Effect Determination for Atrazine

Appendix A. Ecological Effects Characterization

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Appendix A. Ecological Effects Characterization

A.1 Toxicity to Birds / Reptiles

Given a lack of ecotoxicity data for reptiles, avian acute oral, subacute dietary, and chronic reproduction data are used as a surrogate for sea turtles. In addition, open literature data are available for a limited number of reptiles including turtles (red-eared slider [*Pseudemys elegans*] and snapping turtles [*Chelydra* serpentine]) and American alligators (*Alligator mississippiensis*). Ecotoxicity data for birds and reptiles are discussed in Sections A.1.1 through A.1.4.

A.1.1 Birds: Acute Oral Studies

An acute oral toxicity study using the technical grade of the active ingredient (TGAI) is required to establish the toxicity of atrazine to birds. The preferred test species is either mallard duck (*Anas platyrhynchos*; a waterfowl) or bobwhite quail (*Colinus virginianus*; an upland gamebird). Results of this test are summarized below in Table A-1.

Table A-1. Avian Acute Oral Toxicity: Technical Grade and Formulations

Surrogate Species	% ai	LD_{50} (mg/kg) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification[1]
Northern bobwhite quail (*Colinus virginianus*) 14-day old chicks; 8-day test	Tech.	940 slope 3.836	Slightly toxic	000247-21 Fink 1976	Acceptable
Mallard Duck (*Anas platyrhynchos*) 6-months old; 14-day test	76 % 80 WP	> 2,000 slope none	Practically non-toxic	001600-00 Hudson, Tucker & Haegle 1984	Supplemental (only 3 birds) (formulation)
Ring-necked Pheasant (*Phasianus colchicus*) 3-months old; 14-day test	76 % 80 WP	> 2,000 slope none	Practically non-toxic	001600-00 Hudson, Tucker & Haegle 1984	Supplemental (formulation)
Japanese Quail (*Coturnix c. japonica*) 50-60 days old; 14-day test	Tech.	4,237 slope > 6	Practically non-toxic	000247-22 Sachsse and Ullman 1974	Supplemental (species not native)

Since the lowest LD_{50} is in the range of 501 to 2,000 mg/kg, atrazine is categorized as slightly toxic to avian species on an acute oral exposure basis. According to Hudson *et al*. (1984), signs of intoxication in mallards first appeared 1 hour after treatment and persisted up to 11 days. In pheasants, signs of intoxication disappeared by 5 days after treatment. Signs of intoxication included weakness, hyper-excitability, ataxia, tremors; weight loss occurred in mallards.

Degradates: Minor atrazine degradates include deethylatrazine (DEA), deisopropylatrazine (DIA) and diaminochlorotriazine. Acute mammalian LD_{50} values available for deethylatrazine and deisopropylatrazine are both more toxic than the parent atrazine. Therefore, a special (70-1) acute oral toxicity test with the upland gamebird (preferably northern bobwhite) are required to

address the concern for these degradates. Acute avian LD_{50} data for the atrazine degrdates, deethylatrazine (DEA) and deisopropylatrazine(DIA), and hydroxyatrazine (HA) are summarized in Table A-2.

Table A-2. Avian Acute Oral Toxicity: Degradates

Surrogate Species	Degradate % ai	LD_{50} (mg/kg-bw) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification[1]
Northern bobwhite quail (*Colinus virginianus*) 18-week old chicks; 14-day test	Deisopropyl atrazine (DIA) 96%	> 2,000 slope none	Practically non-toxic	465000-07 Stafford, 2005a	Acceptable
Northern bobwhite quail (*Colinus virginianus*) 17-week old chicks; 14-day test	Hyrdroxy atrazine (HA) 97.1%	> 2,000 slope none	Practically non-toxic	465000-08 Stafford, 2005b	Acceptable
Northern bobwhite quail (*Colinus virginianus*) 16-week old chicks; 14-day test	Desethyl Atrazine (DEA) 96%	768 Slope = 6.21 (95% CI = 3.19 – 9.27)	Slightly toxic	465000-09 Stafford, 2005c	Acceptable

The results of the acute avian oral toxicity data with the atrazine degradates shows that DEA is slightly toxic, while HA and DIA are practically non-toxic, to bobwhite quail. It should be noted that the LD_{50} value for DEA (768 mg/kg-bw) is less than the corresponding value for the parent technical grade of atrazine (940 mg/kg-bw), indicating that the DEA degradate is more toxic to birds than the parent on an acute oral exposure basis. In the DEA study, 10, 40, 90, and 100% mortality was observed in quail exposed to DEA at 445, 735, 1212, and 2000 mg/kg-bw by 14 days (MRID # 465000-09). In addition, sublethal treatment-related effects, including reduction in body weight gain and decreased food consumption, were observed at the lowest treatment level of 270 mg/kg-bw as well as the higher doses. Although no treatment-related mortality was observed in the acute oral test using DIA, sublethal effects on reduced body weight gain and food consumption were observed at concentrations of 445 mg/kg-bw (MRID # 465000-08) and higher. No mortality and/or sublethal effects were noted in the acute oral test with HA (MRID # 465000-08).

A.1.2 Birds: Subacute Dietary Studies

Two subacute dietary studies using the TGAI are required to establish the toxicity of atrazine to birds. The preferred test species are mallard duck and bobwhite quail. Results of these tests are tabulated below in Table A-3.

Table A-3. Avian Subacute Dietary Toxicity

Surrogate Species	% ai	5-Day LC_{50} (ppm)[1]	Toxicity Category	MRID No. Author/Year	Study Classification
Northern bobwhite (*Colinus virginianus*) 9-days old chicks	99.0	> 5,000 (no mortality)	Practically non-toxic	000229-23 Hill *et al*. 1975	Acceptable
Northern bobwhite (*Colinus virginianus*) young adults	Tech.	> 10,000	Practically non-toxic	unknown - Gulf South Gough & Shellenberger 1972	Supplemental (Adult birds & no raw data)
Ring-necked pheasant (*Phasianus colchicus*) 10-days old chicks	99.0	> 5,000 (no mortality)	Practically non-toxic	000229-23 Hill *et al*. 1975	Acceptable
Japanese Quail (*Coturnix c. japonica*) 7-days old chicks	99.0	> 5,000 (7 % mortality at 5,000 ppm)	Practically non-toxic	000229-23 Hill *et al*. 1975	Supplemental (species not native)
Mallard duck (*Anas platyrhynchos*) 10-days old ducklings	99.0	**> 5,000** (30 % mortality at 5,000 ppm)	Practically non-toxic	000229-23 Hill *et al*. 1975	Acceptable

[1] Test organisms observed an additional three days while on untreated feed.

Because the LC_{50} values are greater than 5,000 ppm, atrazine is categorized as practically non-toxic to avian species on a subacute dietary exposure basis. In the sub-acute dietary with mallard ducks, 30% mortality was observed at the highest test concentration of 5,000 ppm (MRID # 000229-23). The time to death was Day 3 for the one Japanese quail and Day 5 for three mallard ducks (J. Spann at Patuxent Wildlife Center, 1999, personal communication).

Subacute dietary studies using a typical end-use product (TEP) may be required on a case-by-case basis to establish the toxicity of atrazine formulations to birds. The preferred test species are mallard duck and bobwhite quail. Results of these tests are summarized below in Table A-4.

Table A-4. Formulation Avian Subacute Dietary Toxicity

Surrogate Species	% ai Form	5-Day LC_{50} (ppm ai)[1] Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification
Northern bobwhite (*Colinus virginianus*) (6-weeks old)	76 80 WP	5,760 slope 3.252	Practically non-toxic	000592-14 Beliles & Scott 1965	Supplemental (birds too old)
Mallard duck (*Anas platyrhynchos*)	76 80 WP	19,560 slope 1.807	Practically non-toxic	000592-14 Beliles & Scott 1965	Acceptable for 80W formulation

[1] Test organisms observed an additional three days while on untreated feed.

Because the LC_{50} values exceed 5,000 ppm, atrazine is categorized as practically non-toxic to avian species on a subacute dietary basis for the 80W formulation (76% ai). In the mallard study, a highly noticeable weight loss and emaciated birds were found at all test levels (1,000 to 32,000 ppm) relative to controls.

