered high range field doses of 5 µg/kg had negligible effects on colony health.
- Faucon et al. 2005
    - In a terrestrial toxicity study, the toxic effect of imidacloprid exposure through nectar to honey bees (*Apis mellifera*) was investigated. Honey bees colonies (8/dose) were exposed to imidacloprid (purity not reported) at concentrations of 0, 0.5 and 5 µg/L via saccharose syrup three times per week for four weeks (total 13 administrations). Authors monitored bee activity, mortality, colony weight and honey production, brood production, pathologies and performed imidacloprid residue analysis. Authors observed a non-significant increase in bee activity, a significantly higher frequency of pollen carrying and a larger number of capped brood cells. Activity and pollen carrying was reestablished once imidacloprid was no long being administered to colonies. Authors concluded imidacloprid exposure at field realistic doses does not produce and immediate counter-productive or severe problems in colonies.
- Pettis et al. 2012
    - In a terrestrial toxicity study the impact of imidacloprid exposure on disease susceptibility in honey bees (*Apis mellifera*) was investigated using the gut parasite *Nosema* spp. Honey bee colonies (10/dose) were exposed to imidacloprid (purity not reported) at doses of 5 and 20 ppb for 10 weeks via protein supplement patties. After 5 and 8 weeks of exposure emerging brood were fed a suspension of spore of the known bee pathogens *N. apis* and *N. ceranae* for 2 days of adult life. Bees were sacrificed 10 days later and development of *Nosema* infection in individual bees was determined. Authors observed a significant increase in *Nosema* growth within individual bees from colonies exposed to imidacloprid. Authors concluded pathogen growth is an indirect

effect of exposure to imidacloprid and could be a major contributor to honey bee colony decline.

- Feltham et al. 2014
    - In a terrestrial toxicity study, the impact of imidacloprid exposure at field realistic doses on bumblebee (*Bombus terristrus*) foraging activity was investigated. Authors exposed bumble bee colonies (3/dose) to imidacloprid (purity not reported) at concentrations of 0 (control), or 0.7 µg/kg in sugar water and 6 µg/kg in pollen for four weeks. Radio frequency identification was used to monitor foraging duration, and an automated system recorded weight of bees as they entered and exited the hive. No difference in foraging duration or rate was observed between treated bees and controls; however, the duration of trips increased with longer exposure time and treated bees brought back significantly less pollen than controls resulting in less colony weight gain and fewer production of workers and queens. Authors concluded, over time exposure to imidacloprid can reduce colony success due to less efficient foraging.
- Whitehorn et al. 2012
    - In a terrestrial toxicity study, the effect of field-realistic exposure of imidacloprid on bumble bee (*Bombus terrestris*) colonies was investigated. Colonies (25/dose) were exposed to imidacloprid (purity not reported) at doses of 0, 6 µg/kg in pollen and 0.7 µg/kg in sugar water, or 12 µg/kg in pollen and 1.4 µg/kg in sugar water for 14 days. After exposure, colonies were placed in the field to forage independently and were monitored for 6 weeks. Treated colonies had decreased weight gain, a lower number if empty pupal cells and reduced queen production compared to controls. Authors concluded trace levels of imidacloprid can have strong negative consequences for queen production resulting in a population level impact.
- Laycock et al. 2012
    - In a reproductive toxicity study conducted by Laycock et al., queenless microcolonies of worker bumble bees (*Bombus terrestris*) received doses of dietary imidacloprid (purity unknown) of 0, 0.08, 0.20, 0.51, 1.28, 3.20, 8.00, 20.00, 50.00 or 125 µg/L (15, 6, 5, 7, 17, 7, 5, 5, 3 and 6 microcolonies/dose, respectively) by feeders containing syrup mixed with the test substance. Microcolonies were monitored daily for mortality and brood production. Feeders were weighed daily to determine consumption of syrup. Three trials, each comprising 14 days (1 day of acclimation, 13 days of exposure) were conducted. Bees were sacrificed after 14 days and ovaries were removed for evaluations. Laid eggs and larvae were also collected for evaluation. To measure ovary development, authors measured the length, width and area of each intact terminal oocyte from dissected ovaries and each laid egg. Total mortality for the study comprised one dead worker bee in a single microcolony exposed to a dose of 125 µg/L imidacloprid. Worker fecundity declined significantly with increasing dosage of dietary imidacloprid ($p<0.001$); an exposure level of 1 µg/L resulted in 42% reduction in worker fecundity. Increased dosage of imidacloprid resulted in a decline in *per capita* daily feeding rates; this decline in feeding rates lead to a decline in fecundity. Larger oocytes in ovaries were observed in all but the highest dose of imidacloprid compared to controls. Bees at the highest dose (125 µg/L) had smaller oocytes; however, no dose dependent variation in oocyte size was evident in bees exposed to doses lower than 125 µg/L. A decrease in workers with mature oocytes was observed at 125 µg/L. Bees feeding on the highest dosage (125 µg/L neither developed their ovaries fully nor laid eggs; microcolonies feeding on dosages of 20 µg/L or less developed ovaries fully and were capable of laying eggs. Bees exposed to 50 µg/L developed their ovaries but did not lay eggs. Authors concluded ingestion of

imidacloprid substantively reduced the fecundity of worker bumble bees and dietary ingestion of imidacloprid can cause sub-lethal effects on bumble bee reproduction.

