Appendix G: Simazine Ecological Effects Characterization

This appendix presents available submitted and open literature studies available on simazine. Studies that are submitted to the Agency in support of pesticide registration or re-registration are categorized as either; acceptable, supplemental, or invalid. Acceptable means that all essential information was reported, the data are scientifically valid, and the study was performed according to recommended protocols. Studies in the “acceptable” category fulfill the corresponding data requirement in 40 CFR Part 158 and are appropriate for use in risk assessment. Supplemental studies are also scientifically valid; however, they were either performed under conditions that deviate from recommended guideline protocols or certain data necessary for complete verification are missing. Supplemental studies may be used quantitatively in the risk assessment and can, at the Agency’s discretion, fulfill the corresponding data requirement in 40 CFR Part 158. Invalid studies are not scientifically valid, or deviate substantially from recommended protocols such that they are not useful for risk assessment. Invalid studies do not fulfill the corresponding data requirement in 40 CFR Part 158.

With respect to the open literature, studies may be classified as either; qualitative, quantitative, or invalid. The degree to which open literature data are quantitatively or qualitatively characterized is dependent on whether the information is relevant to the assessment endpoints (i.e., maintenance of the survival, reproduction, and growth of the listed species) identified in the problem formulation. Open literature studies classified as qualitative are not appropriate for quantitative use but are of good quality, address issues of concern to the risk assessment, and, when appropriate, are discussed qualitatively in the risk characterization discussion. Those open literature studies that are classified as quantitative are appropriate for quantitative use in the risk assessment including calculation of RQs. It should be noted that this appendix includes all relevant data taken from the 2005 RED simazine effects appendix. Open literature data in the 2005 RED includes ECOTOX information obtained in November 2004. In addition, an update of the ECOTOX open literature information for simazine was obtained on September 30, 2006. Data that pass the ECOTOX screen described in Section 4.1 of the assessment are evaluated along with the registrant-submitted data, and may be incorporated qualitatively or quantitatively into this endangered species assessment. In general, effects data in the open literature that are more conservative than the registrant-submitted data are considered for quantitative use.

Citations for all open literature not considered as part of this assessment because it was either rejected by the ECOTOX screen or accepted by ECOTOX but not used (e.g., the endpoint is less sensitive and/or not appropriate for use in this assessment) are included in Appendix F. Appendix F also includes a rationale for rejection of those studies that did not pass the ECOTOX screen and those that were not evaluated as part of this endangered species assessment. Further detail on the ECOTOX exclusion categories is provided in the Agency's *Guidance of the Evaluation Criteria for Ecological Toxicity Data in the Open Literature* (U.S. EPA, 2004).

A.1 Toxicity to Freshwater Animals

A.1.1 Freshwater Fish, Acute Submitted Data

Two freshwater fish toxicity studies using the TGAI are required to establish the toxicity of simazine to fish. The preferred test species are rainbow trout (*Oncorhynchus mykiss;* a coldwater fish) and bluegill sunfish (*Lepomis macrochirus;* a warmwater fish). Toxicity data are available for technical grade simazine and two formulations including Aquazine 80W (88.6% a.i.) and a 50% formulation. Results of these tests are summarized below in Table A-10.

Table A-10: Freshwater Fish Acute Toxicity for Technical Grade Simazine and Formulations

Surrogate Species/ Static or Flow-through test	% a.i.	96-hour LC_{50} (mg ai/L) (measured/nominal) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification
Simazine (Technical)					
Fathead minnow (*Pimephales promelas*) Static test	Tech. (NR)	6.4 (4.8 – 8.7) (nominal) No slope data	moderately toxic	000333-09 Sleight, 1971	Supplemental (no raw data, no test concentrations)
Bluegill sunfish (*Lepomis macrochirus*) Static test	99.1	16 (9.9 – 26) (nominal) No slope data	slightly toxic	000254-38 Beliles et al., 1965	Acceptable
Rainbow trout (*Salmo gairdneri*) Static test	97.6	>10 (nominal) No slope data	slightly toxic	001631-35 Thompson and Forbis, 1983	Supplemental (no LC_{50} determined)
Rainbow trout (*Salmo gairdneri*) Static test	Tech. (NR)	NR 28-day subacute study NOAEC = 2.5 (measured) No slope data	NA	00043668 Zak et al., 1973	Supplemental (28-day study; only one concentration tested)

Table A-10: Freshwater Fish Acute Toxicity for Technical Grade Simazine and Formulations					
Surrogate Species/ Static or Flow-through test	**% a.i.**	**96-hour LC_{50} (mg ai/L) (measured/nominal) Probit Slope**	**Toxicity Category**	**MRID No. Author/Year**	**Study Classification**
Goldfish (*Carassius auratus*) Static test	99.1	>32 (nominal) No slope data	slightly toxic	00023322 Woodard Res. Corp., 1965	Supplemental (no LC_{50} determined; unacceptable test species; only 5 fish/treatment level)
Gesatop GW (90% formulated product)					
Seabream (Sparus aurata) yolk-sac larvae	90	72- hour LC50 = 4.19* mg a.i./L	moderately toxic	Arufe *et al*., 2004	Supplemental (72-h study, no measured concentrations and no raw data provided)
Aquazine 80W (formulated product)					
Rainbow trout (*Salmo gairdneri*) Static test	88.6	>82; >72.6* (measured) No slope data	slightly toxic	40245701 Bowman, 1987	Acceptable
Simazine (50% formulated product)					
Emerald shiner (*Notropis cornutus*) Static test	50	>18; >9* (nominal) No slope data	slightly toxic	00025435 Swabey and Schnek, 1963	Supplemental (no LC_{50} determined; non-guidline test species; only 5 fish/treatment level)
Pumpkinseed (*Lepomis gibbosus*) Static test	50	27; 13.5* No slope data	slightly toxic	000254-35 Swabey and Schnek, 1963	Supplemental (no LC_{50} determined; non-guideline test species; only 5 fish/treatment level)
Bluegill sunfish (*Lepomis macrochirus*) Static test	50	35; 17* No slope data	slightly toxic	000254-35 Swabey and Schnek, 1963	Supplemental (no LC_{50} determined; non-guidline test species; only 5 fish/treatment level)
Largemouth bass (*Micropterus salmoides*) Static test	50	46; 23* No slope data	slightly toxic	000254-35 Swabey and Schnek, 1963	Supplemental (no LC_{50} determined; non-guidline test species; only 5 fish/treatment level)
Redear sunfish (*Lepomis microlopus*) Static test	50	54; 27* No slope data	slightly toxic	000254-35 Swabey and Schnek, 1963	Supplemental (no LC_{50} determined; non-guidline test species; only 5 fish/treatment level)
Bluntnose minnow (*Pimephales notatus*) Static test	50	66; 33* No slope data	slightly toxic	000254-35 Swabey and Schnek, 1963	Supplemental (no LC_{50} determined; non-guidline test species; only 5 fish/treatment level)
Channel catfish (*Ictalarus punctatus*) Static test	50	85; 42.5* No slope data	slightly toxic	000254-35 Swabey and Schnek, 1963	Supplemental (no LC_{50} determined; non-guidline test species; only 5 fish/treatment level)

Table A-10: Freshwater Fish Acute Toxicity for Technical Grade Simazine and Formulations					
Surrogate Species/ Static or Flow-through test	**% a.i.**	**96-hour LC_{50} (mg ai/L) (measured/nominal) Probit Slope**	**Toxicity Category**	**MRID No. Author/Year**	**Study Classification**
Yellow bullhead (*Ictalarus natalis*) Static test	50	110; 55* No slope data	slightly toxic	000254-35 Swabey and Schnek, 1963	Supplemental (no LC_{50} determined; non-guidline test species; only 5 fish/treatment level)
* = adjusted for percentage a.i.					

The range of acute freshwater fish LC_{50} values for technical grade simazine is 4.2 mg/L to >32 mg/L; therefore, simazine is categorized as slightly (>10 to 100 mg/L) to moderately (>1 to 10 mg/L) toxic to freshwater fish on an acute exposure basis. The freshwater fish acute nominal LC_{50} value of 6.4 mg/L is based on a static 96-hour toxicity test using fathead minnow (*Pimephales promelas*) (MRID # 000333-09).

Although freshwater fish LC_{50} values for simazine exceed the predicted limit of simazine's solubility in water (3.8 mg/L), a co-solvent was used to increase the limit of simazine's water solubility, and no observation of precipitate was noted in the test chambers. Therefore, the fathead minnow LC_{50} value of 6.4 mg ai/L was used to characterize acute risks to freshwater fish. This test was categorized as supplemental because no raw data or test concentrations were provided in the study. A no effect level of 2.5 mg ai/L was established in the 96-hour fathead minnow study. This no effect level is consistent with the results of a 28-day subacute rainbow trout study (MRID 000436-68). Following 28-days of exposure, no mortality or other toxic symptoms were observed at the 2.5 mg ai/L treatment level. The subacute study was classified as supplemental because the fish were too large (25-40g) and only one treatment level (2.5 ppm ai) was tested. In the acute bluegill sunfish study, which is classified as acceptable, no mortality was observed in treatment groups $\leq$ 5.6 mg ai/L, and 40% mortality was observed in the 10 mg ai/L treatment group.

There is additional uncertainty in all available acute freshwater studies on the TGAI regarding dissolved levels of simazine in water because mean-measured test concentrations were not analyzed. Reported nominal concentration results reflect the concentration after the application and not necessarily the concentration of simazine in water during or at the end of the 96-hour test. A number of the acute studies on both the TGAI and formulated product are classified as invalid because precipitation of the test substance in the test chambers was reported and LC_{50} values exceed the water solubility of simazine by a large margin.

Acute effects data for freshwater fish is available for a number of simazine's formulated products including Aquazine (80% WP), a 50% formulation, and a 4% granular formulation. With the exception of the 4% granular formulation, all ai-adjusted LC_{50} values for Aquazine (>72.6 mg ai/L) and the 50% formulation (13.5 to 55 mg ai/L) exceed the lowest LC_{50} value for the TGAI (6.4 mg ai/L). The available data suggests that Aquazine and the 50% formulation are less toxic to freshwater fish than the TGAI. The lowest reported 96-hour LC_{50} value for one of simazine's formulated products, a 4% granular, is 5 mg/L (MRID 400980-01) for the fathead minnow. However, further review of the raw data showed that precipitate was observed in the test containers and mean-measured concentrations were not available; therefore, the study was classified as invalid.

Degradates. Acute studies in rainbow trout have also been submitted for DACT and DIA degradates. Table A-11 presents freshwater fish toxicity data for DIA and DACT.

Table A-11: Freshwater Fish Acute Toxicity for Simazine Degradates					
Surrogate Species/ Flow-through or Static	**% ai formul.**	**96-hour LC_{50} (mg ai/L) (measured/nominal)**	**Toxicity Category**	**MRID No. Author/Year**	**Study Classification**
DIA					
Rainbow trout (*Oncorhynchus mykiss*); 1.5 grams Static test; 14 °C 165 mg/L hardness	Not reported	17 (measured dissolved)	Slightly toxic	470461-03 Vial, 1991a	Acceptable
DACT					
Rainbow trout (*Oncorhynchus mykiss*); 1.5 g Static test; 14 °C 164 mg/L hardness	Not reported	>100 (measured dissolved)	Practically non-toxic	470461-04 Vial, 1991b	Acceptable

Acute freshwater fish toxicity values for DIA and DACT are 2.6- and 15.6 times less sensitive than acute toxicity values for simazine, respectively.

A.1.2 Freshwater Fish, Chronic Submitted Data

A freshwater fish early life-stage test using the TGAI is required for simazine because the end-use product is expected to be transported to water from the intended use site. Given its intended use, simazine's presence in water is likely to be continuous or recurrent regardless of toxicity. Simazine is applied more than once a year to some crops and is applied directly to water as an aquatic herbicide; therefore, a fish early-life stage test is required. **Table A-12** summarizes the results of chronic toxicity tests with freshwater fish.