A.1.3 Birds: Chronic Studies

Avian reproduction studies using the TGAI are required for atrazine, because the following conditions are met: (1) birds may be subject to repeated or continuous exposure to the pesticide, especially preceding or during the breeding season, (2) the pesticide is stable in the environment to the extent that potentially toxic amounts may persist in animal feed, (3) the pesticide is stored or accumulated in plant or animal tissues, and/or, (4) information derived from mammalian reproduction studies indicates reproduction in terrestrial vertebrates may be adversely affected by the anticipated use of the product. The preferred test species are mallard duck and bobwhite quail. Results of these tests are provided below in Table A-5.

Table A-5. Avian Reproduction

Surrogate Species/ Study Duration	% ai	NOAEC/ LOAEC (ppm ai)	Statistically sign. (p<0.05) LOAEC Endpoints	MRID No. Author/Year	Study Classification
Northern bobwhite (*Colinus virginianus*) 20 weeks	97.1	**NOAEC 225** **LOAEC 675**	29 % red. in egg production 67 % incr. in defective eggs 27 % red. in embryo viability 6-13 % red. in hatchling body wt. 10-16 % red. in 14-day old body wt. 8.2 % red. in 14-day old body wt. (after recovery period)	425471-02 Pedersen & DuCharme 1992	Acceptable
		NOAEC < 75 LOAEC 75	6.7-18 % red. in 14-day old body wt.		
Mallard duck (*Anas platyrhynchos*) 20 weeks	97.1	**NOAEC 225** **LOAEC 675**	49 % red. in egg production 61 % red. in egg hatchability 12-17 % red. in food consumption	425471-01 Pedersen & DuCharme 1992	Acceptable
		NOAEC 75 LOAEC 225	9-13 % red. in food consumption (During 3 of 11 biweekly periods)		

In the bobwhite study, reproductive endpoints were measured after a 3-week recovery period. During the recovery period, there was a 67% percent increase in the number of defective eggs at 675 ppm as compared to controls; the number of defective eggs during the recovery period was consistent with the number of defective eggs during the treatment period at 675 ppm (MRID # 425471-02). Bobwhite and mallard tests show similar toxic effects on reduced egg production and embryo viability/hatchability with LOAEC and NOAEC values of 675 and 225 ppm, respectively. Although the bobwhite test showed a 7 to 18% reduction in 14-day body weight in the 75 ppm treatment group, relative to the control group, the reproductive endpoints were considered to be more biologically significant, given the use of the avian data as a surrogate for sea turtles in the Chesapeake Bay.

In the 8-day subacute LC_{50} test with adult Japanese quail, food consumption and body weight were reduced and egg production stopped after 3 days of exposure to atrazine (Sachsse and Ullman, 1975; MRID 000247-23).

A.1.4 Birds/Reptiles: Open Literature

A.1.4a Birds: New Open Literature Data

Two studies were located in the open literature that evaluated the potential for atrazine to affect endpoints including growth, sexual maturity, liver effects, and endocrine effects in birds (summarized in Table A-6). Wilhelms et al. (2005; Ecotox Reference # 80632) reported that dietary exposure to 1000 ppm atrazine resulted in reduced food consumption (15% reduction compared with controls) and weight gain (31% reduction compared to controls), and elevated testosterone levels (approximately 3-fold increase relative to controls) in male Japanese quail. It is possible that the reduced food intake observed in this study represents taste aversion. Atrazine was not definitively associated with effects on any other endpoint evaluated. Wilhelms et al. (2006; Ecotox Reference # 82035) observed similar types of effects in female Japanese quail at comparable dietary concentrations (Table A-6).

This study suggests that atrazine was associated with evidence of toxicity at dietary concentrations of 1000 ppm in Japanese quail. These open literature studies were less sensitive than the submitted data summarized in Table A-5.

Table A-6. Avian Reproduction/Growth Effects Tests from Open Literature (2006 Review)

Study type/ Test material	Test Organism (Common and Scientific Name) and Age and/or Size	Test Design	Endpoint Concentration in ppb	Citation (EcoRef. #)	Rationale for Use in Risk Assessment[1]
Reproduction dietary studies in birds / Atrazine technical 99.9% ai	Male Japanese quail	Seven separate studies were conducted. Dietary concentrations ranged from 10 to 1000 ppm. Animals were approximately 6-week old males. Endpoints evaluated included growth, liver effects, sexual maturation, and anti-estrogenic effects. Exposure duration was up to 4 weeks. In addition, studies using SC administration and silastic implants were also conducted that evaluated endpoints including growth, liver effects, testes weight, and circulating LH levels. Doses up to 10 mg/kg-bw were tested.	At 1000 ppm, there was a reduction in growth rate and food intake and an elevation in testosterone levels, although the reduction in testosterone leves was not consistently observed across studies. Other statistically significant observations were considered spurious and not related to atrazine treatment.	Wilhelms et al., 2005 (80632)	Qual: Study did not evaluate a comprehensive suite of reproductive effects and was not the most sensitive study in birds.
Reproduction maturation in birds / Atrazine technical, 99.9% ai	Female Japanese quail	Birds were exposed to dietary concentrations that ranged from 1 ppm to 1000 ppm. The following endpoints were evaluated: growth, food intake, liver, ovary, and oviduct weight, and plasma luteinizing hormone and estradiol levels. Exposure was up to 4 weeks.	Growth, food intake, liver weight, and circulating estradiol levels were significantly ($p<0.05$) reduced in birds exposed to atrazine at 1000 ppm, but not at lower levels.	Wilhelms et al., 2006 (82035)	Qual: Study did not evaluate a comprehensive suite of reproductive effects. No concentrations between 100 and 1000 ppm were evaluated. Studies have been submitted that evaluated concentrations between 100 and 1000 ppm; NOAEC/LOAEC values from those studies are considered to be more suitalbe.

[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.1.4b Reptiles: Open Literature Data from 2003 IRED

Atrazine was tested on eggs of the turtle, red-eared slider (*Pseudemys elegans*) and the American alligator (*Alligator mississippiensis*) to determine if atrazine produced endocrine effects on the sex of the young (Gross, 2001). The turtle and alligator eggs were placed in nests constructed of sphagnum moss treated with 0, 10, 50 100 and 500 µg/L for 10 days shortly after being laid. The test temperatures, 27.3 ^{o}C for the turtle and 32.8 ^{o}C for alligators, normally yield all male young. No adverse effects were found. Analysis of the embryonic fluids indicated that no atrazine was present in the eggs at the detection limit (0.5 µg/L). Under these conditions, atrazine does not appear to have permeated the leathery shell of reptiles (MRID 455453-03 and 455453-02).

A.1.4c Reptiles: New Open Literature Data (2006 Literature Review)

Two additional open literature studies on snapping turtle and alligator egg exposures to atrazine are summarized below (De Solla et al., 2005 and Crain et al., 1999) in Table A-7. The results of both of these studies suggest that exposure of reptilian eggs to atrazine does not cause significant alteration in gonadal development and aromatase activity at environmentally relevant concentrations.

Snapping turtles (*Chelydra serpentina*) were used to determine if environmentally relevant exposures to atrazine affected gonadal development (De Solla et al., 2005; Ecotox Reference #: 82032). Eggs were incubated in soil treated with atrazine at a typical field application rate (1.32 lb ai/A), 10-fold this rate (13.2 lb ai/A) and a control rate (no atrazine) for the duration of embryonic development (~117 days). Measured concentrations of atrazine in the low and high atrazine treatment groups were 0.64 and 8.1 ppm, respectively. The incubation temperature (25 ^{o}C) was selected to produce only males. Although some males with testicular oocytes and females were produced in the atrazine-treated groups (3.3 – 3.7%), but not in the control group, no statistical differences were found among the treatment and control groups. In addition, there was no difference in hatching success and thyroid activity among the different atrazine treatments and the control. According to the study authors, observations of other turtles suggest that natural and spontaneous intersexes exist in turtle populations.

Gonadal histology and hepatic steroidogenic activity was measured in American alligator eggs exposed to atrazine at concentrations of 0, 0.014, 0.14, 1.4, and 14 ppm (Crain et al., 1999; Ecotox Reference #: 70208). All atrazine treated eggs incubated at female- and male-determining temperatures produced female and male hatchlings, respectively. No differences in gonadal and reproductive tract histology or hepatic aromatase activity were observed in any of the atrazine-treated or control alligators. The results of the study suggest that embryonic exposure to atrazine does not cause significant alterations in gonadal structure or hepatic steroidogenic enzyme activity of hatchling American alligators.