- Gill et al. 2012
    - In a terrestrial toxicity study, the effects of chronic exposure to imidacloprid on natural foraging behavior and worker mortality were investigated in bumblebees (*Bombus terrestris*). Ten early stage bumblebee colonies were exposed to imidacloprid (purity not reported) at a concentration of 10 ppb for four weeks. Authors tagged foragers and activity was recorded automatically by RFID readers. Foraging behavior was observed for 1 hour per colony per day; size and presence of pollen loads collected were recorded. Colonies were sacrificed at the end of the experiment and the number of workers and brood was counted, and the mass of the nest was measured. Total worker production and brood development at the end of the experiment were significantly lower in treated colonies compared to controls; however, worker production did not become significantly lower until the end of week 2, which coincides with the time taken by workers to develop from egg to adult. Foragers exposed to imidacloprid were significantly less efficient at collecting pollen in the field and the percentage of workers getting lost was significantly higher. Authors concluded that imidacloprid exposure causes impairment to pollen foraging efficiency, leading to increased colony demand for food reducing worker production.
- Scholer and Krischik 2014
    - In a terrestrial toxicity study the effects of imidacloprid on individual behavior and colony health of the American bumble bee (*Bombus impatien*) were investigated. Authors monitored queen health (mortality and movement), worker behavior (movement, and consumption of sugar) and colony health (weight, number of wax pots, brood production, and bee weight). Colonies (8/dose) were exposed to imidacloprid (purity not reported) in 50% sugar syrup at concentrations of 0, 10, 20, 50 and 100 ppb for eleven weeks. Authors recorded queen status weekly, and video recorded movement of queens and workers twice for 30 minutes in weeks 4 and 8. Colonies were sacrificed at the end of 11 weeks and dissected for evaluation. Authors observed significantly higher queen mortality at 6 weeks in the 50 and 100 ppb dose groups and by 11 weeks in the 20, 50 and 100 ppb dose groups. Significant reductions in queen survival, worker movement, colony consumption, and colony weight were observed at doses of 20, 50 and 100 ppb by eleven weeks. Authors concluded that exposure to imidacloprid causes behavior changes resulting in detrimental effects on colonies at exposure levels of 200 ppb and above.

ToxServices' summary and conclusion:

- Available data indicate that imidacloprid may cause sublethal effects on bee health and behavior. However, no score was assigned due to the lack of criteria for this endpoint.

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): *vH*
Imidacloprid was assigned a score of Very High for persistence based on field and laboratory half-lives as high as 1,250 days in soil, which is predicted to be its major compartment. GreenScreen® criteria classify chemicals as a Very High hazard for persistence when the half-life in soil is greater than 180 days (CPA 2012a). Confidence in the score is reduced due to the large variation in half-life values.

- Authoritative and Screening Lists
    - *Authoritative:* EU Risk Phrase R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment[19].
    - *Screening:* H410 - Aquatic Chronic 1 - Very toxic to aquatic life with long lasting effects (does not correspond to GreenScreen® criteria) [20]
- U.S. EPA 2012b
    - The BIOWIN modeling Ready Biodegradable Predictor indicates that imidacloprid is not expected to be readily biodegradable. Fugacity modeling predicts 90% will partition to soil with a half-life of 120 days, 9.46% will partition to water with a half-life of 60 days, and 0.592% will partition to sediment with a half-life of 542 days (Appendix E).
- HSDB 2006
    - Imidacloprid was found to biodegrade more rapidly in soil under vegetation; half-lives of 48 and 190 days were found with and without vegetation, respectively. A half-life of 34 days was reported for imidacloprid using soil where citrus products are grown extensively.
    - The half-lives of imidacloprid in crops containing either no fertilizer, cow manure, pig slurry or a mix of cow manure and pig slurry were 10, 24, 105 and 108 days, respectively. After the first two months imidacloprid concentrations diminished at increased rates.
    - Imidacloprid is degraded under anaerobic aquatic conditions more rapidly than under aerobic soil conditions.
- Fossen 2006
    - *Soil:* Imidacloprid has reported half-lives of 26.5 - 229 days, depending on soil properties, from field studies. The longest half-lives are from field studies in the absence of light. Degradation is more rapid in soil with cover crops than in bare soils. Degradation in soil via photolysis has a half-life of 39 days.
    - *Surface water:* Half-life < 3 hours in presence of sunlight
    - *Ground water:* Half-life ranges from 33 – 44 days at pH 7 and 25°C. It is more stable in acidic and neutral water, and more readily hydrolyzed in alkaline water.
- Goulson 2013
    - *Soil:* A half-life of 1,250 days in loam was calculated from data in a field study.
    - *Soil*: Half-lives of 28 – 1,230 days in loam, alluvial, lateritic, and coastal alkaline soils were reported from laboratory studies.
- Morrissey et al. 2015
    - *Soil:* Half-lives of 104 – 228 days have been reported for soil. No additional details were provided. The compound is more stable in water and soil under anaerobic conditions.
    - *Surface water:* A half-life of < 1 day for water photolysis in the presence of sunlight was reported.
    - *Surface water:* A half-life of > 1 year was reported for hydrolysis at pH 9. Imidacloprid is stable under acidic and neutral conditions.