Table A-12: Chronic Toxicity of Simazine to Fish							
Species	**% a.i.**	**NOAEC (mg/L)**	**LOAEC (mg/L)**	**Study Properties[a]**	**Most sensitive parameter**	**MRID , Author, Year**	**Status**
Aquazine (80% Formulated Product)							
Fathead minnow (*P. promelas*)	80	0.16 / 1.2; 0.13 / 0.96*	0.31 / 2.5; 0.25 / 2.0*	N, F-T (120 day full life cycle test)	increased % hatch / 12% reduc. in fry growth	000436-76, Mayer & Sanders, 1975	Acceptable

Table A-12: Chronic Toxicity of Simazine to Fish							
Species	**% a.i.**	**NOAEC (mg/L)**	**LOAEC (mg/L)**	**Study Properties[a]**	**Most sensitive parameter**	**MRID , Author, Year**	**Status**
Fathead minnow (*P. promelas*)	80	0.31; 0.25*	0.62; 0.50*	N, S[b] (120 day full life cycle test)	increased fry growth (30-day old fry)	000436-76, Mayer & Sanders, 1975	Acceptable

[a] M = mean-measured concentrations; N = nominal chemical concentrations; F-T = flow-through; S = static.
* = adjusted for percentage a.i.

No freshwater fish early life-stage test using the TGAI was submitted for simazine. Two fish life-cycle tests with fathead minnow were submitted for Aquazine, an 80% formulation that is typically applied directly to the water (MRID 000436-76). The studies are classified as acceptable, however, they do not satisfy the §72-5 guideline requirement using the TGAI. One test was conducted with steady concentrations via continuous flow. In the second test, the chemical was applied at the beginning of the test and allowed to decrease at normal degradation rates. Both tests were conducted at the same initial test concentrations. The static test where test concentrations decrease over time is intended to be representative of typical use-pattern exposures of Aquazine. The lowest endpoint values in the continuous and usage-pattern exposures were increase in percent hatched fry (NOAEC = 0.13 ppm ai) and increased fry growth (length) (NOAEC = 0.25 ppm ai), respectively. However, neither of these endpoints are considered as toxicologically relevant for the risk assessment. Therefore, a NOAEC value of 0.96 ppm is used, based on 12% reduction in growth (length) to 30-day old fry at a continuous exposure treatment level of 2.0 ppm ai. The corresponding LOAEC value, based on reduction in fry growth, is 2.0 ppm ai.

A.1.3 Freshwater Fish, Open Literature Data

No additional information is available that indicates greater acute freshwater fish sensitivity to simazine than the submitted data. In addition, no laboratory freshwater fish early life-stage or life-cycle tests using simazine and/or its formulated products were located in the open literature. However, one laboratory study on sublethal effects of simazine to male Atlantic salmon (*Salmo salar* L.) is available. In a study conducted by Moore and Lower (2001; ECOTOX# 67727), simazine inhibited *in vitro* olfactory function in male Atlantic salmon parr. The results of this study are summarized in Table A-13. Following a 5 day exposure period, the reproductive priming effect of the female pheromone prostaglandin $F_{2\alpha}$ on the levels of expressible milt in males was reduced after exposure to simazine at concentrations as low as 0.1 μg/L. Although the hypothesis was not tested, the study authors suggest that exposure of smolts to simazine during the freshwater stage may potentially affect olfactory imprinting to the natal river and subsequent homing of adults. However, no quantitative relationship is established between reduced olfactory response of male epithelial tissue to the female priming hormone in the laboratory and reduction in salmon reproduction (i.e., the ability of male salmon to detect, respond to, and mate with ovulating females). Although this study produced a NOAEC that is lower than the fish full life-cycle test of 960 ppb, this study was not considered appropriate for RQ calculation for the following reasons:

(1) A negative control was not used; therefore, potential solvent effects cannot be evaluated;
(2) The study did not determine whether the decreased response of olfactory epithelium to specific chemical stimuli would likely impair similar responses in intact fish.
(3) A quantitative relationship between the magnitude of reduced olfactory response of males to the female priming hormone observed in the laboratory and reduction in salmon reproduction (i.e., the ability of male salmon to detect, respond to, and mate with ovulating females) in the wild is not established.

Table A-13: Freshwater Fish Sublethal Effect Studies from the Open Literature

Study type/ Test material	Test Design	Test Organism	Effects	Citation (ECOTOX #)	Rationale for Use in Risk Assessment [1]
Olfactory detection of female priming pheromone, protogandin $_{F2\alpha}$ in FW fish 30 min exposure Simazine, Atrazine, and Simazine/ Atrazine mixtures (% a.i. NR)	Skin and cartilage removed to expose olfactory rosettes Olfactory epithelium perfused with control water for 30 min, then to simazine-treated water at nominal concentrations of 0.1, 0.5, 1.0, and 2.0 ug/l for 30 min.	mature male Atlantic salmon (*Salmo salar* L.) parr; length = 140 mm; weight = 34.2 g) source: Environment Agency, Cynrig hatchery, Wales	Responses of olfactory epithelium of mature male salmon parr to a 10^{-9} M concentration of $PGF_{2\alpha}$ were recorded after perfusion of olfactory rosette w/ different treatment levels of simazine. The electrophysiological recordings from the olfactory epithelium in response to $PGF_{2\alpha}$ were significantly reduced after exposure to simazine at nominal concentrations of 1.0 ug/L and 2.0 ug/L. The responses were 88 and 71% of the controls, respectively. Simazine reduced the ability of male salmon parr to detect the female priming pheromone. When the olfactory epithelium was exposed to simazine and atrazine together (concentrations of 0.5:0.5 and 1.0:1.0 ug/L), there was no significant reduction in the olfactory response when compared to the single pesticides at equivalent concentrations of 1.0 and 2.0 ug/L.	Moore, A., and N. Lower, 2001 (67727)	QUAL : A solvent control, but no negative control, was used; therefore, potential solvent effects cannot be evaluated; Study conducted on olfactory epithelium; therefore it is unclear whether response to chemical stimuli would impair similar responses in intact fish. Relationship between the magnitude of effects on the endpoints evaluated and reproduction or survival has not been established.
Priming response of male FW fish to $PGF_{2\alpha}$ 5 day exposure Simazine, Atrazine, and Simazine/ Atrazine mixtures (% a.i. NR)	Nominal simazine concentrations of 0, 1.0, 1.5, 1.0, and 2.0 ug/L. Solutions renewed every 12 h. 5 day exposure to simazine followed by 5 day exposure to $PGF_{2\alpha}$.	Sperminating male Atlantic salmon (*Salmo salar* L.) parr, length = 138 mm; weight = 31.6 g)	Exposure to $PGF_{2\alpha}$ for 5 h significantly increased levels of expressible milt in male salmon parr. Groups of male fish exposed to nominal simazine concentrations of 0.1, 0.5, 1.0, and 2.0 ug/L showed no apparent significant increases in the levels of expressible milt. Although expressible milt levels were reduced in all groups, there were no significant differences between the different simazine treatments. Exposure to simazine and atrazine mixtures had no synergistic effect on the priming response, and plasma levels of testosterone, 11-keto-testosterone and 17,20β-dihydroxy-4-pregnen-3-one were similar in the groups of male parr exposed to individual pesticides.		

[1] QUAL = The paper is not appropriate for quantitative use but is of good quality, addresses issues of concern to the risk assessment and is used in the risk characterization discussion.

A.1.4 Freshwater Invertebrates, Acute Submitted Data

A freshwater aquatic invertebrate toxicity test using the TGAI is required to establish the toxicity of simazine to aquatic invertebrates. Acute freshwater invertebrate data are available for the technical grade of simazine. Results of acute toxicity tests with freshwater invertebrates are tabulated in Table A-14. Based on the available data, simazine is categorized as highly to slightly toxic to freshwater invertebrates on an acute basis.

Acute toxicity data for simazine are available for *Daphnia magna*, as well as seven other freshwater invertebrates including the seed shrimp (*Cypridopsis vidua*), scud (*Gammarus lacustris* and *G. fasciatus*), stonefly (*Pteronarcys californica*), sowbug (*Asellus brevicaudus*), glass shrimp (*Palaemonetes kadiakensis*), and crayfish (*Orconectes nais*).

In a comparative analysis of herbicides on six species of freshwater invertebrates, 48-hr exposure to simazine at concentrations of 1.0 and 3.7 mg ai/L resulted in 50 percent mortality in daphnia and seed shrimp, respectively (MRID 450882-21). In the same analysis, simazine did not appear to have any effect on the scud (*G. fasciatus*), sowbug, glass shrimp, or crayfish, with 48-hr TL_{50} values exceeding 100 mg ai/L. However, as previously mentioned. toxicity values > 100 mg ai/L exceed the water solubility of simazine by a wide margin; therefore, the validity of the data is uncertain. TL_{50} values reported in the study are median tolerance limits, representative of the concentration in water in which 50 percent of the animals exhibit a specific response (i.e., mortality, immobilization) at a given time. It should be noted that no test concentrations or raw data were provided as part of this study; therefore, it was classified as supplemental. In addition, the slope of the dose-response relationship for daphnia could not be determined due to a lack of raw data and test concentrations.

Two additional supplemental 96-hr acute toxicity studies on freshwater invertebrates are available for the technical grade of simazine. In a chemical database of acute toxicity to freshwater animals maintained by the Columbia National Fisheries Research Laboratory of the U.S. Fish and Wildlife Service, 96-hr exposure of the stonefly (*P. californica*) to simazine resulted in an EC_{50} of 1.9 mg ai/L (MRID 400980-01). A 96-hr EC_{50} value of 13 mg ai/L was reported for the scud (*G. lacustris*) (MRID 050092-42) in a study classified as supplemental because no mortality data were provided and test concentrations were not specified.

Table A-14: Acute Toxicity of Simazine to Freshwater Invertebrates

Species	% a.i.	48-hr EC_{50}, mg/L (confid. int.)	NOAEC (mg/L)	Study Properties[a]	Toxicity Classification	MRID, Author, Year	Status
Water flea (*Daphnia magna*) (TL_{50})	98.1	1	NR	N, S	highly toxic	450882-21, Sanders, 1970	Supplemental (No raw data; test concentrations not provided)
					moderately	450882-21,	

Table A-14: Acute Toxicity of Simazine to Freshwater Invertebrates							
Species	**% a.i.**	**48-hr EC_{50}, mg/L (confid. int.)**	**NOAEC (mg/L)**	**Study Properties[a]**	**Toxicity Classification**	**MRID, Author, Year**	**Status**
Seed shrimp (*Cypridopsis vidua*) (TL_{50})	98.1	3.7 (2.6 - 5.3)	NR	N, S	toxic	Sanders, 1970	Supplemental[b]
Corals (*Madracis mirabilis*, *Diplora strigos* and, *Favia fragum*)	Not Given	0.01	NR	N, S	highly toxic	Owen *et al*, 1993	Qualitative (zooxanthele, rather than coral, were actually tested, no purity given and no measured concentrations)
Scud (*Gammarus lacustris*) (96-hr LC_{50})	Tech.	13 (11.4 - 15.0)	NR	N, S	slightly toxic	450882-21, Sanders, 1970	Supplemental[b] (No raw data; test concentrations not provided)
Stonefly (naiads) (*Pteronarcys californica*) (96-hr LC_{50})	98.1	1.9 (0.9 - 4.04)	NR	N, S	moderately toxic	400980-01, Mayer & Ellersieck	Supplemental[b]
Scud (*Gammarus fasciatus*) (TL_{50})	98.1	>100	NR	N, S	practically non-toxic	450882-21, Sanders, 1970	Supplemental[b]
Sowbug (*Asellus brevicaudus*) (TL_{50})	Tech	>100	NR	N, S	practically non-toxic	450882-21, Sanders, 1970	Supplemental[b]
Glass shrimp (*Palaemonetes kadiakensis*) (TL_{50})	Tech	>100	NR	N, S	practically non-toxic	45088-221, Sanders, 1970	Supplemental[b]
Crayfish (*Orconectes nais)* (TL_{50})	Tech	>100	NR	N, S	practically non-toxic	450882-21, Sanders, 1970	Supplemental[b]

[a] M=mean-measured chemical concentrations, N=nominal chemical concentrations; F-T=flow-through; S=static.
TL_{50} = median tolerance limit in water in which 50 percent of the animals exhibit a specific response at a given time.
NR = Not reported.
[b] Non-guideline test species.

Degradates: Acute aquatic invertebrate testing with *Daphnia magna* (72-2) was completed to address degradate concerns for DIA and DACT. Table A-15 presents freshwater invertebrate toxicity data for these degradates.