Table A-7. Reptilian Toxicity Tests from Open Literature (2006 Review)					
Study type/ Test material	**Test Organism (Common and Scientific Name) and Age and/or Size**	**Test Design**	**Endpoint Concentration in ppm**	**Citation (EcoRef. #)**	**Rationale for Use in Risk Assessment[1]**
Chronic lab (117 days) / Atrazine 480 formulation (atrazine content = 481 g/L and unspecified triazines of 29 g/L)	Snapping turtle (*Chelydra serpentine*) eggs	- Eggs incubated in soil treated w/atrazine at 1.32 lb ai/A (measured conc = 0.64 ppm) and 13.2 lb ai/A (measured conc = 8.1 ppm) and control. - 3 replicates (with 23-24 eggs/replication)/treatment group. - Incubator temp = 25° (±1°C) to produce males. - Endpoints: gonadal development (hatching success, gonadal morphology, and thyroid activity)	NOAEC = 13.2 lb ai/A (0.81 ppm) Some males w/testicular oocytes and females produced in atrazine-treated groups (3.3 – 3.7%); however, no significant differences between atrazine treatments and controls were observed. Thyroids from each treatment and control displayed similar levels of activity.	De Solla et al., 2005 (82032)	QUAL: - no raw data provided - 3 PAHs detected at non-toxic levels in control soil, but not analyzed for in the atrazine treatment groups - low incidence of intersex or females precluded ability to differentiate between a low incidence caused by atrazine exposure and random sampling error
Chronic lab (duration NR) / Atrazine (99 % ai)	American alligator (*Alligator mississippiensis*) eggs at stage 21 in embryonic development, just prior to onset of gonadal differentiation	- Eggs were treated w/atrazine at 0, 0.014, 0.14, 1.4, and 14 ppm via topical application to the eggshell in 50 µl of 95% ethanol. - 5 eggs/treatment were incubated at temperatures to produce either 100% males (33 °C) or 100% females (30 °C). - Endpoints: gonadal histology and hepatic steroidogenic activity	NOAEC = 14 ppm All atrazine treated eggs incubated at female- and male-determining temps produced female and male hatchlings, respectively. No differences in gonadal histology (Mullerian duct epithelial cell height and medullary regression) and hepatic aromatase activity was noted between atrazine treated groups and controls.	Crain et al., 1999 (70208)	QUAL: - no raw data provided

[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.2 Toxicity to Freshwater Animals

A.2.1 Freshwater Fish and Amphibia, Acute

Two freshwater fish toxicity studies using the TGAI are required to establish the toxicity of atrazine to fish. The preferred test species are rainbow trout (*Oncorhynchus mykiss;* a coldwater fish) and bluegill sunfish (*Lepomis macrochirus;* a warmwater fish). Results of these tests are summarized below in Table A-8.

Table A-8. Freshwater Fish Acute Toxicity (TGAI)

Surrogate Species/ Static or Flow-through test	% a.i.	96-hour LC_{50} (ppb) (measured/nominal) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification

Table A-8. Freshwater Fish Acute Toxicity (TGAI)

Surrogate Species/ Static or Flow-through test	% a.i.	96-hour LC_{50} (ppb) (measured/nominal) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification
Rainbow trout (*Oncorhynchus mykiss*) Static test	98.8	**5,300** (nominal) slope - 2.723	moderately toxic	000247-16 Beliles & Scott 1965	Acceptable
Brook trout (*Salvelinus tontinalis*) Flow-through test	94	6,300 4,900 (8-day test) not specified	moderately toxic	000243-77 Macek *et al*. 1976	Supplemental (52-gram fish & no raw data)
Fish from the Nile River *Chrysichthyes auratus* Static-renewal - daily 150 mg/L $CaCO_3$; 22EC	96	6,370 (not specified)	moderately toxic	452029-11 Hussein, El-Nasser & Ahmed 1996	Supplemental (non-native sp.; 26-gram fish; no raw data)
Bluegill sunfish (*Lepomis macrochirus*) Flow-through test	94	> 8,000 6,700 (7-day test) (not specified)	moderately toxic	000243-77 Macek *et al*. 1976	Supplemental (6.5-gram fish & no raw data)
Tilapia 38 grams (*Oreochromis niloticus*) Static-renewal - daily 150 mg/L $CaCO_3$: 22EC	96	9,370 (not specified)	moderately toxic	452029-11 Hussein, El-Nasser & Ahmed 1996	Supplemental (non-native sp.; 38-gram fish; no raw data)
Fathead minnow (*Pimephales promelas*) 24-Hour renewal test	94	15,000 (nominal) 15,000 (5-day test)	slightly toxic	000243-77 Macek *et al*. 1976	Supplemental (no raw data)
Carp (*Cyprinus carpio*) Semi-static test	93.7	18,800 (nominal) slope not reported	slightly toxic	452029-13 Neskovic *et al*. 1993	Supplemental (no raw data)
Fathead minnow juvenile (*Pimephales promelas*) Flow-through test; 52 mg/L $CaCO_3$	97.1	20,000 (measured) Slope - 6.889	slightly toxic	425471-03 Dionne 1992	Acceptable
Bluegill sunfish (*Lepomis macrochirus*) Static test	98.8	24,000 (nominal) no slope	slightly toxic	000247-17 Beliles & Scott 1965	Acceptable
Brown trout (*Salmo trutta*) 1.9 gr. Static-Renewal - daily pH 6; 10EC; 11 mg/L $CaCO_3$	NR	27,000 (nominal)	slightly toxic	452029-09 Grande, Anderson & Berge 1994	Supplemental (no raw data; slight aeration & purity unknown)
Zebrafish (*Brachydanio rerio*)	NR	37,000 (NR)	slightly toxic	MRID # NR Korte & Greim 1981	Supplemental (article unavailable)
Bluegill sunfish (*Lepomis macrochirus*) Static test	100	57,000 (nominal)	slightly toxic	001471-25 Buccafusco 1976	Acceptable
Goldfish (*Carassius auratus*) Static test	98.8	60,000 (nominal) Slope - 2.695	slightly toxic	000247-18 Beliles & Scott 1965	Supplemental (not an acceptable species)

The range of acute freshwater fish LC_{50} values for technical grade atrazine is 5,300 to 60,000 ppb; therefore atrazine is categorized as slightly (>10,000 to 100,000 ppb) to moderately (>1,000 to 10,000 ppb) toxic to freshwater fish on an acute exposure basis. The freshwater fish acute

nominal LC_{50} value of 5,300 ppb is based on a static 96-hour toxicity test using rainbow trout (*Oncorhynchus mykiss*) (MRID # 000243-77).

Table A-9 presents fish and amphibian toxicity data for formulated products.

Table A-9. Freshwater Fish and Amphibian Acute Toxicity (Formulated Products)

Surrogate Species/ Flow-through or Static	% ai formul.	96-hour LC_{50} (ppb) (measured/nominal)	Toxicity Category	MRID No. Author/Year	Study Classification
Black Bass - fry (*Micropterus salmoides*) Static test; 20EC 78 mg/L hardness	80 80 W	12,600 (nominal) slope - 5.86	slightly toxic	452277-17 R. O. Jones 1962	Supplemental (48-hours; limited raw data)
Channel Catfish yolk sac (*Ictalurus punctatus*) Static test; 23.3-25.8EC 78 mg/L hardness	80 80 W	16,000 (nominal) slope - 3.36	sightly toxic	452277-17 R. O. Jones 1962	Supplemental (limited raw data)
Bluegill Sunfish fry (*Lepomis macrochirus*) Static test; 25-27EC 78 mg/L hardness	80 80 W	20,000 (nominal) no slope	slightly toxic	452277-17 R. O. Jones 1962	Supplemental (limited raw data)
American Toad - larvae (*Bufo americanus*) Flow-through test	40.8 4L	10,700 late stage 26,500 early stage (nominal)	slightly toxic	452029-10 Howe *et al*. 1998	Supplemental (no raw data)
Northern Leopard Frog larvae (*Rana pipiens*) Flow-through test	40.8 4L	14,500 late stage 47,600 early stage (nominal)	slightly toxic	452029-10 Howe *et al*. 1998	Supplemental (no raw data)
Coho Salmon (*Oncorhynchus kisutch*) Renewal daily; 144 hr	40.8* AAtrex Liquid	> 18,000 25 % mortality (measured)	slightly toxic	452051-07 Lorz *et al*. 1979	Supplemental (no LC_{50} value & 12-17 months old)
Rainbow trout (*Onchorhynchus mykiss*) Flow-through test	40.8 4L	20,500 (nominal)	slightly toxic	452029-10 Howe *et al*. 1998	Supplemental (no raw data)
Channel Catfish (*Ictalurus punctatus*) Flow-through test	40.8 4L	23,800 (nominal)	slightly toxic	452029-10 Howe *et al*. 1998	Supplemental (no raw data)
Rainbow trout (*Oncorhynchus mykiss*) Static test	43 Liquid	24,000 (unknown)	slightly toxic	400980-01 Mayer & Ellersieck 1986	Supplemental (no raw data)
Bluegill sunfish (*Lepomis macrochirus*) Static test	43 Liquid	42,000 (unknown)	slightly toxic	400980-01 Mayer & Ellersieck 1986	Supplemental (no raw data)

* Percent a.i. assumed based on description as a liquid formulation, AAtrex.