[19] This is an authoritative EU R-phrase that addresses a combination of hazards including aquatic toxicity, persistence, and/or bioaccumulation

[20] This is a screening EU H-Statement that addresses a combination of hazards including aquatic toxicity, persistence, and/or bioaccumulation

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of Very High was assigned. There are no standard biodegradation data available for imidacloprid. Available field and laboratory data indicate that it degrades slowly through a variety of biological and chemical mechanisms. Hydrolysis can occur under alkaline, but not neutral and acidic conditions. Photolysis can also occur, and the slowest degradation rates are in anaerobic, low light conditions. Modeling predicts that imidacloprid will partition primarily to soil. Field and experimental half-lives in soil vary greatly depending on soil type, ground cover, pH, and presence of light. Numerous field and laboratory studies report half-lives greater than 180 days, which indicates that imidacloprid has the potential to persist in the environment at least under some environmentally relevant conditions. Therefore a score of Very High was assigned, but confidence in the score is reduced due to the large variation in half-life values.

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

Imidacloprid was assigned a score of Very Low for bioaccumulation based on an experimental log K_{ow} of 0.57 with support from a modeled BCF of 1.026. GreenScreen® criteria classify chemicals as a Very Low hazard for bioaccumulation when the log K_{ow} is less than 4 and the BCF is less than 100 (CPA 2012a). Confidence in the score is high because it is based on an experimental log K_{ow}.

- Authoritative and Screening Lists
  - *Authoritative:* EU Risk Phrase R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment[21].
  - *Screening:* H410 - Aquatic Chronic 1 - Very toxic to aquatic life with long lasting effects (does not correspond to GreenScreen® criteria) [22]
- U.S. EPA 2012b
  - BCFBAF predicts a BCF of 1.026 based on a log K_{ow} of 0.57, indicating this chemical is not likely to bioaccumulate because the BCF is less than 100 based on a log K_{ow} less than 5 (Appendix E).
- HSDB 2006
  - An estimated BCF of 3.2 was calculated for imidacloprid using a log K_{ow} of 0.57 and a regression derived equation. This suggests potential for bioconcentration in aquatic organisms is low.
- ChemIDplus 2016
  - Imidacloprid has an experimental log K_{ow} of 0.57.
- Bacey 2000
  - The moderate K_{ow} of 3.7 (not stated whether this value is predicted or experimental) combined with its rapid photodegredation in water (half-life <3 hours) and on soil (half-life 39 days) suggests low potential for bioaccumulation

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of Very Low was assigned. The experimental log K_{ow} of 0.57 indicates that it is not likely to be bioaccumulative, and this is supported by the predicted BCF of 1.026.

[21] This is an authoritative EU R-phrase that addresses a combination of hazards including aquatic toxicity, persistence, and/or bioaccumulation

[22] This is a screening EU H-Statement that addresses a combination of hazards including aquatic toxicity, persistence, and/or bioaccumulation

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): *L*
Imidacloprid was assigned a score of Low for reactivity based on a lack of structural alerts, an HMIS rating of 0 for physical hazards, and reactivity statements on a product SDS indicating that it is not explosive, self-reactive, a substance that may produce flammable gases on contact with water, or oxidizing. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when the chemical does not warrant GHS classification for any of the reactivity sub-endpoints and the chemical is not present on authoritative or screening lists (CPA 2012a). Confidence in the score is reduced due to the lack of experimental data on the pure target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- UN 2010
  - The structure of imidacloprid contains an alert for explosive properties (nitro group) (Appendix F).
- UN 2013
  - Based on examination of the structure, ToxServices determined that imidacloprid is not an organic peroxide, does not contain reactive groups associated with self-reactive substances, and is not an organometallic substance that may produce flammable gases on contact with water.
- Sigma Aldrich 2015
  - Imidacloprid was assigned an HMIS rating of 0 for physical hazards. This corresponds to "Materials that are normally stable, even under fire conditions, and will NOT react with water, polymerize, decompose, condense, or self-react. NonExplosives (ILPI 2015).
- Bayer Crop Science 2009
  - A formulation containing 70% imidacloprid was not explosive in a test conducted according to 92/69/EEC, A.14 / OECD 113.
  - A formulation containing 70% imidacloprid has no oxidizing properties.

ToxServices' summary and conclusion:

- Based on the weight of evidence, a score of Low was assigned. Imidacloprid contains a structural alert for explosivity, but it has received an HMIS rating of 0 for physical hazards which indicates that it is not explosive, and a formulation containing 70% imidacloprid was not explosive in a guideline test. It does not contain alerts for self-reactivity, or substances that may produce flammable gases on contact with water. In addition, a formulation containing 70% imidacloprid has no oxidizing properties. Therefore a score of Low was assigned, but the confidence in the score is reduced due to the lack of experimental data on the neat target chemical.

Flammability (F) Score (vH, H, M, or L): *L*
Imidacloprid was assigned a score of Low for flammability based on an HMIS rating of 0 which indicates that it will not burn. GreenScreen® criteria classify chemicals as a Low hazard for flammability when available data indicate that the chemical does not warrant GHS classification for flammability and the chemical is not present on authoritative or screening lists (CPA 2012a). Confidence in the score is reduced due to the lack of experimental data on the neat target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Sigma Aldrich 2015
  - Imidacloprid was assigned an HMIS rating of 0 for flammability. This corresponds to "Materials that will not burn (ILPI 2015)."
- Bayer Crop Science 2009
  - A formulation containing 70% imidacloprid is not highly flammable.