Table A-15. Freshwater Invertebrate Acute Toxicity of Simazine Degradates					
Surrogate Species/ Flow-through or Static	**% ai form.**	**48-hour EC_{50} (mg/L) (measured/ nominal)**	**Toxicity Category**	**MRID No. Author/Year**	**Study Classification**
DIA					
Waterflea (*Daphnia magna*); 1st instar (6-24 h old) Static test	Not reported	>100 (measured dissolved)	Practically non-toxic	470461-02 Vial, 1991d	Supplemental (no raw data were provided)
DACT					

Table A-15. Freshwater Invertebrate Acute Toxicity of Simazine Degradates					
Surrogate Species/ Flow-through or Static	**% ai form.**	**48-hour EC_{50} (mg/L) (measured/ nominal)**	**Toxicity Category**	**MRID No. Author/Year**	**Study Classification**
Waterflea (*Daphnia magna*); 1st instar (6-24 h old) Static test	Not reported	>100 (measured dissolved)	Practically non-toxic	470461-01 Vial, 1991c	Acceptable

Acute freshwater invertebrate toxicity values for DIA and DACT are greater than 100 times less sensitive than acute toxicity values for simazine.

A.1.5 Freshwater Invertebrate, Chronic Submitted Data

A freshwater aquatic invertebrate life-cycle test using the TGAI is required for simazine because the end-use product may be applied directly to the freshwater environment or is expected to be transported to water from the intended use site and the following conditions are met: the pesticide is intended for use such that its presence in water is likely to be continuous, and the pesticide is persistent in water (i.e., half-life greater than 4 days). The preferred test species is *Daphnia magna*. Results of these tests are summarized below in Table A-16.

No freshwater invertebrate life-cycle test using the TGAI was submitted for simazine. A freshwater aquatic invertebrate life-cycle test using the formulated product Aquazine (80% formulation) was submitted for simazine (MRID 000436-76) using the preferred species *D. magna*. No treatment-related adverse effects to parental mortality and production of offspring occurred during the 21-day study at the highest test concentration of 2.0 mg ai/L. The only treatment-related effect was a significant stimulation of offspring produced at the 0.08 mg ai/L test concentration. Therefore, the NOAEC value is 2.0 mg ai/L. The study is classified as acceptable and satisfies the data requirement for freshwater aquatic invertebrate life-cycle testing with the TGAI.

Table A-16: Chronic (Life-cycle) Toxicity of Simazine to Freshwater Invertebrates							
Species	**% ai**	**NOAEC (mg/L)**	**LOAEC (mg/L)**	**Study Properties[a]**	**Most sensitive parameter**	**MRID, Author, Year**	**Status**
Aquazine (80% Formulated Product)							
Water flea (*Daphnia magna*)	80	2.5 (highest test level); 2.0*	>2.5; >2.0*	M, F-T	No adverse effects	000436-76, Mayer & Sanders, 1975	Supplmental: No effect at the highest test concentration
[a] M=mean-measured chemical concentrations, N=nominal chemical concentrations; F-T=flow-through; S=static, S-R = static renewal.							

The range of acute freshwater fish LC_{50} values for technical grade simazine is 6.4 mg/L to >32 mg/L; therefore, simazine is categorized as slightly (>10 to 100 mg/L) to moderately (>1 to 10 mg/L) toxic to freshwater fish on an acute exposure basis. The freshwater fish acute nominal LC_{50} value of 6.4 mg/L is based on a static 96-hour toxicity test using fathead minnow (*Pimephales promelas*) (MRID # 000333-09).

Although freshwater fish LC_{50} values for simazine exceed the predicted limit of simazine's solubility in water (3.8 mg/L), a co-solvent was used to increase the limit of simazine's water solubility, and no observation of precipitate was noted in the test chambers. Therefore, the fathead minnow LC_{50} value of 6.4 mg ai/L was used to characterize acute risks to freshwater fish. This test was categorized as supplemental because no raw data or test concentrations were provided in the study. A no effect level of 2.5 mg ai/L was established in the 96-hour fathead minnow study. This no effect level is consistent with the results of a 28-day subacute rainbow trout study (MRID 000436-68). Following 28-days of exposure, no mortality or other toxic symptoms were observed at the 2.5 mg ai/L treatment level. The subacute study was classified as supplemental because the fish were too large (25-40g) and only one treatment level (2.5 ppm ai) was tested. In the acute bluegill sunfish study, which is classified as acceptable, no mortality was observed in treatment groups $\leq$ 5.6 mg ai/L, and 40% mortality was observed in the 10 mg ai/L treatment group.

A.1.6 Freshwater Invertebrates, Open Literature Data

No additional data on the acute toxicity of simazine or its degradates to freshwater invertebrates are located in the open literature.

Only one chronic toxicity study on freshwater invertebrates from the open literature is available. The results of this study are summarized in **Table A-17.** The effects of technical grade simazine (98% ai) on growth and reproduction of *Daphnia pulex* were evaluated using both *Chalmydomas reinhardii* (green alga) and a mixed bacterial culture as a food source (Carter, 1981; ECOTOX#: 70902). Using green alga as a food source, simazine concentrations at the highest treatment level (5.0 ppm) were shown to enhance reproduction and growth in *D. pulex* neonates following 14 days of exposure. These results are similar to those reported in the registrant-submitted study, where stimulation of offspring produced was observed at the 0.8 ppm treatment level. Reproduction in egg-bearing adults fed green alga was not affected at the highest simazine exposure concentration of 5.0 ppm, following 16 days of exposure. Conversely, increasing simazine concentrations depressed reproduction in egg-bearing adults when mixed bacterial cultures were used as a food source. Specifically, reproduction in control populations (46 young/individual) was significantly higher than in populations exposed to 5.0 ppm (29 young/individual) and 1.0 ppm (31 young/individual). However, no significant differences in the number of offspring per individual were observed at treatment levels of 0.1, 0.2, and 2.0 ppm. According to the study author, the responses observed in the group fed mixed bacterial cultures were erratic and not dose-dependent. In summary, it appears that *D. pulex* fed a diet of green alga are less sensitive to the effects of simazine, as compared with those that are fed mixed bacterial cultures.

However, given the variability in reproductive responses between *D. pulex*, based on diet and uncertainties associated with the erratic responses in *D. pulex* fed mixed bacterial cultures, the data are addressed in a qualitative fashion only. In addition, chronic guideline studies specify that freshwater invertebrates be fed a diet of either synthetic food or algae; therefore, effects observed in invertebrates fed mixed bacterial cultures are not comparable to the results of guideline studies.

Table A-17: Freshwater Fish Sublethal Effect Studies from the Open Literature					
Study type/ Test material	**Test Organism (Common and Scientific Name) and Age and/or Size**	**Test Design**	**Endpoint Concentration in ppm**	**Citation (ECOTOX #)**	**Rationale for Use in Risk Assessment[(1)]**
Chronic (14 day) 98% a.i.	Water flea *(Daphnia pulex)* (adult egg-bearing)	Static renewal; 6 treatment levels; 9 reps/treatment level; bacterial food source	NOAEC = 0.5 LOAEC = 1.0 (reproduction)	Carter, 1981 (70902)	QUAL (responses erratic and not dose-dependent; not comparable to guideline study because of food source)
Chronic (16 day) 98% a.i.	Water flea *(Daphnia pulex)* (adult egg-bearing)	Static renewal; 6 treatment levels; 8 reps/treatment level; Green alga (*Chalmydomas reinhardii)* food source	NOAEC = >5.0 (no effect at highest treatment level)		
Chronic (14 day) 98% a.i.	Water flea *(Daphnia pulex)* (neonates, age not specified)	Static renewal; 6 treatment levels; 8 reps/treatment level; Green alga (*Chalmydomas reinhardii)* food source	Reproduction and growth significantly higher (α = 0.05) in 5.0 ppm treatment group than all other treatment groups and the control		
[(1)] QUAL = The paper is not appropriate for quantitative use but is of good quality, addresses issues of concern to the risk assessment and is used in the risk characterization discussion. NR = not reported					

A.1.7 Freshwater Microcosm/Field Studies

A summary of all the freshwater aquatic microcosm, mesocosm, and field studies for simazine that provide endpoints more sensitive than the submitted data, and/or provide information regarding recovery, succession, and resistance is included in Table A-18 and provided below.

Gilderhaus (1969; MRID 000254-33) conducted simulated field tests on nine 0.01-acre, rectangular concrete pools filled with Mississippi River water to a depth of three feet. Pool bottoms were covered with a three-inch layer of loam soil to support benthic organisms and rooted vegetation. Four pounds of *Elodea canadensis* were placed in each pool to test the efficacy of simazine treatments. Three pools were treated only once at 1.0, 2.5, and 5.0 ppm. Three pools were treated with 1.0, 2.5, and 5.0 ppm, respectively, once every four weeks for a total of five treatments each. The remaining three pools were controls. Each pool was stocked with 150 goldfish (3 inches in length and 8.6 g), 150 fingerlings bluegills (1.4 inches and 0.7 g), and 10 adult bluegills (averaging 6.5 inches and 104 g). It should be noted that all of the tested fish were larger than the recommended size (0.5 g) for acute toxicity studies, and the more sensitive life stages (*e.g.*, larval and fry) were not tested in this study. The reported results indicated that *Elodea* and algae were eliminated from all treated pools for the duration of the study. Levels of fish survival were generally erratic. Goldfish survivors generally showed dose-related reductions in the monthly- and yearly-treated pools at simazine treatments of 2.5 and 5.0 ppm. Survival levels were generally higher in the pools treated monthly rather than the pools treated only once a year. In the case of bluegills, reduced survival was dose-related in pools treated only once, while survival levels were similar (no dose-related effect) in the three pools treated monthly. Reductions in bluegill survival were higher than the control groups in both pools treated at 5.0 ppm and in the monthly-treated pool at 2.5 ppm. Given the level of erratic response in fish survival, it is not possible to derive NOAEC or LOAEC values from the submitted field study. In addition, it is unclear whether differences in fish survival were due to the direct effects of simazine or indirect effects associated with low DO and loss of food/habitat resources. The mean number of zooplankton and benthic fauna showed no dose-response effects following simazine treatments of 1.0, 2.5, and 5.0 ppm to concrete ponds; however no analysis of individual invertebrate populations by species was completed.

McGinty (1984; ECOTOX#: 10969) studied the effects of periodic applications of simazine on the growth of *Tilapia nilotica* swim-up fry (< 12 mm in length) in circular fiberglass pools (4.12 m^2) with an average depth of 45 cm. Four out of 8 pools were treated with 1 mg/L of simazine (% a.i. unspecified) at 0, 24, 56, 87, and 106 days after the pools were stocked with 100 *Tilapia* fry each. The other 4 pools, which were untreated, served as controls. The duration of the study was 126 days. Survival in control ponds (91%) was not significantly different than survival in treated pools (87%); however, after only 42 days of exposure, the average weight per 100 fish was significantly less for treated pools (269 g) than for control pools (388 g). The yield of *Tilapia* fingerlings after 126 days was 52% less in fiberglass pools treated periodically with 1 mg/L simazine than in untreated control pools. The study author attributes approximately 32% of this reduction to the low abundance of natural foods (phytoplankton) in treated pools. The additional 20% reduction in yield is attributed to a combination of the direct effect of simazine and poor water quality. It is unclear, however, how the author derived the percentage of reduction in yield impacts due to indirect effects (i.e., loss of fish diet due to reduced phytoplankton from simazine application) and direct toxic effects of simazine. In addition, no water quality data were provided; therefore, reduction in yield as a result of "poor" water quality could not be evaluated by the reviewer. Furthermore, concentrations of simazine in the pond water over time were not provided.

Gordon *et al.* (1982; ECOTOX#: 15428) also studied the effects of simazine on pond ecosystems (< 4.0 ha) for a duration of 210 days following a single simazine application (%a.i.unspecified) of 1 mg/L. System components studied were fish, macrophytes, benthic macroinvertebrates, phytoplankton, zooplankton, bacteria, and water chemistry. Pre- and post-treatment samples were collected in order determine direct and indirect effects. Following application of simazine to the pond, macrophyte death occurred, resulting in decreased DO, and increased CO_2, total suspended solids, total carbon, and specific conductivity. DO decreased from 8 to 3 mg/L within 3 days following application. Decreases in DO were concurrent with increased mortality of phytoplankton and macrophytes. Zooplankton biomass decreased in the post-treatment period. No significant differences were seen in total abundance of biomass of benthic macroinvertebrates, with the exception of one taxon, *Ostracoda*, which increased in the post period treatment. No significant differences were observed in bacterial numbers. Significantly fewer yield of young-of-the-year (YOY) bluegills survived in the simazine-treated ponds, although the mean weight increase of those survivors was three-fold greater than that of YOY bluegills in the control pond. YOY largemouth bass showed comparable growth in the simazine-treated and control ponds; however, juvenile and adult bass grew significantly slower ($\alpha < 0.05$) in the pond treated with simazine. The study author attributed decreases in growth and survival rates of sport fishes in the simazine-treated pond to oxygen depletion, reduced forage availability, and generally decreased ecosystem productivity in the absence of macrophytes.