All toxicity values for the atrazine formulations are > 10 and 100 ppm; therefore, the formulated products are classified as slightly toxic to aquatic invertebrates on an acute exposure basis. Based on comparison of acute toxicity data for technical grade atrazine and formulated products of atrazine, it appears that freshwater fish are more sensitive to the TGAI. It should be noted that available formulated product (40.8% ai for 4L) data for amphibians reports LC_{50} values >10,000 ppb.

Degradates Acute fish testing with bluegill and rainbow trout are required to address degradate concerns. Table A-10 presents freshwater fish toxicity data for hydroxyatrazine.

Table A-10. Freshwater Fish Acute Toxicity (Hydroxyatrazine)

Surrogate Species/ Flow-through or Static	% ai formul.	96-hour LC_{50} (ppb) (measured/nominal)	Toxicity Category	MRID No. Author/Year	Study Classification
Bluegill sunfish (*Lepomis macrochirus*); 1.15 g Static test; 20.8 – 21.6 °C 125 mg/L hardness	98	>3,800 (measured dissolved)	moderately toxic*	465000-05 Peither, 2005b	Acceptable
Rainbow trout (*Oncorhynchus mykiss*); 0.75 g Static test; 13.2 – 14.1 °C 125 mg/L hardness	98	>3,000 (measured dissolved)	moderately toxic*	465000-04 Peither, 2005a	Acceptable

* Biological results for both studies were based on the mean-measured concentration of dissolved Hydroxyatrazine, which remained constant at the limit of its water solubility throughout the duration of the tests. Therefore, hydroxyatrazine is not acutely toxic to bluegill sunfish and rainbow trout at the limit of its water solubility.

Although the freshwater fish LC_{50} values (>3,000 to >3,800 ppb) for the degradate, hydroxyatrazine, are within the range classifying it as moderately toxic, the biological results for both studies were based on dissolved (filtered) mean-measured concentrations of hydroxyatrazine, which remained constant at the limit of its water solubility (3-4 ppm ai) throughout the duration of the tests. No mortalities were reported in either study at the maximum test concentration. Therefore, hydroxyatrazine is technically classified as moderately toxic to fish on an acute exposure basis; however, given that its solubility limit is close to the maximum concentration tested, hydroxyatrazine is not likely to be acutely toxic to freshwater fish at the limit of its water solubility.

A.2.2 Freshwater Fish, Chronic

A freshwater fish early life-stage test using the TGAI is required for atrazine because the end-use product 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 recurrent; an aquatic acute EC_{50} is less than 1 mg/L (i.e., *Chironomus tentans* LC_{50} 0.72 ppm); and the pesticide is persistent in water (*i.e.*, half-life greater than 4 days). The preferred test species is rainbow trout. Table A-11 presents the chronic toxicity data for freshwater fish.

Table A-11. Freshwater Fish Early Life Stage Toxicity

Surrogate Species/ Study Duration/ Flow-through or Static Renewal	% ai	NOAEC/LOAEC ug/L (ppb) (measured or nominal)	Statistically sign. (p=0.05) Endpoints Affected	MRID No. Author/Year	Study Classification

Table A-11. Freshwater Fish Early Life Stage Toxicity

Surrogate Species/ Study Duration/ Flow-through or Static Renewal	% ai	NOAEC/LOAEC ug/L (ppb) (measured or nominal)	Statistically sign. (p=0.05) Endpoints Affected	MRID No. Author/Year	Study Classification
Rainbow trout (*Oncorhynchus mykiss*) 86 days, flow-through 50 mg/L $CaCO_3$	Tech.	NOAEC 410 LOAEC 1,100 (measured)	sign. delays in hatching @ 1,100 and 3,800 µg/L sign. red. wet wt. at 30 & 58 days @ 1,100 & 3,800 µg/L sign. red. dry wt. @ 3,800 µg/L 58.8 % mortality @ 3,800 µg/L at swim-up	452083-04 Whale *et al*. 1994	Invalid (DMSO used as solvent, which aids in transport of chemicals across cell membranes)
Rainbow trout embryo-larvae (*Oncorhynchus mykiss*) 27 days; flow-through	80 WP	Hardness 50 mg/L: LC50 660 LC01 29 Slope 1.2 Hardness 200 mg/L: LC50 810 LC01 77 Slope 1.38	% normal survival 50/200 mg/L 19 µg/L - 94 98 54 - 88 90 54 ** - 68 74 5,020 ** - 10 9 50,900 ** - 0 0	452029-02 Birge, Black & Bruser 1979	Supplemental (short test; no raw data for statistical analyses)
Channel catfish embryo-larvae (*Ictalurus punctatus*) 8 days; flow-through	80 WP	Hardness 50 mg/L: LC50 220 Slope 0.977 Hardness 200 mg/L: LC50 230 Slope 0.84	highly teratogenic in all tests; no results for soft water 420 µg/L - 16% terata 830 µg/L - 47 % terata 46,700 µg/L - 86 % terata	452029-02 Birge, Black & Bruser 1979	Supplemental (short test; no raw data for statistical analyses)
Zebrafish (*Brachydanio rerio*) 35 Days; pH 8; 27±1EC Flow-through test Hardness 24 mg/L	98	NOAEC 300 LOAEC 1,300 (measured) 35-Day LC50 890 Slope 1.25	2 - 3 % sign. incr. in edema 45-62 % mortality	452029-08 Gorge & Nagel 1990	Supplemental (no raw data)

In addition to affecting survival of rainbow trout and catfish embryo-larvae, Birge *et al*. (1979) also reported that "Atrazine was highly teratogenic in all tests." The frequency of teratogenicity was reported for channel catfish in hard water and is included in the table above; no data on frequency was reported for soft water or for rainbow trout. (MRID # 452029-02).

A freshwater fish life-cycle test using the TGAI is required for atrazine because the end-use product is expected to be transported to water from the intended use site and studies of other organisms indicate that the reproductive physiology of fish may be affected. The preferred test species is fathead minnow. Results of four fish life-cycle tests are tabulated below in Table A-12. Following 44 weeks of exposure to atrazine in a flow-through system, brook trout mean length and body weight were reduced by 7.2% and 16% at concentrations of 120 ppb, as compared to the control (MRID 000243-77). The corresponding NOAEC for this study is 65 ppb.