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APPENDIX A: Hazard Benchmark Acronyms
(in alphabetical order)

(AA) Acute Aquatic Toxicity

(AFI) Acute Foliar Invertebrates and Pollinators Toxicity

(AT) Acute Mammalian Toxicity

(ATV) Acute Terrestrial Vertebrates Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(CFI) Chronic Foliar Invertebrates and Pollinators Toxicity

(CTV) Chronic Terrestrial Vertebrates 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 Imidacloprid (CAS #138261-41-3)

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

GreenScreen® Score Inspector

Table 1: Hazard Table

			Group I Human					Group II and II* Human								Ecotox[1]		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 Ecotoxicity	Chronic Ecotoxicity	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	Imidacloprid	138261-41-3	*M*	*L*	*M*	M	*M*	*H*	vH	M	vH	*H*	L	DG	L	L	vH	vH	*vH*	vL	*L*	*L*

Table 3: Hazard Summary Table

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

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
Imidacloprid	1

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

Table 6

Chemical Name	Final GreenScreen® Benchmark Score
Imidacloprid	1

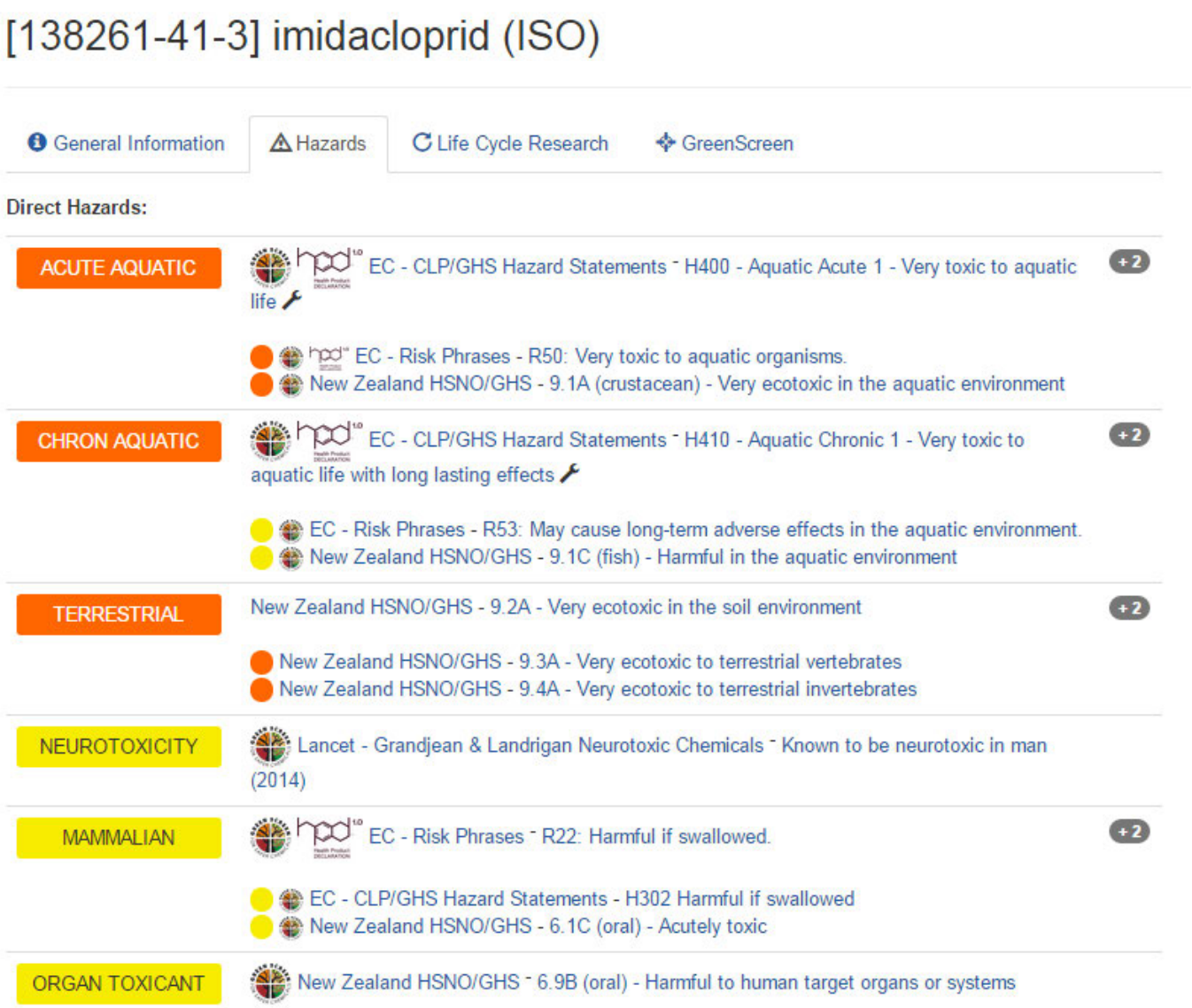
After Data gap Assessment
Note: No Data gap Assessment Done if Preliminary GS Benchmark Score is 1.

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												

[1]The ecotoxicity scores presented in the table and used in the score calculation represent the most conservative score of the aquatic and terrestrial endpoints.