The effects of single simazine treatments on channel catfish and bluegill ponds were studied by Tucker and Boyd in 1978 (ECOTOX#: 71314) and by Tucker *et al.* in 1983 (ECOTOX#: 10669). The objective of the 1978 study was to determine if a single preflooding treatment of Aquazine (80% ai) controlled algae in channel catfish ponds without resulting in a reduction to fish yields. Fourteen earthen ponds (0.04 - 0.06 ha) were used; 6 ponds were stocked with 7,400 fingerling channel catfish, and 8 ponds were stocked with 5,000 bluegill. Prior to filling the catfish ponds with water, 3 of the 6 ponds were treated with Aquazine at a rate of 13.4 kg/ha (11.9 mg/L). Aquazine in a water suspension was applied evenly over the pond bottom. Four of the 8 bluegill ponds were treated with 1.5 mg/L simazine (post-flooding) by applying a slurry of the chemical over the pond surface. In catfish ponds treated with simazine prior to flooding, simazine concentrations in water remained above 0.2 mg/L (200 ppb) for more than 4 months. Use of simazine resulted in an extended period of decreased DO as compared to the control ponds. The yield of catfish from treated ponds was 19% less ($P < 0.01$) than that from control ponds. Feed conversion efficiency of fish from treated ponds was also poorer than the controls. The study author attributed the adverse effects to prolonged exposure to lowered DO. In the bluegill ponds, DO also decreased rapidly following Aquazine application. The average yield of bluegills from simazine-treated ponds was 11% less than the controls; however not significant ($P > 0.1$). Although bluegill production was not reduced as much as catfish production by simazine treatment, the single application of simazine to the water did not result in season-long control of macrophytes. Simazine concentrations in the surface water of the bluegill ponds following application were not provided by the study author.

In the 1983 study by Tucker *et al.*, channel catfish ponds infested with *Chara vulgaris* were treated with 1.3 mg/L simazine. Earthen ponds (~0.6 ha) were stocked with channel catfish (55 g average weight) at 12,350 fish/ha. Four heavily infested ponds were treated with simazine (% a.i. unspecified), and 4 ponds containing little or no *Chara* were monitored for changes in water quality before and after herbicide treatment. The ponds were equipped with emergency aeration that was initiated when DO fell below 2.5 mg/L.

Water quality changes following treatment included decreased DO and pH, and increased total ammonia-nitrogen (TA-N), nitrite-nitrogen, and CO_2. The magnitude of effects was greatest in 2 weeks following treatment. Temporal changes in DO, CO_2, pH, and TA-N in treated ponds are related to the response of the plant community to simazine. Decreased DO and increased CO_2 and TA-N are the result of the death and decomposition of plants. Decreased pH (from elevated CO_2) compensated for the increased TA-N concentrations; therefore, un-ionized ammonia-nitrogen (UA-N) concentrations remained at moderate levels. This is important because UA-N is considered the principal toxic species of ammonia to catfish. As simazine concentrations decreased with time, phytoplankton species became established. Increases in planktonic chlorophyll *a* were accompanied by increases in pH and DO and decreases in CO_2 and TA-N. These changes are the result of photosynthetic oxygen production and CO_2 uptake, which moderated water quality parameters as well as pH. Although water quality variables did not reach lethal levels (due to emergency aeration at DO levels of < 2.5 mg/L), fish production was reduced 20% compared to untreated ponds. The feeding response of fish stopped immediately after the simazine application. While water quality changes after herbicide treatment undoubtedly affected feeding response, the reduced response persisted even after water quality variables returned to control pond levels. This suggests a possible direct effect of the simazine on feeding response. The study authors recommend the use of emergency aeration equipment to avoid low DO following simazine application, however potential problems of increased CO_2 and nitrite concentrations may result in fish growth reduction from even a single simazine application.

Table A-18: Simazine Field and Microcosm Studies (2006 RED Summary)

Study type/ Test material	Study Design	Test Organism	Effects	Citation (ECOTOX #)	Rationale for Use in Risk Assessment[1]
Field study 210 days Simazine (% a.i. NR)	1.0 mg/L simazine applied to <0.4 ha pond (control pond also tested). Biological and water quality components measured 3, 5, 7, 10, and 18 days after application, and biweekly and monthly (210 d).	Phytoplankton Macrophytes Zooplankton Macroinvertebrates Largemouth bass (*Micropterus salmoides*) Bluegill (*Lepomis macrochirus*)	The simazine application rate of 1.0 mg/L caused significant reduction in the growth and survival rate of freshwater fish, although the effects are attributed to a combination of low DO and reduced food resources. Application of simazine to the pond produced die-off of the macrophytes, which resulted in decreased D.O., and increase in CO_2, TSS, total carbon, and specific conductivity. Decreases in DO were concurrent with increased mortality of both phytoplankton and macrophytes. Zooplankton biomass decreased. No significant differences were seen in total abundance or biomass of macroinvertebrates, although the taxa of Ostracoda increased. Significantly fewer yield of young-of-the-year (YOY) bluegills survived in the simazine-treated ponds, although the mean	Gordon, R.W., *et al.*, 1982 (15428)	QUAL (field study; application rates higher than those currently allowed under label requirements for direct applications; simazine concentrations over time not provided)

Table A-18: Simazine Field and Microcosm Studies (2006 RED Summary)					
Study type/ Test material	**Study Design**	**Test Organism**	**Effects**	**Citation (ECOTOX #)**	**Rationale for Use in Risk Assessment(1)**
			weight increase of the survivors was 3x greater than YOY in the control pond. YOY largemouth bass showed comparable growth in simazine-treated and control ponds; however juvenile and adult bass grew slower.		
Field study 20 wks Simazine (% a.i. NR)	- -9 x 0.01-A rectangular concrete pools w/MS river water (3 ft deep). - Bottom covered w/3" layer loam soil. - 4 lbs *Elodea canadensis* in each pool. - 3 pools treated once at 1, 2.5, and 5 ppm; 3 pools treated w/1, 2.5 and 5 ppm every 4 wks for total of 5 treatments/each; and 3 controls (untreated)	Algae *Elodea candensis* Zooplankton Macroinvertebrates Goldfish Bluegill (*Lepomis macrochirus*)	*Elodea candensis* and algae eliminated from all treated pools for duration of study. Levels of fish survival were erratic. Goldfish survivors showed reduction at 2.5 and 5.0 ppm treatment levels; survival was higher in monthly-treated pools than pools treated 1x/yr. Reduction in bluegill survival was dose-related in pools treated once; survival was similar (not dose-related) in pools treated monthly Bluegill survival was significantly reduced as compared to control in pools treated at 5 ppm and in the monthly-treated pool at 2.5 ppm. The mean number of zooplankton and benthic fauna showed no dose-response effects following simazine treatments of 1, 2.5, and 5 ppm.	Gilderhaus, 1969 (MRID# 000254-33)	QUAL (no water quality parameters reported; tested fish were larger than recommended size for acute studies and sensitive life stages were not tested; given erratic response in fish survival, it is not possible to derive NOAEC or LOAEC values; no analysis of individual invertebrate populations by species was conducted).
Microcosm 126 days Simazine (% a.i. NR)	1.0 mg/L simazine applied to 4 circular fiberglass pools (4.12 m^2, depth = 45 cm) at 0, 24, 56, 87, and 106 days after pools were stocked with 100 *Tilapia nilotica* swim-up fry each. Four untreated pools were used as controls. Pools were not cleaned and only water lost by evaporation was replaced.	Nile tilapia (*Tilapia nilotica*) (swim-up fry, <12mm in length)	Yield of tilapia fingerlings after 126 days was 52% less in fiberglass pools treated periodically with 1 mg/L simazine as compared to the untreated ponds. Study author attributes 32% of this reduction to the low abundance of natural foods (phytoplankton) in treated pools. The additional 20% reduction is attributed to a combination of the direct effect of simazine and poor water quality. There were no significant differences in survival between the control pools (91%) and the treated pools (87%). After 42 days, the average weight per 10 fish was significantly less in the treated pools (269 g) as compared to the control pools (388 g).	McGinty, 1984 (10969)	QUAL (field study; application rates higher than those currently allowed under label requirements for direct applications; simazine concentrations over time not provided; no water quality data)
Field study of catfish ponds 60 days Simazine (% a.i. NR)	1.3 mg/L simazine applied to channel catfish ponds infested with *Chara vulgaris*. Catfish were stocked into 0.06 ha earthen ponds at 12,350 fish /ha. Four heavily infested ponds were treated w/simazine and 4 ponds containing little or no *Chara* were monitored for changes in water quality before and after simazine treatment. The	Channel catfish (*Ictalurus* punctatus) (average weight = 55 g)	Fish production was reduced 20% compared to untreated ponds, although DO levels did not reach lethal levels due to emergency aeration. The feeding response of fish stopped immediately after simazine application. Reduced feeding persisted even after water quality variables in treated ponds returned to control pond levels. This suggests a possible direct effect of simazine on the feeding response. Water quality changes following treatment included decreased DO, increased ammonia-nitrogen, nitrite-nitrogen, and CO_2 (and decreased pH). The magnitude of	Tucker *et al.*, 1983 (10669)	QUAL (field study; application rates higher than those currently allowed under label requirements for direct applications; simazine concentrations over time not provided; emergency aeration provided)

Table A-18: Simazine Field and Microcosm Studies (2006 RED Summary)					
Study type/ Test material	**Study Design**	**Test Organism**	**Effects**	**Citation (ECOTOX #)**	**Rationale for Use in Risk Assessment[(1)]**
	ponds were equipped with emergency aeration that was initiated when DO fell below 2.5 mg/L.		effects was greatest in 2 weeks following treatment. Temporal changes in DO, CO_2, pH, TA-N in treated pools are related to the response of the plant community to simazine.		
Field study of catfish and bluegill ponds ~120 days Aquazine (80% a.i.)	14 earthen ponds (0.04 - 0.06 ha) used; 6 ponds stocked w/7400 channel catfish fingerlings, and 8 ponds stocked with 5000 bluegill. Prior to filling ponds w/water, 3 of the 6 catfish ponds treated with Aquazine at rate of 13.4 kg/ha (~12 lb/A). Aquazine was applied as a suspension in water and applied evenly over the entire pond bottom. Four of the 8 bluegill ponds were treated w/ 1.5 mg/L (1.88 mg/L Aquazine 80W). A slurry of the chemical was dispersed over the pond surface.	Channel catfish (*Ictalurus* punctatus) Bluegill (*Lepomis macrochirus*)	<u>Catfish ponds</u>: In catfish ponds treated w/Aquazine prior to flooding, simazine concentrations in water remained above 200 ppb for more than 4 months. Persistence resulted in lower chlorophyll *a* and percentage of pond bottoms covered by macrophytes in treated ponds as compared to controls. Use of simazine resulted in an extended period of decreased DO as compared to control ponds. Catfish yield from treated ponds was 19% less (P<0.01) than control ponds. Feed conversion efficiency of fish from treated ponds was poorer than controls. Prolonged exposure to lowered DO may be responsible for adverse effects. <u>Bluegill ponds</u>: DO decreased rapidly following Aquazine application. The average yield of bluegills from treated ponds was 11% less than the controls, however not significant (P> 0.1). Although bluegill production was not reduced as much as catfish production by simazine, the single application of simazine to the water did not result in season-long control of macrophytes.	Tucker and Boyd, 1978 (71314)	QUAL (field study; application rates higher than those currently allowed under label requirements for direct applications)
In situ enclosures of marsh water 27 days Simazine (>98%)	Littoral enclosures (240 x 120 cm sheets of 1.5-mm PVC plastic on long axis w/ends cemented together) placed in water ~ 60-cm depth and embedded into sediment to depth of 45 cm. Artificial substrata placed upright in sediments. Concentrations of 0.1, 1.0, and 5.0 mg/L simazine dispensed in 300-l enclosure volume. Sampled substrata 9 days following simazine application and at weekly intervals for 5 wks. Colonization of substrata by periphyton was monitored by measuring chlorophyll *a* and carbon assimilation rate.	Periphyton	Data suggests that the EC_{50} of chlorophyll synthesis by marsh periphyton must lie between 0.1 and 1.0 mg/L simazine. No change to chlorophyll a accumulation and carbon assimilation rate were observed at simazine concentrations of 0.1 mg/L, relative to the control. Algal biomass increased over time in all treatments w/the most notable increases in treated enclosures following flooding. Secondary effects include reduction in DO and pH, and increases in dissolved Ca, Mg, K, ammonia, nitrate, and phosphate. No detrimental long-term effect on productivity of periphyton may be predicted.	Goldsborough and Robinson, 1983 (11289)	QUAL (field study, simazine concentrations over time not provided)
In situ marsh enclosures	Littoral enclosures (diameter = 78 cm;	Periphyton	No reduction in total biovolume was observed at the 0.1 mg/L simazine	Goldsborough and Robinson,	QUAL (field study, simazine