Table A-12. Freshwater Fish Life-Cycle Toxicity

Surrogate Species/ Study Duration/ Flow-through or Static Renewal	% ai	NOAEC/LOAEC µg/L (ppb) (measured or nominal)	Statistically sign. ($p \leq 0.05$) Endpoints Affected	MRID No. Author/Year	Study Classification
Brook trout (*Salvelinus frontinalis*) 44 weeks, flow-through	94	**NOAEC 65** **LOAEC 120** (measured)	7.2 % red. mean length 16 % red. mean body weight	000243-77 Macek *et al*. 1976	Acceptable
Bluegill sunfish (*Lepomis macrochirus*) 6-18 months, flow-through	94	NOAEC 95 LOAEC 500 (measured)	LOAEC based on loss of equilibrium in a 28-day test conducted at the same lab.	000243-77 Macek *et al*. 1976	Supplemental (Low survival in the controls)
Fathead minnow (*Pimephales promelas*) 39 weeks; flow-through	97.1	NOAEC < 150 LOAEC 150 (measured)	6.7 % red. in F_1 length 22 % red. in F_1 body wt. (sign. diff. from neg. control)	425471-03 Dionne 1992	Supplemental (Failed to identify a NOAEC)
Fathead minnow (*Pimephales promelas*) 43 weeks, static-renewal	94	NOAEC 210 LOAEC 870 (measured)	LOAEC based on 25% mortality in a 96-hour test conducted at the same lab.	00024377 Macek *et al*. 1976	Supplemental (High mortality in control adults)

A.2.3 Freshwater Fish/Amphibians, Open Literature Data on Mortality/Survivorship

Open literature data on the effects of atrazine to mortality/survivorship of amphibians is summarized in Table A-14. Additional open literature data on amphibian mortality/survivorship is also included as part of the discussion on sublethal effects for amphibians in Section A.2.4 and Table A-16. Available acute data for amphibians indicate that they are relatively insensitive to technical grade atrazine with acute LC_{50} values > 20,000 ppb. Chronic mortality data for amphibians confirms that exposure to atrazine does not cause direct mortality to frogs and salamanders at concentrations ranging from approximately 200 to 2000 ppb; these concentrations represent the highest tested atrazine treatment levels within each of the studies. Only one study (Storrs and Kiesecker, 2004; reviewed below) shows counterintuitive patterns of survivorship (lower survivorship at low atrazine doses as compared to higher doses of atrazine); however, there are a large number of uncertainties associated with the study, including possible surfactant effects and variable sampling sizes, which confound the ability to discern a atrazine treatment-related survivorship effect. Further review of the open literature studies containing chronic mortality data is included as part of discussion for sublethal effects to amphibians.

Three species of amphibian larvae (tadpoles) were tested with technical grade atrazine (Table A-14). The leopard frog (*Rana pipiens*), wood frog (*Rana sylvatica*), and American toad (*Bufo americanus*) tadpoles each have LC_{50} values of >20,000 ppb atrazine (Allran and Karasov, Ecotox Reference # 59251). Based on these values, the amphibians evaluated are relatively insensitive to atrazine on an acute exposure basis. However, sublethal effects were observed at 4.3 mg/L and higher. These effects included elevated ventilation rates (4.3 mg/L and higher) and reduced feeding (20 mg/L only) in adults and increased incidences of deformities in survivors at 4.3 mg/L and higher (approximately 19% incidence). Deformities included wavy tail (54%),

lateral tail flexure (27%), facial edema (12%), axial shortening (3.5%), dorsal tail flexure (3.3%), and blistering (0.3%). Similar incidences of deformities were observed for all species tested.

Birge et al. (1983; Ecotox Reference # 19124) tested the effects of atrazine exposure on developing embryos of bullfrogs and American toads under flow through conditions from fertilization to 4-days after hatching. Incidences of "gross debilitating" abnormalities were evaluated. LC_{50}s (mortality + malformation incidences) for atrazine were 410 ug/L and >4800 ug/L in bullfrogs and American toads, respectively. Specific information on the abnormalities associated with atrazine was not included in the study report, although defects of the head and vertebral column, dwarfed bodies, partial twining, microcephaly, absent or reduced eyes and fins, and amphiarthrodic jaws were most commonly reported across the treatments. Also, insufficient information was included in the study report to allow for an independent evaluation of data and verification of the statistical analyses. Although an LC_{50} of 410 ug/L was reported in bullfrogs, 92% survival was observed at 410 ug/L in the study. Survival did not fall below 50% until atrazine concentrations exceeded 15,000 ug/L. Therefore, there is considerable uncertainty in the LC_{50} reported by Birge et al. (1983) of 0.41 mg/L (410 ug/L). Nonetheless, the data provide evidence that atrazine exposure to embryo-larvae stages may produce developmental abnormalities. Developmental abnormalities were generally observed at atrazine levels that also induced mortality.

Long-term (32 days) static renewal exposure of a commercial formulation of atrazine (Aatrex Nine-O; 85.5% ai) to four species of tadpole frogs including spring peepers (*Pseudacris crucifer*), American toads (*Bufo americanus*), green frogs (*Rana clamitans*), and wood frogs (*Rana sylvatica*) was studied at early (Gosner stages 25-27) and late (stages 29-36) developmental stages (Storrs and Kiesecker, 2004; Ecotox Reference # 78290). Nominal atrazine concentrations were 3, 30, and 100 ppb; measured concentrations at Day 1 were 2.8, 25, and 64 ppb. With the exception of late stages of the toad and wood frog, there was significantly lower survival for animals exposed to 2.84 ppb as compared with either the higher treatment groups. Significant differences in survivorship within the 2.84 treatment group relative to the control were observed for late stages of the toad and both stages of the green frog. However, no significant survivorship differences between any of the treatment levels and the control were observed for late spring peepers, early toads, and late wood frogs. The study author suggests that greater mortality at lower doses than higher doses is associated with a U-shaped dose-response pattern characteristic of many endocrine disruptors. However, the reference to the U-shaped dose-response curve cannot be substantiated with only one statistically significant point. In addition, there are also many uncertainties associated with the study. Possible impacts related to the surfactant of the commercial grade of atrazine confound the ability to demonstrate treatment-related effects. In addition, statistical patterns reported by the study authors may have been influeced by variable sample sizes, both within treatment levels and between different stages of tadpole species. In the case of the late stage toad, the sample size was extremely low ($\leq$ 7 for each treatment and control). Finally, evidence of survivorship patterns observed in this study has not been replicated in any other available studies (although different atrazine formulations were used). Survivorship patterns were presented as survival probability; therefore, it was not possible to determine or quantify the number of days until death or the overall mortality at the end of the experiment.

Table A-14. Amphibian Mortality/Survivorship Toxicity Tests from Open Literature (2006 Review)					
Study type/ Test material	**Test Organism (Common and Scientific Name) and Age and/or Size**	**Test Design**	**Endpoint Concentration in ppb**	**Citation (EcoRef. #)**	**Rationale for Use in Risk Assessment[1]**
Acute lab (14 days) / 99% ai	- Leopard frog (*Rana pipiens*) - Wood frog (*Rana sylvaticas*) - American toad (*Bufo americanus*)	- Renewal - Hardness (mg/L as $CaCO_3$) = 290 Target Temp: 22 Deg. C Animals were exposed in the embryonic stage.	LC_{50} for all 3 species= >20,000 (measured). Effects included increased incidence of deformities in embryos exposed for 4 days after hatching and elevated ventilation rate in exposed adults at 4.3 mg/L and higher.	Allran and Karasov, 2001 (59251)	QUAL. Study may provide insight into effect levels of atrazine exposed adults and embryos; however, reporting limitations were noted, and study did not provide sensitive endpoint.
Chronic (32 d) lab study / Atrazine commercial-grade (Aatrex Nine-O; 85.5% ai)	- Spring peeper (*Pseudacris crucifer*) - American toad (*Bufo americanus*) - Green frog (*Rana clamitans*) - Wood frog (*Rana sylvatica*) - All tadpoles at early (Gosner stages 25-27) and late (stages 29-36) developmental stages	- Static renewal (water replaced every 3 d) at nominal concentrations of 0, 3, 30, and 100 ppb. Measured conc (after 1 d = ND, 2.84, 25.2, and 64.8 ppb) - Peepers, toads, and early-stage green frogs kept in 120 ml polypropylene cups w/100 ml (treatment in dechlorinated water); late wood and green frogs kept in 750 ml poly cups w/ 500 ml water; # tadpoles/treatment varied - Temperature = 22 ºC - Photoperiod = 12 h light/dark - Feeding: crushed alfalfa every 3 d - Endpoints: Surivorship	Early spring peeper: LOAEL = 64.8; NOAEL = 25.2 Late spring peeper: NOAEL = 64.8 Early A. toad: NOAEL = 64.8 Late A. toad: LOAEL = 2.84 NOAEL = <2.84 Early green frog: LOAEL = 2.84 NOAEL = <2.84 Late green frog: LOAEL = 2.84 NOAEL = <2.84 Late wood frog: NOAEL = 64.8	Storrs and Kiesecker, 2004 (78290)	QUAL: - no raw data provided - time to mortality, relative to control, was not discussed - with exception of green frogs, sample sizes varied; sample size for late American toads was ≤ 7 animals - statistical patterns likely influenced by variable sample sizes - possible surfactant effects - suvivorship patterns observed have not been replicated in any other study - survivorship patterns expressed as survival probability; therefore, parameters such as number of days until death and overall mortality were not presented
Acute, developmental study; Atrazine technical unspecified purity	Bullfrog and American toad embryos	Eggs were exposed from fertilization to 4 days post hatch. **Atz Concs:** 28 to 4800 ug/L **Exposure:** flow through **Endpoints:** Presence of gross debilitating anomalies. **Temp**: 12-14 DegC **pH:** 7 – 7.8	Bullfrog LC_{50}: 410 ug/L American toad LC_{50}: >4800 ug/L	Birge et al., 1983. (19124)	No raw data provided and reporting deficiencies were noted. LC_{50}s were not based on mortality per se, but on abnormalities that would presumably preclude survival under natural conditions.