APPENDIX C: Pharos Output for Imidacloprid (CAS #138261-41-3)



APPENDIX D: ECOSAR Modeling Results for Imidacloprid (CAS #138261-41-3)

ECOSAR Version 1.11 Results Page

SMILES: c1nc(CL)ccc1CN2C(=NN(=O)(=O))NCC2
CHEM: Imidacloprid
CAS Num: 138261-41-3
ChemID1:
MOL FOR: C9 H10 CL1 N5 O2
MOL WT: 255.67
Log K_{ow}: 0.555 (EPISuite K_{ow}win v1.68 Estimate)
Log K_{ow}: (User Entered)
Log K_{ow}: 0.57 (PhysProp DB exp value - for comparison only)
Melt Pt: (User Entered for Wat Sol estimate)
Melt Pt: 144.00 (deg C, PhysProp DB exp value for Wat Sol est)
Wat Sol: 4528 (mg/L, EPISuite WSK_{ow}win v1.43 Estimate)
Wat Sol: (User Entered)
Wat Sol: 610 (mg/L, PhysProp DB exp value)

Values used to Generate ECOSAR Profile

Log K_{ow}: 0.555 (EPISuite K_{ow}win v1.68 Estimate)
Wat Sol: 610 (mg/L, PhysProp DB exp value)

Available Measured Data from ECOSAR Training Set

CAS No	Organism	Duration	End Pt	Measured mg/L (ppm)	Ecosar Class	Reference
138261-41-3	Daphnid		ChV	2.5	Neonicotinoids	OPP Pesticide Ecotoxicity DB; Supplame
138261-41-3	Daphnid	48-hr.	LC50	85.2	Neonicotinoids	OPP Pesticide Ecotoxicity DB
138261-41-3	Fish (SW)	96-hr.	LC50	163	Neonicotinoids	OPP Pesticide Ecotoxicity DB

ECOSAR v1.1 Class-specific Estimations

Aliphatic Amines
Halopyrdines
Neonicotinoids

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Aliphatic Amines	: Fish	96-hr.	LC50	436.989
Aliphatic Amines	: Daphnid	48-hr.	LC50	44.036
Aliphatic Amines	: Green Algae	96-hr.	EC50	50.658
Aliphatic Amines	: Fish		ChV	42.456
Aliphatic Amines	: Daphnid		ChV	3.072
Aliphatic Amines	: Green Algae		ChV	14.899
Halopyrdines	: Fish	96-hr.	LC50	5.132
Halopyrdines	: Daphnid	48-hr.	LC50	2.812
Halopyrdines	: Fish		ChV	5.207
Halopyrdines	: Daphnid		ChV	0.218 !
Neonicotinoids	: Fish	96-hr.	LC50	416.753
Neonicotinoids	: Daphnid	48-hr.	LC50	121.455
Neonicotinoids	: Green Algae	96-hr.	EC50	73.402
Neonicotinoids	: Fish		ChV	214.497
Neonicotinoids	: Daphnid		ChV	6.745
Neonicotinoids	: Green Algae		ChV	3.856
Neonicotinoids	: Fish (SW)	96-hr.	LC50	370.091
Neonicotinoids	: Invertebrate (SW)	96-hr.	LC50	0.647
Neonicotinoids	: Invertebrate		ChV	0.008
Neutral Organic SAR (Baseline Toxicity)	: Fish	96-hr.	LC50	4168.083 *
	: Daphnid	48-hr.	LC50	2071.075 *
	: Green Algae	96-hr.	EC50	888.651 *
	: Fish		ChV	348.091
	: Daphnid		ChV	139.330
	: Green Algae		ChV	172.903

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

NOTE: ! = exclamation designates: The toxicity value was estimated through application of acute-to-chronic ratios per methods outlined in the ECOSAR Methodology Document provided in the ECOSAR Help Menu.

Class Specific LogK_{ow} Cut-Offs

If the log K_{ow} of the chemical is greater than the endpoint specific cut-offs

presented below, then no effects at saturation are expected for those endpoints.

Aliphatic Amines:

Maximum LogK_{ow}: 6.0 (Fish, Mysid LC50)
Maximum LogK_{ow}: 5.0 (Daphnid LC50)
Maximum LogK_{ow}: 7.0 (Green Algae EC50)
Maximum LogK_{ow}: 8.0 (Fish, Daphnid ChV)
Maximum LogK_{ow}: 7.0 (Green Algae ChV)

Halopyrdines :

Maximum LogK_{ow}: 5.0 (LC50)
Maximum LogK_{ow}: 6.4 (EC50)
Maximum LogK_{ow}: 8.0 (ChV)

Neonicotinoids:

Maximum LogK_{ow}: 5.0 (Fish 96-hr LC50; Daphnid LC50)
Maximum LogK_{ow}: 5.0 (Invertebrate (SW) LC50)
Maximum LogK_{ow}: 6.4 (Green Algae EC50)
Maximum LogK_{ow}: 8.0 (Chronic Values)

Baseline Toxicity SAR Limitations:

Maximum LogK_{ow}: 5.0 (Fish 96-hr LC50; Daphnid LC50)
Maximum LogK_{ow}: 6.4 (Green Algae EC50)
Maximum LogK_{ow}: 8.0 (ChV)

APPENDIX E: EPISuite Modeling Results for Imidacloprid (CAS #138261-41-3)