Table A-18: Simazine Field and Microcosm Studies (2006 RED Summary)					
Study type/ Test material	**Study Design**	**Test Organism**	**Effects**	**Citation (ECOTOX #)**	**Rationale for Use in Risk Assessment[(1)]**
42 days Simazine (>98%)	volume ~300 l) situated in a marsh. Rods used as substrata for periphyton growth were positioned vertically. Simazine added to enclosures at concentrations of 0.1, 1.0, and 5.0 mg/L (plus one control). Substrata collected 9 days after simazine application and at weekly intervals for 6 weeks. Measurements included carbon assimilation, chlorophyll a concentration, densities of algal taxa, and total algal biovolume. Flooding during the experiment provided opportunity to monitor extent and rate of recovery of the community.		concentration, with increasing inhibition (94 - 98%) at pre-flood concentrations of 1.0 and 5.0 mg/L. This suggests that the community LC_{50} (herbicide concentration yielding 50% reduction in biovolume) lies between 0.1 and 1.0 mg/L simazine. Following flooding and removal of herbicide, increases in biovolume were observed in all but the highest treatment levels, with rates of colonization similar to control. After flooding, substratum colonization dominated by *Cocconeis placentula*. There was no evidence that a clearly herbicide resistant/tolerant community had developed in the 2.5 week period prior to flooding, although the lower relative abundance of filamentous green algae at 5.0 mg/L suggests that this taxa were selectively inhibited to a greater extent than the others. High abundance of periphytic blue-green alga suggests that this taxon possesses some means of herbicide tolerance.	1986 (12264)	concentrations over time not provided)
In situ enclosures of marsh water 18 days Simazine (>97.7%)	Cylindrical enclosures placed in marsh water ~ 60-cm depth and embedded into sediment to depth of 45 cm. Artificial substrata placed vertically in enclosures. Simazine dispensed to give ~1.0 mg/L in 300-l enclosure volume. Treatment consisted of 7-day exposure before flooding, and an 11-day exposure following readdition of simazine 9 days after the flood. Sampled substrata @ 1, 3, and 5 week intervals. Substrata segments received 0.1, 0.5, 1.0, 2.5, or 5.0 mg/L (3 reps/ treatment plus 3 controls). Colonization of substrata by periphyton was monitored by measuring chlorophyll *a* and carbon assimilation rate.	Periphyton	Rates of specific photosynthesis (carbon fixed per unit chlorophyll) of periphyton samples from simazine treated enclosures were generally equal to or greater than corresponding rates of samples from control enclosures. The findings indicate that herbicide resistance can develop in lentic periphyton after short (7 days) exposure; however, this can occur only at simazine concentrations $\geq$ 0.8 mg/L (comparisons of treated enclosure EC_{50}s w/ ambient concentrations show that significant increases in EC_{50} occur when simazine concentration was greater than 0.8 mg/L.	Goldsborough and Robinson, 1988 (3136)	QUAL (field study, simazine concentrations over time not provided; application rates higher than those currently allowed under label requirements for direct applications)
Mesocosm study on succession of aquatic plants 6 months	25 lb does of granular simazine applied to alternate halves of a 1/5 acre (3-ft deep) pond on 2 consecutive weekends. Changes in aquatic plant	Aquatic plants	Specific endpoints or effect values were not reported. A 4-yr old farm pond containing *Najas flexilis* and *Potamogeton foliosus* was treated in the spring. After decay of the	Crawford, 1981 (MRID 450882-03)	QUAL (no endpoints reported; use of simazine granular formulations has been cancelled;

Table A-18: Simazine Field and Microcosm Studies (2006 RED Summary)					
Study type/ Test material	**Study Design**	**Test Organism**	**Effects**	**Citation (ECOTOX #)**	**Rationale for Use in Risk Assessment[(1)]**
Simazine granules (%a.i.NR)	communities over time were observed.		higher plants, phytoplankton did not dominate, but instead herbicide resistant seeds and subsurface structures of *Potamogeton foliosus* developed. Benthic algae covered and stabilized the bottom. Following stabilization, the water cleared and *Chlara vulgaris* became established in a portion of the pond where the substrate was firm. Treatment of the pond with simazine resulted in death of the majority of macrophytes. However, recovery of the macrophytes was noted within two to three months post application. Seeds and tubers of *P. foliosus* maybe resistant to simazine.		paper discusses succession, recovery, and possible resistance of aquatic plant species in a natural farm pond)
Mesocosm study of algal succession 85 days Simazine (Princep) (%a.i.NR)	Microcosms consisted of 12 x 3 liter Erlenmeyer flask plugged w/cotton. Algal cultures were obtained from a chicken processing oxidation pond allowed to grow to stationary phase. Nominal concentrations of 50, 150, 400 ppb simazine were used. Photosynthesis, respiration, dry weights, diversity, species dominance, and chlorophyll a were measured.	Aquatic plants	Simazine caused a shift in time of highest productivity peaks by about 2 weeks at 150 and 400 ppb. A lag in net productivity, but larger peaks of productivity, were seen in the higher doses. Pigments and dry weights were relatively unaffected. Although it was stated that successional sequence was affected, there were very few organisms on which to base this observation. Algal species exposed to the highest concentration had delayed net and gross productivity and respiration, which was followed by rate increases in both that exceeded the same rates for algal species exposed to lower concentrations. Gross productivity was greatest for the high exposure group at the end of the bioassay. Algal biomass in control and lower treatment groups were not different. The type of successsional sequence of species was affected by treatment level with Chlorella dominating at the higher levels. Uncertainty exists as to whether this community shift remains in the absence of simazine.	Bryfogle and McDiffett, 1979 (MRID 450882-05)	QUAL (paper discusses succession and recovery of algae; endpoints are less sensitive; measured concentrations of simazine over time are not reported)
(1) QUAL = The paper is not appropriate for quantitative use but is of good quality, addresses issues of concern to the risk assessment and is used in the risk characterization discussion.					

A.2 Toxicity to Saltwater Animals

Not very much toxicity data is available for saltwater organisms, though several taxa are represented, three fish species and five invertebrate species (three of these being corals). Since the Delta smelt inhabits both saltwater and freshwater, data were pooled and the most sensitive data used from either freshwater or saltwater organisms.

Table E3: Acute Toxicity of Simazine to Estuarine/Marine Fish							
Species	% a.i.	96-hr LC_{50} mg/L (confid. int.)	NOAEC (mg/L)	Study Properties[a]	Toxicity Classification	MRID, Author, Year	Status
EPA PC Code: 080807 - Simazine (Technical)							
Sheephead minnow *Cyprinodon variegatus*	96.9	>4.3	4.3	M, S	moderately toxic	42503702, Murphy, 1992	Acceptable
Gesatop GW (90% formulated product)							
Seabream (*Sparus aurata*) yolk-sac larvae	90	72- hour LC_{50} = 4.190*		N, S-R	moderately Toxic	Arufe *et al*., 2004	Supplemental: Nominal concentrations; no raw data provided; no way to confirm tha simazine stayed in solution
EPA PC Code: 080807 - Simazine (80 WP)							
Striped bass (*Morone saxatilis*)	80	>3.0; >2.4*	3; 2.4*	N, S-R	moderately toxic	00043667, Cook & Smith, 1976	Supplemental (erratic mortality response)

[a] M=mean-measured chemical concentrations, N=nominal chemical concentrations; F-T=flow-through; S=static.
* Adjusted for % ai.

Table E4: Acute Toxicity of Simazine to Estuarine/Marine Invertebrates							
Species	% a.i.	96-hr EC_{50}/ LC_{50} mg/L (confid. int.)	NOAEC (mg/L)	Study Properties[a]	Toxicity Classification	MRID, Author, Year	Status
EPA PC Code: 080807 - Simazine (Technical)							
Eastern oyster (*Crassostrea virginica*)	96.9	>3.7	3.7	M, F-T	moderately toxic	42503703, Murphy, 1992	Acceptable
Eastern oyster (*Crassostrea virginica*) (7 day weight	99.1	>1.0 (only test concen	1.0	N, F-T	moderately toxic	00043677, Wright & Beliles,	Supplemental (non-standard endpoint)

Table E4: Acute Toxicity of Simazine to Estuarine/Marine Invertebrates							
Species	% a.i.	96-hr EC_{50}/ LC_{50} mg/L (confid. int.)	NOAEC (mg/L)	Study Properties[a]	Toxicity Classification	MRID, Author, Year	Status
gain study)		tration)				1966	
Collembola, *Proisotoma minuta* (7 day mortality study	Tech. product but %ai not given	>1000	1000	N,S	practically nontoxic	Park and Lees, 2005	Supplemental (non-standard endpoint; >solubility limit; no confirmation of test concentrations; and no LOAEC)
Corals (*Madracis mirabilis*, *Diplora strigos* and, *Favia fragum*)	Not Given	0.01	NR	N, S	highly toxic	Owen et al, 1993	Quantitative

[a] M=mean-measured chemical concentrations, N=nominal chemical concentrations; F-T=flow-through; S=static.

A.3 Toxicity to Non-target Plants

A.3.1 Non-Target Terrestrial Plants: Submitted Data

Terrestrial plant testing (seedling emergence and vegetative vigor) is required for herbicides that have terrestrial non-residential outdoor use patterns and that may move off the application site through either volatilization (vapor pressure > 1.0 x 10^{-5} mm Hg at 25^{o}C) or drift (aerial or irrigation), and/or that may have listed species associated with the application site.

For seedling emergence and vegetative vigor testing, the following plant species and groups should be tested: (1) six species of at least four dicotyledonous families, one species of which is soybean (*Glycine max*) and the second crop is a root crop; and (2) four species of at least two monocotyledonous families, one of which is corn (*Zea mays*).

Terrestrial Tier II studies are required for all low dose herbicides (those with the maximum use rate of 0.5 lbs ai/A or less) and any pesticide showing a negative response equal to or greater than 25% in Tier I tests. Tier II terrestrial plant testing is required for simazine because it is a herbicide with numerous agricultural uses.

The results of the Tier II seedling emergence and vegetative vigor toxicity tests on non-target plants are summarized below in Tables A-19 and A-20, respectively. Seedling emergence and vegetative vigor were studied on ten non-target crops (including soybean, lettuce, radish, tomato, cucumber, cabbage, oat, ryegrass, corn, and onion) following application of Princep 4L herbicide (simazine) at 4 lb ai/A (MRIDs 426346-03 and 426346-04). Both studies are scientifically sound and fulfill the guideline requirements for Tier II seedling emergence and vegetative vigor studies (Subdivision J, §123-1a & b). Based on the results of the tests, it appears that emerging seedlings are more sensitive to simazine via soil/root uptake exposure than emerged plants via foliar routes of exposure. However, all tested plants, with the exception of corn, exhibited adverse effects in both the seedling emergence and vegetative vigor toxicity tests, following exposure to Princep 4L at 4 lb ai/A.

For seedling emergence, the most sensitive species was lettuce (a dicot), based on dry weight, with an EC_{25} of 0.009 lb ai/A; the NOAEC and EC_{05} for lettuce dry weight were 0.0018 and 0.0027 lb ai/A, respectively. After 21 days, lettuce dry weight was reduced, as compared to the control, by 25%, 25%, and 79% at respective treatment levels of 0.0054, 0.016, and 0.049 lb ai/A. The most sensitive monocot in the seedling emergence test was onion, based on plant height, with an EC_{25} of 0.02 lb ai/A, and respective NOAEC and EC_{05} values of 0.049 and 0.0017 ai/A. The EC_{05}, rather than the NOAEC value, was chosen as the appropriate "no effect level" endpoint for the onion because the reported NOAEC value exceeds the EC_{25}.