[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.2.4 Sublethal Effects, Freshwater Fish and Amphibians (Open Literature)

A.2.4a Sublethal Effects: Freshwater Fish (2003 IRED Data):

A number of open literature studies were reviewed as part of the 2003 IRED. The results of these studies, which are summarized below, show sublethal effects to olfaction, behavior, kidney histology, and tissue growth at atrazine concentrations ranging from 0.1 to 3000 ppb.

Adult largemouth bass (*Micropterus salmoides*) were exposed to nominal concentrations of technical grade atrazine (purity 97.1%) at 0, 25, 35, 50, 75, and 100 µg/L for 20 days to determine the potential effects on endocrine-mediated functions (Wieser and Gross, 2002) . Additionally, bass were exposed to commercial grade (purity 42.1%) atrazine at 100 µg/L. After 20 days, plasma concentrations of estradiol, 11-ketotestosterone, testosterone, and vitellogenin (a protein that serves in yolk formation) were measured. Female bass treated with 100 µg/L formulated atrazine contained significantly higher plasma estradiol and exhibited plasma vitellogenin roughly 37 times greater (260 µg/ml) than controls (7 µg/ml). Male bass treated with 100 µg/L formulated atrazine contained significantly lower plasma 11-ketotestosterone levels. While not statistically significant, plasma testosterone (286 pg/ml) was lower than controls (433 pg/ml) and plasma vitellogenin (42 µg/ml) was 7 times greater than control (6 µg/ml). Although there was considerable variability in plasma vitellogenin levels, atrazine-treated fish appeared to have elevated plasma vitellogenin relative to controls at 50 and 100 µg/L of atrazine. Plasma 11-ketotestosterone was significantly lower in fish exposed to atrazine concentrations greater than 35 µg/L. Treatment of fish with commercial grade atrazine resulted in a significant increase in plasma estradiol in female fish and a significant decrease in 11-ketotestosterone in male fish. Although not statistically significant, plasma vitellogenin in both female and male fish appeared to be increased in fish treated with technical and commercial grade atrazine.

Although high variability confounds this study's ability to resolve the effects of atrazine on plasma steroids and vitellogenesis, the study has demonstrated that technical grade atrazine affects plasma 11-ketotestosterone in males and that the formulated product affects plasma estradiol in females. The non-guideline study is classified as supplemental and provides useful information on the potential effects of atrazine (MRID 456223-04).

Effects on behavior were found to be significant ($p < 0.0001$) in zebrafish (*Brachydanio rerio*) following 1-week exposures at 5 to 3125 µg/L atrazine (Steinberg *et al*., 1995). Fish exposed to atrazine for 1-week showed a pronounced preference ($p < 0.0001$) for the dark part of the aquarium compared to the control. Because no significant differences were found between the effects at the various test concentrations (5 µg/L: 79%; 25 µg/L: 85%; 125 µg/L: 83%; 625 µg/L: 81%; 3125 µg/L: 81%), these changes in swimming behavior appears to be threshold effects. After 4 weeks at the above exposures, 15 to 24 % more of the treated fish preferred dark habitats than did the controls. The authors concluded that atrazine may have an affect on the sensory organs and the nervous system at atrazine concentrations commonly found in surface waters (MRID # 452049-10).

Saglio and Trijase (1998) measured 5 behavioral activities in goldfish following 24-hour exposures to 0.5, 5 and 50 µg/L atrazine. A number of behavioral measurements were

statistically significant ($p < 0.05$) from controls, but in most instances the significance was inconsistent and failed to show a dose-related effect. The only behavioral effect showing a consistent, dose-related effect was reduction in grouping (i.e., significant at 5 µg/L (31% reduction) and 50 µg/L (39% reduction). Other behaviors with statistically significant effects were surfacing at 5 µg/L (341% increase), burst swimming at 0.5 and 50 µg/L (1.00 and 2.25 units, respectively, the controls showed no effect). Following the introduction of skin extract, 5 µg/L of atrazine significantly ($p < 0.05$) reduced sheltering (81%) and grouping (60%), but these effects showed no consistency with effects at 0.5 and 50 µg/L. This study shows that a 24-hour exposure at 5 µg/L atrazine significantly affected aspects of swimming, positioning in water column, increased number of mouth openings at the surface, and social behaviors, allow the results of the study appear to be rather subjective. (MRID # 452029-14).

Fischer-Scherl *et al*. (1991) reported acute and chronic atrazine-induced alterations in rainbow trout kidneys affecting renal corpuscles, renal tubules, renal interstitium, and glomerular filtration. Compared to control fish, chronic 28-day exposures at 5, 10 and 20 µg/L reduced Bowman's space due to a proliferation of podocytes. At higher chronic concentrations (40 and 80 µg/L) renal corpuscles appeared hypercellular and enlarged (i.e., hypertrophy) due to a proliferation of podocytes and mesangial cells. Also, the amount of membrane-bound vesicles with varying electron-dense contents had increased in the urinary space of renal corpuscles. Fibrillar structures and fibrocytes were found around Bowman's capsule indicating beginning periglomerular fibrosis. Acute 96-hour exposures at 1.4 and 2.8 mg/L caused a more pronounced obliteration of Bowman's space due to the proliferation of mesangial cells and more renal corpuscles were affected. Increasing amounts of cellular debris accumulated in Bowman's space. Simultaneously, epithelial cells of the parietal layer of Bowman's capsule displayed an increased number of lysosomes and swollen mitochondria. Also, the number of glomerular endothelial cells exhibiting vacuolar degeneration increased. Furthermore, light microscopy shows minor alterations to renal tubules, but electron micrographs revel considerable changes. First, obvious alterations of tubules appeared at 10 µg/L. Basilar labyrinth was dilated and irregularly arranged. The mitochondria were electron-dense and showed club-shaped ends of circular structure. At 40 µg/L, part of the endoplasmic reticulum appeared foamy and fragments of endoplasmic reticulum were heavily distended. At 80 µg/L in proximal and distal tubular epithelia lysis of the cytoplasm with formation of vacuoles and vesicles and condension of mitochondria was prominent. In many tubular epithelia, only remnants of the former parallel-arranged tubular system were present, mitochondria were swollen, lysosomal structures as well as a vacuolization of the cytoplasm were detectable. In proximal tubules, lysomes had increased in number and size. At acute exposures (1,400 and 2,800 µg/L), tubular structural lesions similar to those described at 80 µg/L were present, but a distinctly higher number of renal tubules was affected. Extensive cytoplasmic vacuolization was evident and the parallel arrangement of the basilar labyrinth was completely lost, some mitochondria were dark and condensed. Tubules of the basilar labyrinth appeared foggy, partly involving mitochondria. Except for an increase in cells with mitotic figures at concentrations of 5, 10, 20 µg/L, no conspicuous alterations in basic interstitial architecture could be detected. Beginning at 40 µg/L, a loosening of the hemopoietic tissue was evident. Cells, preumably macrophages and phagocytizing material, had increased in number. In addition to these effects, sinusendothelial cells were severely damaged at a concentration of 80 µg/L. They separated from the basement membrane and exhibited numerous

vesicular and lysosomal structures as well as swollen degenerating mitochondria. Alterations in renal interstitium were considerable at acute exposures with 1,400 and 2,800 µg/L. Interstitial tissue was loosened and a state of spongiosus was indicated. Numerous macrophages were present. Nuclei of interstitial cells were pyknotic or karyorhectic, mitochondria were swollen and the cytoplasm displayed lytic areas. Cell boundaries in some parts of the interstitium were lost. Cell organelles were scarce, but lysosomal structures abundant. (MRID # 452029-07)

Davies *et al*. (1994) exposed three fish species to 0.9, 3.0, 10, 50 and 340 µg/L atrazine for a period of 10 days and measured effects on growth and properties of various tissues, such as blood, muscle and liver. Statistically significant ($p < 0.05$) effects occurred at levels as low as 0.9 and 3.0 µg/L. The most sensitive, consistent statistically significant effect was with the species *Galaxias maculatus* at 10 µg/L (i.e., 144% increase in muscle RNA/DNA levels), and the DNA levels were significantly reduced 25%. In *Pseudaphritis urvillii* consistent significant effects were found on glutathione (GSH) in the liver at 50 µg/L (24% reduction) and 340 µg/L (13% reduction). Consistent, significant effects with rainbow trout were found at 50 and 340 µg/L (i.e., reductions of 15% and 14%, respectively, in protein levels in muscle); and at 350 µg/L (159% reduction in growth and a 23% increase in glucose levels) (MRID # 452029-04).