CAS Number: 138261-41-3
SMILES: c1nc(CL)ccc1CN2C(=NN(=O)(=O))NCC2
CHEM: Imidacloprid
MOL FOR: C9 H10 CL1 N5 O2
MOL WT: 255.67
---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log K_{ow} (octanol-water): ------
Boiling Point (deg C): ------
Melting Point (deg C): ------
Vapor Pressure (mm Hg): ------
Water Solubility (mg/L): ------
Henry LC (atm-m^3/mole): ------

Log Octanol-Water Partition Coef (SRC):
Log K_{ow} (K_{ow}WIN v1.68 estimate) = 0.56
Log K_{ow} (Exper. database match) = 0.57
Exper. Ref: TOMLIN, C. (2003)

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 378.84 (Adapted Stein & Brown method)
Melting Pt (deg C): 156.00 (Mean or Weighted MP)
VP (mm Hg,25 deg C): 1.68E-006 (Modified Grain method)
VP (Pa, 25 deg C): 0.000225 (Modified Grain method)
MP (exp database): 144 deg C
Subcooled liquid VP: 2.7E-005 mm Hg (25 deg C, Mod-Grain method)
: 0.0036 Pa (25 deg C, Mod-Grain method)

Water Solubility Estimate from Log K_{ow} (WSK_{ow} v1.42):
Water Solubility at 25 deg C (mg/L): 7172
log K_{ow} used: 0.57 ($expk_{ow}$ database)
no-melting pt equation used
Water Sol (Exper. database match) = 610 mg/L (20 deg C)
Exper. Ref: TOMLIN, C. (2003)

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 1e+006 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Aliphatic Amines
Halopyrdines
Neonicotinoids

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method: 1.04E-013 atm-m^3/mole (1.05E-008 Pa-m^3/mole)

Group Method: Incomplete
Exper Database: 1.65E-15 atm-m^3/mole (1.67E-010 Pa-m^3/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: 7.880E-011 atm-m^3/mole (7.985E-006 Pa-m^3/mole)
VP: 1.68E-006 mm Hg (source: MPBPVP)
WS: 7.17E+003 mg/L (source: $WSK_{ow}WIN$)

Log Octanol-Air Partition Coefficient (25 deg C) [K_{oa}WIN v1.10]:
Log K_{ow} used: 0.57 (exp database)
Log K_{aw} used: -13.171 (exp database)
Log K_{oa} (K_{oa}WIN v1.10 estimate): 13.741
Log K_{oa} (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model): 0.2888
Biowin2 (Non-Linear Model): 0.0137
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 2.2134 (months)
Biowin4 (Primary Survey Model): 3.2948 (days-weeks)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model): -0.0674
Biowin6 (MITI Non-Linear Model): 0.0080
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 0.5412
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): 0.0036 Pa (2.7E-005 mm Hg)
Log K_{oa} (K_{oa}win est): 13.741
Kp (particle/gas partition coef. (m^3/µg)):
Mackay model: 0.000833
Octanol/air (K_{oa}) model: 13.5
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model: 0.0292
Mackay model: 0.0625
Octanol/air (K_{oa}) model: 0.999

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 151.6789 E-12 cm^3/molecule-sec
Half-Life = 0.071 Days (12-hr day; 1.5E6 OH/cm^3)
Half-Life = 0.846 Hrs.
Ozone Reaction:
No Ozone Reaction Estimation

Fraction sorbed to airborne particulates (phi):
0.0459 (Junge-Pankow, Mackay avg)
0.999 (K_{oa} method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (K_{oc}WIN v2.00):
K_{oc}: 969.9 L/kg (MCI method)
Log K_{oc}: 2.987 (MCI method)
K_{oc}: 33.64 L/kg (K_{ow} method)
Log K_{oc}: 1.527 (K_{ow} 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 = 0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -1.8866 days (HL = 0.01298 days)
Log BCF Arnot-Gobas method (upper trophic) = 0.011 (BCF = 1.026)
Log BAF Arnot-Gobas method (upper trophic) = 0.011 (BAF = 1.026)
log K_{ow} used: 0.57 (expkow database)

Volatilization from Water:
Henry LC: 1.65E-015 atm-m^3/mole (Henry experimental database)
Half-Life from Model River: 5.674E+011 hours (2.364E+010 days)
Half-Life from Model Lake: 6.19E+012 hours (2.579E+011 days)

Removal in Wastewater Treatment:
Total removal: 1.86 percent
Total biodegradation: 0.09 percent
Total sludge adsorption: 1.77 percent
Total to Air: 0.00 percent
(using 10000 hr. Bio P,A,S)