In the vegetative vigor test, lettuce (a dicot) and oat (a monocot) were determined to be equally sensitive to treatment, based on dry weight, with an EC_{25} of 0.033 lb ai/A for both species; the NOAEC for both was 0.016 lb ai/A, while the EC_{05} values were 0.016 and 0.018 lb ai/A for lettuce and oat, respectively. Following 21 days of exposure, lettuce and oat dry weight was reduced 45 to 51% at a treatment level of 0.049 lb ai/A, and 7 to 11% at a treatment level of 0.016 lb ai/A.

Table A-19: Nontarget Terrestrial Plant Seedling Emergence Toxicity (Tier II)

Species	% a.i.	Endpoints	EC25/EC05 (lbs ai/A)	MRID, Author, Year	Status
Monocot - Corn	45.06	Emergence Survival Shoot Height Dry Weight Phytoxicity NOEC	>4.0 / ND >4.0 / >4.0 >4.0 / >4.0 >4.0 / >4.0 4.0	426346-03, Chetram, 1993a	Acceptable
Monocot - Oats	45.06	Emergence Survival Shoot Height Dry Weight Phytoxicity NOEC	>4.0 / >4.0 >0.049 / ND >0.049 / 0.022 0.031 / 0.018 0.016	426346-03, Chetram, 1993a	Acceptable

Table A-19: Nontarget Terrestrial Plant Seedling Emergence Toxicity (Tier II)

Monocot -Onion	45.06	Emergence Survival Shoot Height Dry Weight Phytoxicity NOEC	>4.0 / >4.0 ND / 4.0 0.02 / 0.0017 >0.016 /ND 0.049	426346-03, Chetram, 1993a	Acceptable
Monocot - Ryegrass	45.06	Emergence Survival Shoot Height Dry Weight Phytoxicity NOEC	>4.0 / >4.0 0.12 / 0.044 0.073 / 0.017 0.045 / 0.022 0.15	426346-03, Chetram, 1993a	Acceptable
Dicot - Radish	45.06	Emergence Survival Shoot Height Dry Weight Phytoxicity NOEC	>4.0 / >4.0 >0.049 / >0.049 >0.049 / <0.15 >0.049 / 0.00041 0.049	426346-03, Chetram, 1993a	Acceptable
Dicot - Soybean	45.06	Emergence Survival Shoot Height Dry Weight Phytoxicity NOEC	>4.0 / ND ND / ND 0.17 / 0.052 0.057 / 0.018 <0.049	426346-03, Chetram, 1993a	Acceptable
Dicot - Lettuce	45.06	Emergence Survival Shoot Height Dry Weight Phytoxicity NOEC	>4.0 / >4.0 ND / ND 0.032 / 0.016 0.009 / 0.0027 0.0018	426346-03, Chetram, 1993a	Acceptable
Dicot - Tomato	45.06	Emergence Survival Shoot Height Dry Weight Phytoxicity NOEC	>4.0 / >4.0 0.074 / 0.039 0.057 / 0.02 0.038 / 0.021 0.016	426346-03, Chetram, 1993a	Acceptable
Dicot - Cucumber	45.06	Emergence Survival Shoot Height Dry Weight Phytoxicity NOEC	>4.0 / 0.0062 >0.049 / ND >0.049 / 0.037 0.046 / 0.016 0.016	426346-03, Chetram, 1993a	Acceptable
Dicot - Cabbage	45.06	Emergence Survival Shoot Height Dry Weight Phytoxicity NOEC	>4.0 / ND >0.049 / ND 0.079 / 0.022 0.034 / 0.011 0.049	426346-03, Chetram, 1993a	Acceptable

ND = Non-monotonic data not suitable for probit model fit.

Table A-20: Nontarget Terrestrial Plant Vegetative Vigor Toxicity (Tier II)

Species	% a.i.	Endpoints	EC25/EC05 (lbs ai/A)	MRID, Author, Year	Status
Monocot - Corn	40.8	Survival Shoot Height Dry Weight Phytoxicity NOEC	>4.0 / >4.0 >4.0 / >4.0 >4.0 / >4.0 4.0	426346-04, Chetram, 1993b	Acceptable
Monocot - Oats	40.8	Survival Shoot Height Dry Weight	ND / ND ND / ND 0.033 / 0.018	426346-04, Chetram, 1993b	Acceptable

Table A-20: Nontarget Terrestrial Plant Vegetative Vigor Toxicity (Tier II)					
		Phytoxicity NOEC	0.016		
Monocot - Onion	40.8	Survival Shoot Height Dry Weight Phytoxicity NOEC	0.62 / 0.39 0.22 / 0.098 0.039 / 0.014 0.016	426346-04, Chetram, 1993b	Acceptable
Monocot - Ryegrass	40.8	Survival Shoot Height Dry Weight Phytoxicity NOEC	0.44 / 0.28 0.26 / 0.13 >0.016 /ND 0.049	426346-04, Chetram, 1993b	Acceptable
Dicot - Radish	40.8	Survival Shoot Height Dry Weight Phytoxicity NOEC	>0.15 / >0.15 ND / ND 0.063 / 0.026 0.049	426346-04, Chetram, 1993b	Acceptable
Dicot - Soybean	40.8	Survival Shoot Height Dry Weight Phytoxicity NOEC	ND / ND 0.13 / 0.066 0.085 / 0.039 0.049	426346-04, Chetram, 1993b	Acceptable
Dicot - Lettuce	40.8	Survival Shoot Height Dry Weight Phytoxicity NOEC	>0.049 / ND >4.0 / 0.0011 0.033 / 0.016 0.016	426346-04, Chetram, 1993b	Acceptable
Dicot - Tomato	40.8	Survival Shoot Height Dry Weight Phytoxicity NOEC	ND / ND ND / ND 0.037 / 0.022 0.031	426346-04, Chetram, 1993b	Acceptable
Dicot - Cucumber	40.8	Survival Shoot Height Dry Weight Phytoxicity NOEC	0.056 / 0.033 0.049 / 0.028 0.036 / 0.0071 0.016	426346-04, Chetram, 1993b	Acceptable
Dicot - Cabbage	40.8	Survival Shoot Height Dry Weight Phytoxicity NOEC	ND / ND 0.09 / 0.06 0.041 / 0.013 0.016	426346-04, Chetram, 1993b	Acceptable
ND = Non-monotonic data not suitable for probit model fit.					

A summary of available data evaluating the phytoxicity of simazine to woody plants was submitted to the Agency in 2007 (Wall, 2007). A total of 79 species were tested in 110 separate trials at application rates of 0.5 to 12 lbs a.i./Acre. Signs of phytotoxicity were summarized and reported. Fifty-four species exhibited either no or negligible (<10%) phytotoxicity. Further examination of data for the remaining 25 woody species showing phytotoxicity values > 10% indicates that the species were exposed to simazine concentrations greater than those expected to be present at environmentally relevant concentrations. These data are summarized in Table A-20b below.

The data indicate that simazine is not likely to have an adverse effect on woody plants when used at labeled application rates (or even at higher rates, which is often tested in field phytotoxicity trials). The species were exposed to simazine in a direct application, which represents a worst case exposure scenario. It is expected that woody plant species adjacent to treated areas would not be exposed to simazine at the tested rates. Potential exposure is expected to much lower, as estimated using the TerrPlant model following a ground application. Furthermore, simazine is labeled for use around numerous wood species including citrus, tree nuts, grapes. Based on the available data and expected lower predicted concentrations away from the treated field, it is unlikely that simazine will cause adverse effects to non-target woody plant species.

Table A-20b. Summary of Simazine Woody Plant Data (Hall, 2007)		
Species	**Application Rate (lbs a.i./Acre)**	**Phytotoxicity (%)**
Abies balsamea	2	5%
	4	0%
Abies fraseri	0.8	0%
	1.6	0%
	3	0%
	4	0%
Almonds	1.8	0%
Almonds: nonpareil	2.7	IS rating = 0[a]
	2.8	0%
Andromeda sp.	1	0%
	2	0%
Apples	0.5	0%
	1	0%
	1.5	0%
	2	0%
	3	0%
Apples: Empire	4	0%
Apples: Golden Delicious	3	10%
	3	13%[b]
	3	73%[b]
Apples: Granny Smith	1	0%
	2	0%
Apples: Red Delicious	0.5	0%
	1	0%
	2	0%
	3	13%[b]
	4	0%
Apricots	2	15%
Betula papyrifera	2	3%
	4	33%
Blueberries	1.5	0%
	2	5%
	2	1%
	2	0%
	2.5	0%
	2.5	0%

Table A-20b. Summary of Simazine Woody Plant Data (Hall, 2007)		
Species	**Application Rate (lbs a.i./Acre)**	**Phytotoxicity (%)**
	2.5	0%
	3	7.5%
	4	0%
	4	0%
	4	5%
	4	3%
	4	0%
Boxwood	2	IS rating = 0[a]
	4	IS rating = 0[a]
	8	IS rating = 0[a]
Buxus sp.	0.75	0%
	1.5	0%
	3	0%
	6	0%
	12	0%
Callistemon rigidus	4	0%
Cherries	1	0%
	1.5	0%
	2	10%
	8.1	IS rating = 1[a]
Cherries: Montmorency	2	0%
	4	0%
Cotoneaster	1	0%
	2	0%
Cotoneaster horizontalis	1.6	0%
	3.2	0%
Cotoneaster salicifus	1.5	3 - 4%
Cranberries	2	0%
	2	0%
	4	0%
Enkianthus campanulatus	1.6	0%
	3.2	0%
Euonymus alatus	1.6	3%
	3.2	3%
Euonymus alatus compactus	0.8	3%
	0.8	5%
	1.6	2 – 18%
	1.6	0%
Euonymus atropurpurea	0.8	18 – 43%
	1.6	10 – 33%
Euonymus sp.	0.7	0%[c]

Table A-20b. Summary of Simazine Woody Plant Data (Hall, 2007)		
Species	**Application Rate (lbs a.i./Acre)**	**Phytotoxicity (%)**
	1.5	20%[c]
	3	25%[c]
	6	30%[c]
	12	10%[c]
Fir, Fraser	2	IS rating = 1[a]
	2	IS rating = 2[a]
	2	IS rating = 1[a]
	2	IS rating = 2[a]
Forsythia intermedia	1.5	9 - 45%[d]
Grape	0.5	0%
	1	5%
	1	0.5%
	1.5	0%
	3	0%
Grape: Chardonnay	1.5	0%
	2.3	0%
Grape: Concord	2	0%
	3	0%
	4	0%
Grape: French Columbard	1.8	8.3%
	2.7	18.3%
	4	0%
Grape: Ruby Cabernet	2.7	15.8 – 21.7%
Grape: Thompson Seedless	1	0%
	2.5	0%
	3	0%
	5	0%
Hops	1	0%
Hydrangea macrophylla	1.5	36 – 100%[d]
Ilex cornuta	2	11.3%
Ilex glabra	0.76	0%
	1.5	0%
	3	0%
	6	0%
	12	0%
Juniperis horizontalis	1	0%
	2	0%
Kalmia latifolia	4	1 – 4%
Nectarines: Fantasia	2	22.5 – 35%
Oak, Black	1	IS rating = 2[a]

Table A-20b. Summary of Simazine Woody Plant Data (Hall, 2007)		
Species	**Application Rate (lbs a.i./Acre)**	**Phytotoxicity (%)**
Oranges	4	0%
Oranges: Hamlin	2	0 – 2.5%
Oranges: Navel	2	0%
Oranges: Valencia	2.7	0%
Ornamentals	2	6%
Pachysandra terminalis	4	2 – 5%
Palm trees	3	IS rating = 5[e]
	6	IS rating = 5[e]
Peach	1	0%
	1	0%
	1	0%
	1	0%
	2	0%
	2	0%
	3	0%
	12	20%
	12	0%
	12	0 – 5%
	12	0%
	12	0 – 5%
Peaches: Red Haven	4	0%
Pears	2	0%
Photinia	2	8%
Picea pungens	2.5	0%
Pinus nigra	1	0%
	2	0%
Pinus strobis	3	10 – 13%
	4	0%
Pinus sylvestris	1	0%
	2	0%
	2.5	13%
Pinus virginiana	4	5 – 10%
Plums	2	50%[f]
	1	10%
	1.5	10%
	2	10%
Podocarpus sp.	4	0%
Prunes	1.6	0%
	4	0%
	8	0%