Alazemi *et al*. (1996) reported gill damage to a freshwater fish; the damage was characterized by the presence of breaks in the gill epithelium at 500 µg/L which developed into deep pits at 5,000 µg/L (MRID 452029-05).

Hussein *et al*. (1996) exposed two Nile River fish (*Oreochromis niloticus* and *Chrysichthyes auratus*) to 3,000 and 6,000 µg/L atrazine for up to 28 days. Fish exposed to these concentrations showed some clinical signs of toxicity, such as rapid respiration and increased rate of gill cover movements; slower reflexes and swimming movements; reduction in feeding activities; and loss of equibrium and death. These signs were more pronounced in *C. auratus* than *O. niloticus*. About 25 percent of the treated fish had abdominal swelling (ascites) in the two species. Exposure to 3,000 and 6,000 µg/L resulted in significant ($p < 0.01$) decreases in the number of red blood cells (RBC), hemoglobin and haematocrit levels compared to controls in both species. While the data appear to show clear differences from controls, these conclusions could not be verified from the data given in the article . The authors also reported significant ($p < 0.01$) changes in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin (MCHC), serum components, and brain and serum AChE levels. While some of these measurements also appear to show clear differences between 3,000 and 6,000 µg/L and the controls, such as brain and serum AChE, whether the effects are significantly different than the controls could not be confirmed from the data presented in the study. (MRID # 452029-11).

Neskovic *et al*. (1993) exposed carp to atrazine concentrations of 1,500, 3,000 and 6,000 µg/L and found changes in the activity of some enzyme activity levels in serum and some organs. Serum alkaline phosphatase levels were significantly ($p < 0.05$) higher at all test levels than in controls. The greatest drop in alkaline phosphatase activity was found in the liver and ranged from 26.1% (1,500 µg/L) to 50.2% (6,000 µg/L). Somewhat weaker effects were found on glutamic-oxaloacetic (GOT) in the liver and kidney ($p < 0.1$). No statistically significant ($p <$

0.01) effects were found on glutamic-pyruvic transaminase (GPT). Histopathological effects include damage to gills (≥ 1,500 µg/L), liver (almost normal at 1,500 µg/L and vacuolization of hepatocytes at ≥ 3,000 µg/L), kidney (more or leµµg/L) and intestine (slightly greater lymphocyte infiltration and stronger mucous secretion at 6,000 µg/L) (MRID # 452029-13).

In addition, effects on olfactory function of Atlantic salmon (Salmo salar) were reported by Moore and Waring (1998) when mature male Atlantic salmon (*Salmo salar* L.) parr were exposed to nominal concentrations of 0.5, 5, 10, and 20 µg/L atrazine. Measured exposure concentrations in the study were 0.04, 3.6, 6.0 and 14.0 µg/L and represented 8, 72, 60, and 70 percent of nominal concentrations, respectively. There appears to be uncertainty about actual exposure concentrations because the water samples were collected only after the test period, and the authors concluded that atrazine in the water samples suffered rapid degradation as the result of an unavoidable delay in being analyzed (MRID # 452049-06).

A.2.4b Sublethal Effects: Freshwater Fish (New (2006) Open Literature Data)

Three open literature studies on the potential of atrazine to induce sublethal effects in fish, including salmon, rainbow trout, and channel catfish, are summarized in Table A-15. Waring and Moore (2004; Ecotox Reference # 72625) exposed salmon smolts to atrazine under flow-through conditions for 7 days. Effects on gill physiology were evaluated. Also, effects on survival from exposure in freshwater and subsequent transfer to atrazine free saltwater were evaluated. These data suggest that gill physiology, represented by changes in Na K ATPase activity and increased sodium and potassium levels, was altered at 1 ug/L and higher. In addition, transfer of fish exposed to atrazine in freshwater at 1 ug/L and higher into atrazine-free saltwater resulted in mortality; 43% mortality was observed at 5 ug/L and higher after 24 hours; 15% of fish exposed at 1 ug/L died (all controls survived). However, it is uncertain if the effects observed in this study are applicable to environmental conditions. For example, salmon were exposed to atrazine in freshwater then moved directly to full salinity sea water. It is uncertain if more gradual changes in salinity after freshwater exposures would also produce similar effects. Also, insufficient information was available for an independent evaluation of data adequacy and verification of statistical analyses. Taken together, these data provide evidence that atrazine exposure may affect gill physiology; however, toxicity values from this study are not used to quantify potential risks due to uncertainties in the correlation between the effects reported from this study in salmon and survival or reproductive effects in fish (and amphibians) considered in this assessment. There are additional uncertainties associated with the extrapolation of effects observed in the laboratory to more variable exposures and conditions in the field. Therefore, these data will be used to qualitiatvely characterize potential risks.

Moore and Lower (2001; Ecotox Reference # 67727) studied effects of simazine and atrazine and mixtures of the two triazines on pheromone-mediated endocrine function in the male salmon parr. This study suggests that short-term exposure of the olfactory epithelium of mature male Atlantic salmon parr to atrazine (1.0 ug/L) significantly reduced the olfactory response to the female priming pheromone, prostaglandin ($PGF_{2\alpha}$). After parr were exposed to atrazine, the

levels of plasma testosterone and 7,20β-dihydroxy-4-pregnen-3-one (17,20BP) were statistically elevated above the control groups. The study authors suggest that exposure resulted in modified androgen secretion within the testes. Atrazine exposure decreased the olfactory epithelium response to the amino acid L-serine. Although the hypothesis was not tested, exposure of smolts to the pesticides during the freshwater stage may potentially affect olfactory imprinting to the natal river and subsequent homing of the adults. Overall, the relationship between reduced olfactory response of males 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) in the wild is not established. In addition, EPA (2001) did not use these data in the development of aquatic life water quality criteria for atrazine because the test material was not adequately described or translated. Furthermore, the study did not determine whether the decreased response of olfactory epithelium to specific chemical stimuli would likely impair similar responses in intact fish. Therefore, the results of the study will be qualitatively discussed only.

Birge et al. (1983; Ecotox Reference # 19124) suggests that atrazine exposure to developing fish may induce abnormalities. Specific types of abnormalities associated with atrazine exposure were not listed by Birge et al. (1983), although the report notes that defects of the head and vertebral column, dwarfed bodies, partial twining, microcephaly, absent or reduced eyes and fins, and amphiarthrodic jaws were reportedly most common across the studies and species. Effect levels (e.g., EC_{50}) for incidences of abnormalities were not presented; however, the LC_{50} (calculated using mortality + terata incidence) for rainbow trout and channel catfish were 870 ug/L and 220 ug/L, respectively. Comparison to the LC_{50}s in rainbow trout in submitted studies (no studies in channed catfish have been submitted) suggests that embryo-larval stages may be more sensitive than more developed life stages of trout, which are typically exposed in acute toxicity studies. Developmental abnormalities were generally observed at atrazine levels that also induced mortality. Overall, sufficient information was not included in the study report to allow for an independent evaluation of data adequacy or verification of statistical analyses. Therefore, these data are used in a qualitative manner to provide additional characterization of potential risks to atrazine exposure in the current ecological risk assessment. These data were not used in the calculation of risk quotients.