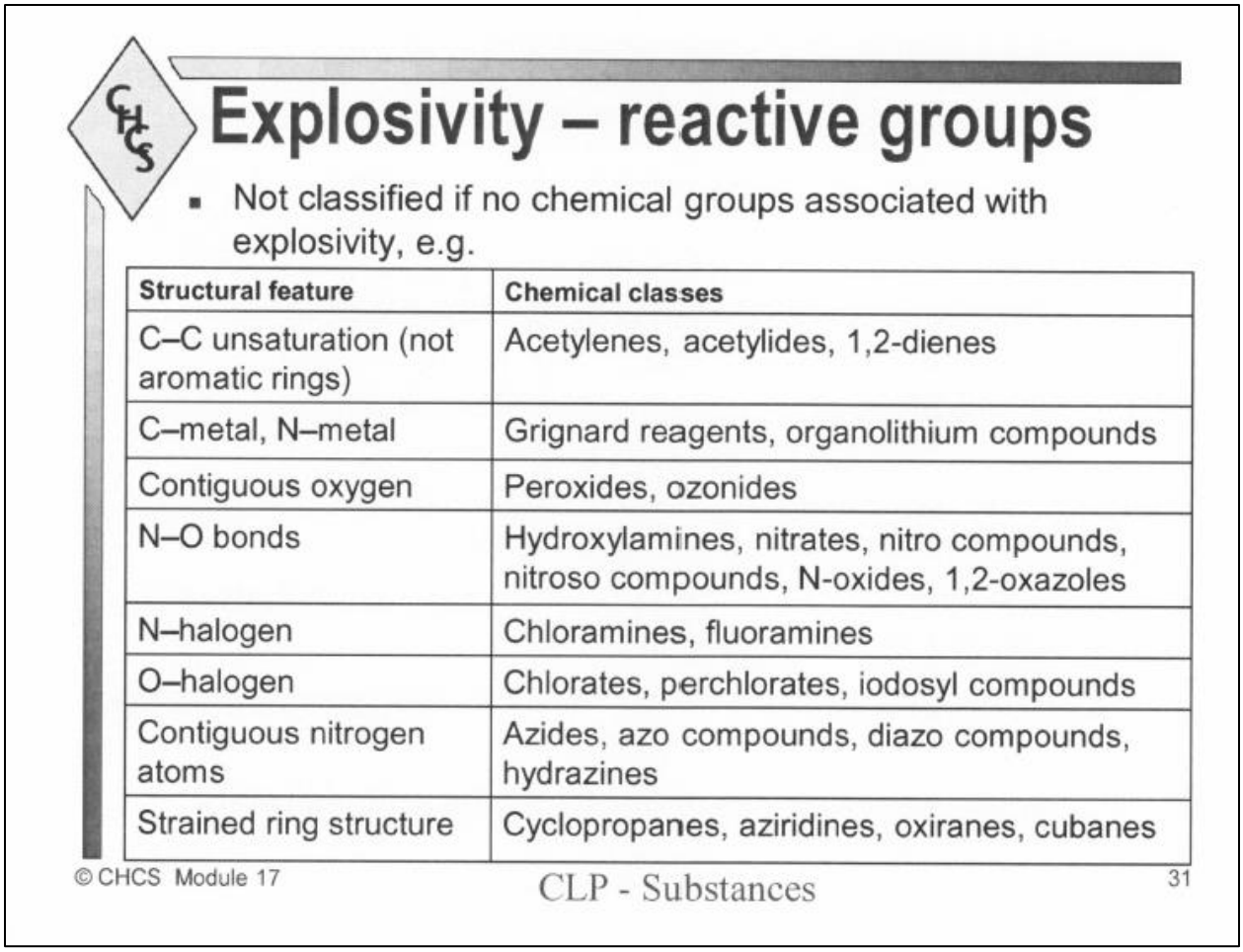
Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr.)	Emissions (kg/hr.)
Air	8.25e-009	1.69	1000
Water	9.46	1.44e+003	1000
Soil	90	2.88e+003	1000
Sediment	0.592	1.3e+004	0

Persistence Time: 2.8e+003 hr.

APPENDIX F: Known Structural Alerts for Reactivity

Explosivity – Abbreviated List



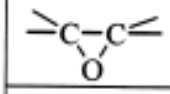
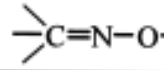
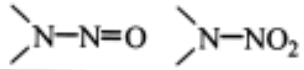
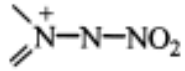
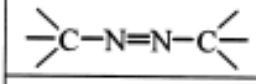
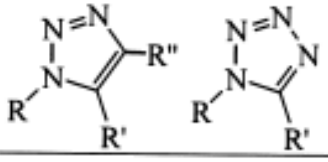
Explosivity – reactive groups

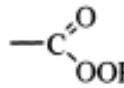
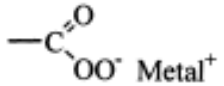
- Not classified if no chemical groups associated with explosivity, e.g.

Structural feature	Chemical classes
C–C unsaturation (not aromatic rings)	Acetylenes, acetylides, 1,2-dienes
C–metal, N–metal	Grignard reagents, organolithium compounds
Contiguous oxygen	Peroxides, ozonides
N–O bonds	Hydroxylamines, nitrates, nitro compounds, nitroso compounds, N-oxides, 1,2-oxazoles
N–halogen	Chloramines, fluoramines
O–halogen	Chlorates, perchlorates, iodosyl compounds
Contiguous nitrogen atoms	Azides, azo compounds, diazo compounds, hydrazines
Strained ring structure	Cyclopropanes, aziridines, oxiranes, cubanes