Table A-20b. Summary of Simazine Woody Plant Data (Hall, 2007)		
Species	**Application Rate (lbs a.i./Acre)**	**Phytotoxicity (%)**
Pseudotsuga menziesii	1.5	0%
	2	0%
	3	0%
	2	0%
	3	0%
	4	0%
	4	0%
Pyracantha	2	0%
Rasberries	1	0%
	2	20%
Rhododendron calendulaceum	4	18%
Rhododendron sp.	1	0%
	1.6	0%
	3.2	0%
Spruce	1.5	0%
	2	0%
	3	0%
	2	0%
	3	0%
	4	0%
	2	0%
	4	0%
	8	0%
	4	0%
	8	13%
Sweet Cherries	3	0%
Taxas baccata	0.8	0%
	1.6	0%
	1.6	3%
	2.4	0%
Taxus cuspidata	0.8	0%
	0.8	0%
	1.6	0%
	1.6	0%
Taxus media	1.6	0%
	2.4	0%
Taxus sp.	0.7	0%
	1.5	0%
	3	0%
	6	0%

Table A-20b. Summary of Simazine Woody Plant Data (Hall, 2007)		
Species	**Application Rate (lbs a.i./Acre)**	**Phytotoxicity (%)**
	12	0%
Thuja occidentalis	0.8	0%
	1.6	0%
Thuja sp.	0.8	0%
	1.6	0%
	1	0%
	2	3.3%
Viburnum	2	IS rating = 0[a]
	4	IS rating = 0[a]
	8	IS rating = 0[a]
Viburnum rhytidophyllum	2	0%
	4	43%
Weigela: Florida	1.5	3 – 35%

[a] IS rating grades chlorosis severity (normal to excessive color) and ranges from 0 to 10. 0 used to indicate no injury
[b] No untreated check for comparison available; effect was noted as being atypical for apples.
[c] 0 to 30% phytotoxicity observed in untreated checks.
[d] Trial included two applications of 1.5 lb ai/A within a 1.5 month period, which is not allowed on simazine label.
[e] IS rating scale from 1-5; 5 used to indicate no injury.
[f] 20% phytotoxicity in untreated checks.

A.3.2 Non-Target Terrestrial Plants: Open Literature Data

Based on a review of the open literature, no additional information is available that indicates greater non-target terrestrial plant sensitivity to simazine than the submitted data.

A.3.3 Aquatic Plants: Submitted Data

Aquatic plant testing is required for any herbicide that has outdoor non-residential terrestrial uses that may move off-site by runoff (solubility >10 ppm in water), by drift (aerial or irrigation), or that is applied directly to aquatic use sites (except residential). Aquatic Tier II studies are required for all herbicides and any pesticide showing a negative response equal to or greater than 50% in Tier I tests.

A summary of acute toxicity of simazine to aquatic plants is provided in **Table A-21.** Tier II toxicity data for technical grade simazine is available for vascular duckweed (*Lemna gibba*) and the following non-vascular plants: blue-green algae (*Anabaena flos-aquae*), marine diatom (*Skeletonema costatum* and *Phaeodactylum tricornutum*), freshwater alga (*Selenastrum capricornutum*), freshwater diatom (*Navicula pelliculosa*), marine algae (*Isochrysis galbana*), and marine green algae (*Chlorococcum* sp. and *Dunaliella tertiolecta*).

One Tier II study of the freshwater aquatic vascular plant, duckweed, was completed using the TGAI of simazine (MRID 425037-04). Frond number was the most sensitive endpoint with an EC_{50} value of 0.14 mg ai/L. NOAEC and LOAEC values, based on reduction in frond number and growth rate inhibition were 0.054 and 0.11 mg ai/L, respectively. Growth was reduced by 9.1% in plants in the 0.11 mg ai/L treatment group. By days 6-9 and onward, there was an increase in colony breakup, smallness of frond, and root destruction in test solutions of $\geq$ 0.23 mg ai/L. The duckweed study was scientifically sound and satisfied the U.S. EPA Guideline Subdivision J, §123-2 for aquatic vascular plant studies with *L. gibba*.

The Tier II results indicate that blue-green algae (*Anabaena*) is the most sensitive non-vascular plant to simazine (MRID 426624-01). The EC_{50} for *Anabaena* is 0.036 mg ai/L, as compared to EC_{50} values ranging from 0.09 to 4 mg ai/L for other non-vascular plants. The Tier II aquatic plant study with the freshwater alga, *Anabaena*, was scientifically valid, but could not be classified as acceptable because a NOAEC value was not determined. In an Agency 1993 memo, dated October 18, 1993, EPA agreed that existing growth data be used to derive an EC_{10} value for use as the NOAEC. However, current Agency policy specifies that the EC_{05} be used to derive the NOAEC in order to protect listed species. The resulting NOAEC value based on the EC_{05} is 0.0054 mg ai/L. Reduction in growth rates of 36.8, 80.1, 97.6, and 107% were observed by day 5 at respective test concentrations of 0.078, 0.17, 0.32, and 0.66 mg ai/L. In addition, a 28% reduction in cell density was observed at the lowest test concentration of 0.02 mg ai/L. Although the study remains supplemental, it may be used to fulfill guideline requirements for an aquatic phytotoxicity test with *Anabaena flos-aquae* (§123-2).

Table A-21: Acute Toxicity of Simazine to Aquatic Plants

Species	%a.i.	EC_{50}, mg/L (confid. int.)	NOAEC (mg/L) a.i.	Most sensitive parameter	Initial/mean measured concentrations	MRID, Author, Year	Status
Vascular Plant							
Duckweed (*Lemna gibba*)	96.9	0.14 (0.12-0.15); slope = 2.6	0.054	frond number	mean	425037-04, Thompson , 1992	Acceptable
Seagrass (*Halophila ovalis*)	99.0	Not determined	<0.001	chlorophyll a activity		Ralph, 2000	Supplemental
Nonvascular Plants							
Green alga *Pseudokirchneriella subcapitata*	Not given	EC50 = 0.297 (0.176-0.427)	NOAEC = 0.002- 0.100		nominal	Sbrilli *et al*, 2004	Supplemental (No purity given; no raw data; test concentrations not provided)
Blue-green algae (*Anabaena flos-aquae*)	96.9	0.036 (0.030-0.042); slope = 2.1	0.0054 (0.0033-0.0076)[a]	growth rate	mean	426624-01, Thompson & Swigert, 1992	Supplemental[a]
Marine diatom (*Skeletonema costatum*)	96.9	0.60 (0.56-0.65); slope = 5.62	0.25	cell density	mean	425037-05, Thompson & Swigert, 1992	Acceptable
Marine diatom (*Phaeodactylum*	98	0.5	NR	NR	nominal	402284-91, Mayer, 1996	

Table A-21: Acute Toxicity of Simazine to Aquatic Plants							
Species	**%a.i.**	**EC_{50}, mg/L (confid. int.)**	**NOAEC (mg/L) a.i.**	**Most sensitive parameter**	**Initial/mean measured concentrations**	**MRID, Author, Year**	**Status**
tricornutum)							Acceptable
Freshwater alga (*Selenastrum capricornutum*)	96.9	0.10 (0.09-0.11); slope = 3.37	0.034	cell density	mean	425037-06, Thompson & Swigert, 1992	Acceptable
Freshwater diatom (*Navicula pelliculosa*)	96.9	0.09 (0.08-0.10); slope = 2.94	0.03	cell density	mean	425037-07, Thompson & Swigert, 1992	Acceptable
Marine algae (*Isochrysis galbana*)	98	0.5	NR	NR	nominal	402284-91, Mayer, 1996	Acceptable
Marine green algae (*Chlorococcum* sp.)	98	2	NR	NR	nominal	402284-91, Mayer, 1996	Acceptable
Marine green algae (*Dunaliella tertiolecta*)	98	4	NR	NR	nominal	402284-91, Mayer, 1996	Acceptable

[a] The study is classified as supplemental because a NOAEC value was not determined based on cell density. Based on an Agency memo dated October 18, 1993, it was determined that the existing growth data be used to calculate an EC_{10} value for use as the NOAEC. However, current Agency policy specifies that the EC_{05} be used to derive the NOAEC in order to protect listed species. The resulting value based on the EC_{05} is 0.0054 mg ai/L.

Degradates: Special tests are required for algal and vascular plant species (123-2) to address concerns for the toxicity of simazine degradates to aquatic plants. A summary of the degradate aquatic plant toxicity data for deisopropylatrazine (DIA) and diamino-atraine (DACT) is provided in Tables A-21 and A-22, respectively.

Table A-21: Degradate Deisopropylatrazine (DIA) Nontarget Aquatic Plant Toxicity (Tier II)					
Species/ Duration/Measured/ nominal	**% ai**	**Conc. (ppb) Probit slope**	**% Response**	**MRID No. Author/Year**	**Study Classification**
Fresh. Blue-Green - Cyanophyceae *Anabaena inaequalis* (12-14 days[1]; nominal)	> 95	2,500 7,000 9,000	50% red. cell count 50% red. growth rate 50% red. photosynthesis	450874-01, Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Freshwater Green - Chlorophyceae *Scenedesmus quadricauda* (12-14 days; nominal)	> 95	6,900 6.500 4,000	50% red. cell count 50% red. Growth rate 50% red. photosynthesis	450874-01, Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Freshwater Green - Chlorophyceae *Chlorella pyrenoidosa* (12-14 days[1]; nominal)	> 95	> 10,000 > 10,000 3,600	50% red. cell count 50% red. growth rate 50% red. photosynthesis	450874-01, Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Fresh. Blue-Green - Cyanophyceae *Anabaena variabilis* (12-14 days; nominal)	> 95	5,500 9,200 4,700	50% red. cell count 50% red. growth rate 50 % red. photosynthesis	450874-01, Stratton 1984	Supplemental (NOAEC and raw data unavailable)

Table A-21: Degradate Deisopropylatrazine (DIA) Nontarget Aquatic Plant Toxicity (Tier II)					
Species/ Duration/Measured/ nominal	**% ai**	**Conc. (ppb) Probit slope**	**% Response**	**MRID No. Author/Year**	**Study Classification**
Fresh. Blue-Green - Cyanophyceae *Anabaena cylindrica* (12-14 days; nominal)	> 95	> 10,000 > 10,000 9,300	50% red. cell count 50% red. growth rate 50% red. photosynthesis	450874-01, Stratton 1984	Supplemental (NOAEC and raw data unavailable)

Table A-22: Degradate Diamino-chlorotriazine (DACT) Nontarget Aquatic Plant Toxicity (Tier II)					
Species/ Duration/Measured/ nominal	**% ai**	**Conc. (ppb) Probit slope**	**% Response**	**MRID No. Author/Year**	**Study Classification**
Fresh. Blue-Green - Cyanophyceae *Anabaena inaequalis* (12-14 days[1]; nominal)	> 95	7,000 >10,000 >100,000	50% red. cell count 50% red. growth rate 50% red. photosynthesis	450874-01, Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Freshwater Green - Chlorophyceae *Scenedesmus quadricauda* (12-14 days; nominal)	> 95	4,600 10,000 >100,000	50% red. cell count 50% red. Growth rate 50% red. photosynthesis	450874-01, Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Freshwater Green - Chlorophyceae *Chlorella pyrenoidosa* (12-14 days[1]; nominal)	> 95	>10,000 >10,000 >100,000	50% red. cell count 50% red. growth rate 50% red. photosynthesis	450874-01, Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Fresh. Blue-Green - Cyanophyceae *Anabaena variabilis* (12-14 days; nominal)	> 95	>10,000 >10,000 100,000	50% red. cell count 50% red. growth rate 50 % red. photosynthesis	450874-01, Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Fresh. Blue-Green - Cyanophyceae *Anabaena cylindrica* (12-14 days; nominal)	> 95	>10,000 >10,000 >100,000	50% red. cell count 50% red. growth rate 50% red. photosynthesis	450874-01, Stratton 1984	Supplemental (NOAEC and raw data unavailable)

The Tier II results for atrazine degradates indicate that DIA is more toxic than DACT, and the most sensitive algae of the five species is generally the blue-green alga *Anabaena inaequalis* with EC_{50} values ranging from 2,500 to > 100,000 ppb. Simazine is more toxic to these algal species than any degradate. The order of descending toxicity for these algal species are simazine > DIA > DACT. Comparison of EC_{50} values from the open literature studies with the EC_{50} value for non-vascular aquatic plants (0.036 mg/L) indicates that DIA and DACT are approximately 70 and 130 times less toxic than simazine, respectively.

A.3.3 Aquatic Plants: Open Literature Data

The open literature contains a large amount of information on the toxicity of simazine to aquatic plants; however, the majority of data report toxicity values that are higher (i.e., not as sensitive) than the endpoints reported in the submitted studies. A number of open literature papers that characterize unique endpoints to aquatic plants, present data with endpoint values that are more sensitive than the submitted endpoints, or discuss aquatic plant succession and recovery following simazine application are discussed below. **Tables A-23 and A-18** provide a summary of the open literature laboratory and *in situ* studies, respectively, on the effects of simazine to aquatic plants. Based on the results of the *in situ* and laboratory studies, it appears that simazine results in a reduction of chlorophyll *a* in periphyton and phytoplankton at simazine levels between 0.5 and 1.0 mg/L. Other studies show increased chlorophyll *a* production at simazine concentrations of $\leq$0.05 ug/L. In addition, despite the apparent sensitivity of the blue-green algae *Anabaena flos-aquae* to simazine, the results of one open literature study suggest possible resistance and shifts in the aquatic periphytic plant community to blue-green alga at the higher simazine treatment levels of 5.0 mg/L. Simazine resistance has also been reported in seeds and tubers of *Potamogeton foliosus*. There is evidence to suggest that recovery occurs in algae upon removal of simazine from the site of action, with the recovery inversely proportional to the prior exposure level. In one study, recovery of macrophytes was noted within two to three months following application of simazine granules at 25 lb doses (% a.i. was not reported). Further detail on the open literature data for aquatic plants is discussed below.

Laboratory studies

Torres and O'Flaherty (1976) (ECOTOX# 4993, MRID# 000235-44) investigated the influence of simazine on the growth of six representative algae (*Chlorella vulgaris*, *Chlorococcum hypnosporum, Oscillatoria lutea*, *Stigeoclonium tenue*, *Tribonema* sp., and *Vaucheria geminate*) and as measured by chlorophyll production. In addition, the interaction of simazine with atrazine and malathion was studied to determine if mixtures of these pesticides interact to produce more inhibition or stimulation of growth than when they are present individually. Following 7 days of exposure, simazine caused chlorophyll inhibition in five of the six tested algal species, ranging from 0 to 37% inhibition at 1 ppb to 36 to 100% inhibition at 1000 ppb. At concentrations of $\leq$ 0.05 ppb, simazine tended to increase chlorophyll concentration, and effects with atrazine and malathion at the same concentrations were additive (atrazine) or mildly synergistic (malathion). At concentrations $\geq$ 1 ppb, the effects were generally additively inhibitory. Simazine has an inhibitory effect on chlorophyll production at concentrations > 1 ppb, and a stimulatory effect at lower concentrations. This effect is similar among other pesticides (atrazine, malathion). In combination, the chemicals are interactive. Below 1 ppb, the interaction is to increase production, and above 1 ppb, the interaction is to inhibit production. This study is classified as qualitative because the endpoint based on chlorophyll inhibition is less sensitive than the most sensitive endpoint from a registrant-submitted study. In addition, this study is qualitatively considered based on the results of pesticide interactions for simazine, atrazine, and malathion.

O'Brien and Prendeville (1979) (ECOTOX#:6963) studied the effects of simazine on membrane permeability in duckweed. The results of this study are summarized in **Table A-23.** Technical grade simazine was used, although the % a.i. was not reported. Plants were floated for different times on a range of simazine solutions before a 5 hr incubation in deionized water. Leakage of electrolytes, as measured by changes in electrical conductance, was used as an indicator of changes in cell membrane permeability. At 0.002 mg/L, simazine increased cell membrane permeability after 12 hours. The data indicate that increased cell membrane permeability may precede the usual phytotoxic symptoms of simazine, such as foliar chlorosis followed by necrosis, although a direct relationship between increased cell permeability and phytotoxic effects associated with exposure to simazine in duckweed is unclear. Little data is available on the effects of simazine on vascular aquatic plant cell membranes. This study was evaluated qualitatively because the cell permeability endpoint cannot be quantitatively linked to the assessment endpoint for vascular plants.

In Situ Studies

A summary of the aquatic plant *in situ* studies is provided in **Table A-18.** Three of the four *in situ* studies discuss effects to freshwater periphyton following simazine exposure to marsh enclosures. Goldsborough and Robinson (1983, 1988, and 1986) (ECOTOX#s: 11289, 12264, and 3136) co-authored all three papers describing functional responses and changes in periphytic algal community structure as a consequence of simazine exposure. Periphyton is benthic algae that grows attached to surfaces such as rocks or plants. They are primary producers and are sensitive indicators of environmental change in lotic waters. The fourth *in situ* study provides a comparison of algaecide effectiveness on phytoplankton.

In all three of the Goldsborough and Robinson studies, varying concentrations of technical grade simazine (from 0.1 to 5.0 mg/L) were added to *in-situ* enclosures of marsh water. Acrylic rods positioned vertically in each enclosure were used as substrata for periphyton colonization and growth. In the 1983 study, colonization of acrylic substrata by periphyton was monitored by measuring chlorophyll *a* accumulation and carbon assimilation rate. No change in either of the endpoints, relative to an untreated control, was observed following 18 days of exposure to 0.1 mg/L simazine, with increasing inhibition (to ~ 95%) at 1.0 and 5.0 mg/L treatment levels. The data suggest that the EC_{50} of chlorophyll synthesis by the marsh periphyton is between 0.1 and 1.0 mg/L simazine. Following a single simazine application, algal biomass (using chlorophyll *a* level as a crude indicator of the photosynthetically-active portion of biomass) increased over time in all treatments with the most notable increases in treated enclosures following flooding. Flooding during the experiment provides an opportunity to monitor the extent and rate of ‘recovery’ of the community. Periphytic productivity was correlated with water chemistry, light availability, time, and the experimental simazine treatment, suggesting that herbicidal effects result from a complex interaction of several parameters rather than herbicide concentration alone. Secondary effects associated with simazine treatment include reduction in DO and pH, and increases in dissolved calcium, magnesium, potassium, ammonia, nitrate, and phosphate. Recovery of communities following decreased herbicide concentrations began within 1 week, with growth rate equal to or greater than the control. The results suggest that the long-term impact of a single dose of simazine on the periphyton community may be minimal.
In the 1986 study, Goldsborough and Robinson evaluated the effects of technical grade simazine on total biovolume and community structure of periphytic algal communities within *in situ* marsh enclosures over 42 days. Measurements of total biovolume are indicative of algal success. No reduction in total biovolume was observed at 0.1 mg/L simazine; however, increasing inhibition (94 to 98%) was observed at pre-flood simazine concentrations of 1.0 and 5.0 mg/L. Similar to the EC_{50} values discussed as part of the

1983 study for chlorophyll synthesis, the data suggest that the community LC_{50} (simazine concentration yielding 50% reduction in biovolume) lies between 0.1 and 1.0 mg/L simazine. Pre-flood community structure of periphyton in simazine-treated enclosures was qualitatively similar to the control. After flooding, substratum colonization in most experimental enclosures was dominated by the diatom, *Cocconeis placentula*. This taxon accounted for 24% of the total biovolume on substrata from the control and the 0.1 mg/L simazine enclosures. Increases in biovolume were also observed in all but the highest simazine treatment level of 5.0 mg/L, following enclosure flooding. Based on the results of the study, there was no major evidence that a clearly herbicide-resistant or tolerant community had developed in the 2.5 week period prior to enclosure flooding, although the lower relative abundance of filamentous green algae at 5.0 mg/L indicates that these taxa were selectively inhibited to a greater extent than the others. High abundance of periphytic blue-green alga at the higher simazine treatment levels suggests that this taxon possesses some means of herbicide resistance. However, disappearance of blue-green alga from the 0.1 mg/L and reduced abundance in 1.0 mg/L treatments following flooding indicates that this taxon is a poor competitor for resources with less tolerant species, and that herbicide tolerance may be achieved at the expense of ecological fitness.

In the 1988 study, Golsborough and Robinson reported that simazine resistance can develop in lentic periphyton after short 7 day exposures; however, resistance can occur only at relatively high ambient simazine levels of 0.8 mg/L or greater. Comparison of the treated enclosure EC_{50}s with ambient simazine concentrations showed that significant increases in EC_{50} values occurred only when the ambient simazine concentration was $\geq$ 0.8 mg/L. This level is generally higher than is found in streamwater following terrestrial runoff or used to control nuisance aquatic vegetation.

Table A-23: Aquatic Plant Toxicity Tests (Laboratory)

Study type/ Test material	Test Organism (Common and Scientific Name)	Test Design	Endpoint Concentration / Results	Citation (ECOTOX #)	Rationale for Use in Risk Assessment[1]
7 day lab study Simazine (% a.i. NR)	*Chlorella vulgaris*, *Chlorococcum hypnosporum*, *Oscillatoria lutea*, *Stigeoclonium tenue*, *Tribonema* sp., and *Vucheria geminata*	Total chlorophyll production measured in 6 species of algae exposed to nominal concentrations of simazine at 0.1, 0.5, 1.0, and 1,000 ppb. Also evaluated influence of atrazine and malathion to determine if pesticides interact to produce more inhibition or stimulation of growth than when present individually. Factorial design with atrazine, simazine, and malathion tested individually and in pairs.	At 1 and 1000 ppb, respectively, the following % reduction in chlorophyll production was reported: 25 – 36% in *Chlorella*; 2 – 100% in *Vucheria*; 37 – 74% in *Oscillatoria;* 0 – 100% in *Tribonema*; No effect to *Stigeoclonium*. At concentrations $\leq$ 0.5 ppb, simazine tended to increase chlorophyll concentrations, and effects w/atrazine or malathion at same concentrations were additive (atrazine) or mildly synergistic (malathion). At concentrations $\geq$ 1 ppb, the effects were generally	Torres and O'Flaherty, 1976 (MRID# 000235-44; ECOTOX Ref# 4993)	QUAL (less sensitive endpoint; papers addresses potential mixture-related effects to non-vascular aquatic plants)

Table A-23: Aquatic Plant Toxicity Tests (Laboratory)					
Study type/ Test material	**Test Organism (Common and Scientific Name)**	**Test Design**	**Endpoint Concentration / Results**	**Citation (ECOTOX #)**	**Rationale for Use in Risk Assessment[1]**
			additively inhibitory.		
Acute (96 hour) cell membrane permeability test Simazine (% a.i. NR)	Duckweed (*Lemna minor*)	100 duckweed fronds floated in simazine solution (conc. NR) from 6 - 9 hours, then incubated in deionized water for 5 hours. Leakage of electrolytes as measured by changes in electrical conductance was taken as an indicator of changes in cell membrane permeability	LOAEL = 0.002 mg/L	O'Brien and Prendeville, 1979 (6963)	QUAL (endpoint cannot be quantitatively linked to the endpoint for vascular plants)
[1] QUAL = The paper is not appropriate for quantitative use but is of good quality, addresses issues of concern to the risk assessment and is used in the risk characterization discussion.					

A.4 Simazine Toxicity Pesticide Toxicity Interactions

Additive toxic interactions between simazine and other triazines, including atrazine have been reported for aquatic plants. Torres and O'Flaherty (1976) claim additive toxicity of atrazine with simazine at concentrations of 1.0 ug/L and 1 mg/L for *Chlorella vulgaris*, *Stigeoclonium tenue*, *Tribonema* sp., *Vaucheria geminata*, and *Oscillatoria lutea*. Combinations of atrazine and simazine resulted in less chlorophyll production than would be expected if these herbicides were acting independently. Mixtures of atrazine, simazine, and malathion at concentrations of 0.1 and 0.5 ug/L usually enhanced the production of chlorophyll. The results of a study by Faust et al. (2001) show that the toxic effects of s-triazine mixtures exceed that of the most active component alone; they demonstrate that low, non-significant effect concentrations of single s-triazines contribute to the overall toxicity, and that the concept of concentration addition provides highly accurate predictions of s-triazine mixture toxicity, regardless of the effect level under consideration and the concentration ratio of the mixture components.

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