Explosivity – Full List

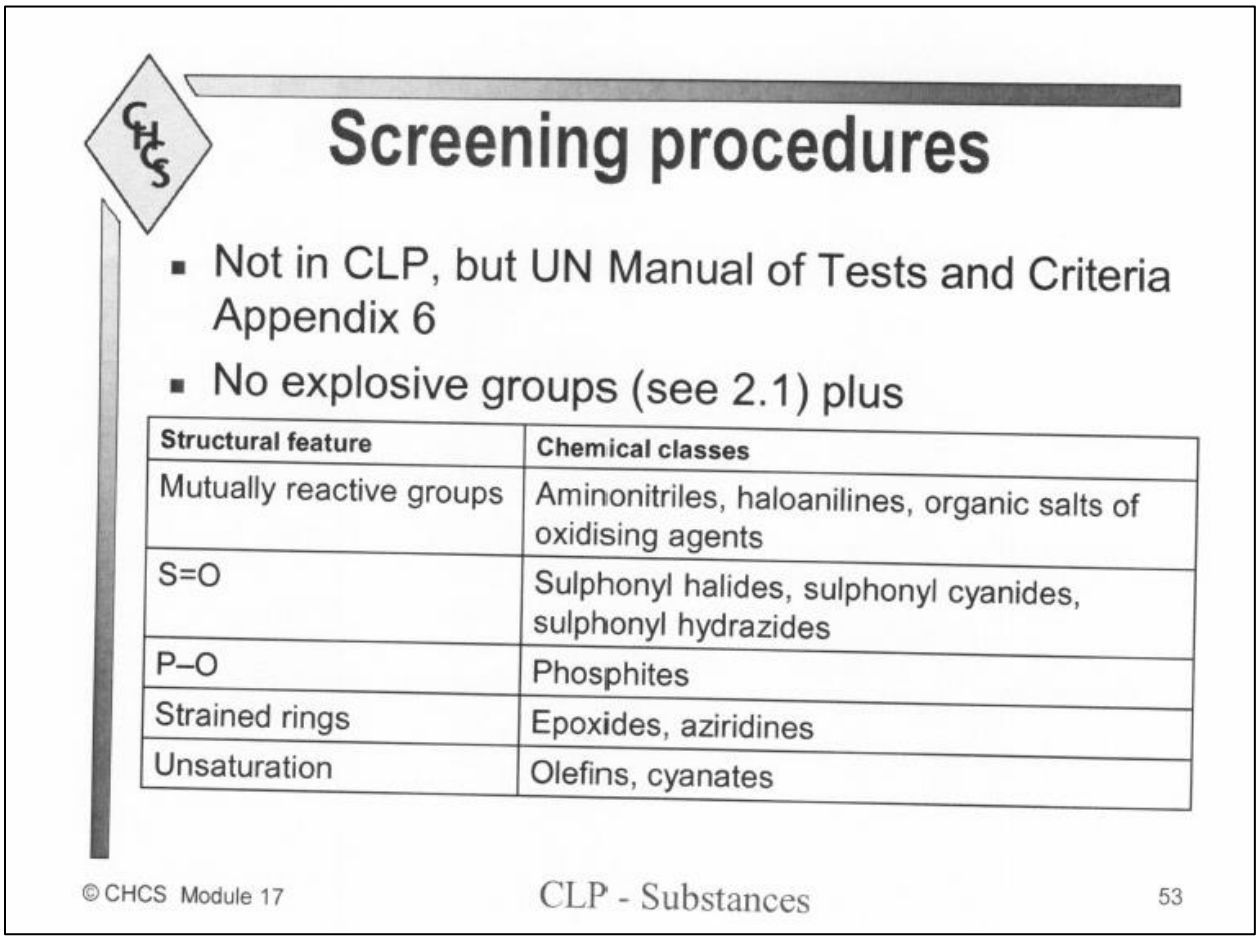
Table R.7.1-28 Chemical groups associated with explosive properties

Chemical group	Chemical Class
-C≡C- -C≡C-Metal -C≡C-Halogen	Acetylenic Compounds Metal Acetylides Haloacetylene Derivatives
	Diazo Compounds
-N=O -NO_2	Nitroso and Nitro Compounds,
R-O-N=O R-O-NO_2	Acyl or Alkyl Nitrites and Nitrates
	1,2-Epoxides
	Metal Fulminates or *aci*-Nitro Salts
	N-Metal Derivatives (especially heavy metals)
	N-Nitroso and N-Nitro Compounds
	N-Azolium Nitroimidates
	Azo Compounds
Ar-N=N-O-Ar	Arene Diazoates
$(ArN=N)_2O$, $(ArN=N)_2S$	Bis-Arenediazo Oxides and Sulfides
RN=N-NR'R''	Triazines
	High-nitrogen Compounds: e.g. Triazoles, Tetrazoles

Chemical group	Chemical Class
[1] ROOR', [2] 	Peroxy Compounds: [1] Alkyl hydroperoxides (R'=H), Peroxides (R'=organic); [2] Peroxo acids (R'=H), Peroxyesters (R'=organic)
[1] ROOMetal, [2] 	Metal peroxides, Peroxoacids salts
$-N_3$	Azides e.g. PbN_6, CH_3N_3
$^{-}O-C-N_2^{+}$	Arenediazonium oxides i.e. inner diazonium salts in which the counter ion is an oxide
Ar-N=N-S- Ar-N=N-S-Ar	Diazonium sulfides and derivatives, Arenediazo Aryl Sulfides
XO_n	Halogen Oxide: e.g. percholrates, bromates, etc
NX_3 e.g. $NC1_3$, $RNC1_2$	N-Halogen Compounds

Adapted from Bretherick (Bretherick's Handbook of Reactive Chemical Hazards 6th Ed., 1999, Butterworths, London)

Self-Reactive Substances



Licensed GreenScreen® Profilers

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