Table A-15. Freshwater Fish Sublethal Effects Tests from Open Literature (2006 Review)					
Study type/ Test material	**Test Organism (Common and Scientific Name) and Age and/or Size**	**Test Design**	**Endpoint Concentration in ppb**	**Citation (EcoRef. #)**	**Rationale for Use in Risk Assessment[1]**
Physiological changes in gill and survival	Salmon smolts	Fish were exposed to atrazine for 7 days at atrazine concentrations of 1 – 23 ug/L under flow through conditions. Emndpoints evaluated included gill physiology and survival. **Temp**: 10-12.5 deg. C **pH:** 7.6 **Solvent:** Industrial methylated spirits	Effects on gill physiology were observed in at least one experiment at 2 ug/L and higher. Effects included altered Na K ATPase activity, increased sodium levels, and increased potassium levels. Transfer of fish exposed to atrazine in freshwater at 1 ug/L and higher into atrazine-free saltwater resulted in mortality; 43% mortality was observed at 5 ug/L and higher after 24 hours.	Waring and Moore 2004 (72625)	Qual: These data suggest that atrazine exposure in freshwater at sublethal levels in salmon smolts may compromise their ability to survive in saltwater. However, uncertainties in this study compared with field conditions, reporting deficiencies, and use of unacceptable solvent preclude its use in quantifying potential risks.
Olfactory detection of female priming pheromone, protogandin $_{F2\alpha}$ in FW fish 30 min exposure Simazine, Atrazine, and Simazine/ Atrazine mixtures (% a.i. NR)	Mature male Atlantic salmon (*Salmo salar* L.) parr; length = 140 mm; weight = 34.2 g) source: Environment Agency, Cynrig hatchery, Wales	Skin and cartilage removed to expose olfactory rosettes Olfactory epithelium perfused with control water for 30 min, then to atrazine-treated water at nominal concentrations of 0.1, 0.5, and 2.0 ug/l for 30 min [results from 0.1 ug/L not reported presumably due to lack of atrazine detection at this concentration].	Significant reduction in the priming response of male salmon to $PGF_{2\alpha}$ (increased levels of expressible milt not present following exposure to PGF) were observed at 1.0 ug/L (possible effects were also observed at 0.5 ug/L).	Moore, A., and N. Lower, 2001 (67727)	Qual: Endpoint not clearly directly relevant to assessment endpoint for freshwater fish.
Developmental study; Atrazine technical unspecified purity	Rainbow trout	Eggs were exposed for 24 days then hatchlings were exposed for 4 days at atrazine concentrations of 28 to 4800 ug/L under flow through conditions. Incidence of "gross debilitating" anomalies was evaluated. **Temp**: 12-14 DegC **pH:** 7 – 7.8	LC_{50} (combined mortality + terata incidences) in rainbow trout was 870 ug/L.	Birge et al., 1983. , (19124)	Qual: No raw data were provided and reporting deficiencies were noted. LC_{50}s were based on combination of abnormalities and mortality.

A.2.4c Sublethal Effects: Amphibians (Summary of the White Paper):

Since the January 2003 IRED, the Agency has conducted an evaluation and review of atrazine effects data on amphibian gonadal development. This information was presented in the form of a white paper for external peer review to a FIFRA Scientific Advisory Panel (SAP) in June 2003. In its white paper (EPA, 2003) dated May 29, 2003, the Agency summarized 17 studies consisting of both open literature and registrant-submitted studies involving both native and non-native frog species

(http://www.epa.gov/oscpmont/sap/2003/june/finaljune2002telconfreport.pdf). Of the 17 studies, seven were laboratory-based, and ten were field studies. All studies were individually evaluated with regard to the following parameters: experimental design, protocols and data quality assurance, strength of cause-effect and/or dose-response relationships, mechanistic plausibility, and ecological relevancy of measured endpoints.

Based on this assessment, the Agency concluded and the SAP concurred that there is sufficient evidence to formulate a hypothesis that atrazine exposure may impact gonadal development in amphibians; however, there are currently insufficient data to confirm or refute this hypothesis. Overall, the weight-of-evidence, based on review of the 17 studies, does not show that atrazine produces consistent, reproducible effects across the range of exposure concentrations and amphibians tested. Deficiencies and uncertainties associated with the reviewed studies limit their usefulness in interpreting potential atrazine effects. Specifically, the demasculinzing (i.e., decreased laryngeal dilator muscle area) effects were not replicated in multiple laboratories. Additionally, the feminizing effects (i.e., intersex, hemaphroditism, and presence of ovotestes) of atrazine were observed in three laboratory studies whose experimental designs could not be reconciled and that reported significant effects at different concentrations: one at 25 µg/L atrazine and the other two at 0.1 µg/L. While the feminizing effects observed in these different studies were consistent qualitatively, there was no consistency across the studies in the reported dose-response relationships. That inconsistency, together with the limitations in methodology in each study, does not allow a reliable determination of causality or the nature of any dose-response relationship. Although the Florida cane toads (*Bufo* marinus) monitored in the field exhibited both demasculinizing effects (genetic males with female coloration) and feminizing effects (oogenesis in male Bidder's organ), there were insufficient data to conclusively link atrazine exposure to the phenomena. Thus, the available data do not establish a concordance of information to indicate that atrazine will or will not cause adverse developmental effects in amphibians.

Because of the inconsistency and lack of reproducibility across studies and an absence of a dose-response relationship in the data, the Agency determined that the conclusions reached in the January 2003 IRED regarding uncertainties related to atrazine's effects on amphibians have not changed. The SAP supported EPA in seeking additional data to reduce uncertatinties regarding potential risk to amphibians (Scientific Advisory Panel, 2003). The data collection for additional amphibian toxicity data has followed the multi-tiered process outlined in the Agency's white paper presented to the SAP. In addition to addressing uncertainty regarding the potential of atrazine to cause these effects, these studies will be helpful in characterizing the nature of any potential dose-response relationship. A data call-in for the first tier of amphibian studies was issued in 2005, and the studies are currently underway, although not yet complete. Therefore, the results of the amphibian toxicity testing, which are expected to become available in 2007, are not available for inclusion in this endangered species risk assessment.

A.2.4d Sublethal Effects: Amphibians (New Open Literature Data)

Open literature data on sublethal effects of atrazine to amphibians, including frogs and salamanders, are summarized in Tables A-16 and A-17 and discussed in the following subsections. The following information includes studies identified as part of the 2006 open literature search that were not reviewed as part the white paper discussed above.

Frogs (Anurans)

A total of eight studies on potential sublethal effects of atrazine to frogs were reviewed as part of the open literature. Four of the eight studies were classified as acceptable to use in qualitative sense and the other four were classified as unacceptable. Two of the four qualitative studies are microcosm/mesocosm tests (one of which includes data for both frogs and salamanders), and two are chronic lab studies. A review of the qualitative studies is provided below and summarized in Table A-16. Studies were classified as qualitative because they address issues of concern to the risk assessment, but are not appropriate for quantitative use due to uncertainties related to a lack of raw data and limitations in the study design. In summary, the microcosm/mesocosm and chronic lab data for frogs indicate that sublethal effects to amphibians, such as reduced mass and length at metamorphosis, may occur at exposure concentrations of approximately 200 ppb and higher under the conditions tested. Decreased frog weight (and length) at metamorphosis is hypothesized to result from atrazine's effect on algal populations, which are a primary source of food for developing anurans. Other factors, such as decreasing DO, pH, and macrophyte biomass following atrazine exposure may also contribute to observed sublethal effects. In the lab, plasma testosterone was reduced in male frogs at atrazine concentrations of 259 ppb; however, an increase in aromatase activity (aromatase increases synthesis of 17β-estradiol resulting in depletion of testosterone levels) was not observed. Therefore, the mechanism associated with decreased testorsterone levels in adult males is unclear. The observed effect level of ~200 ppb is greater than the aquatic community-level effect of 10-20 ppb documented in the 2003 atrazine IRED. In addition, uncertainties and associated limitations in the design of the reviewed studies are similar to the conclusions of the amphibian white paper.

The effects of technical grade atrazine (% ai unspecified) on survival, mass, and length at metamorphosis, and days to metamorphosis of larval gray tree frogs (*Hyla versicolor*) inhabiting artificial pond microcosms was studied by Diana et al. (2000; Ecotox Reference # 59818). The interrelationship of these parameters and DO concentrations, water pH, and estimates of phytoplankton, periphyton, and macrophyte biomass were also evaluated. Gray tree frog larvae (40 larvae/treatment; 4 replicates/treatment) were exposed to nominal atrazine concentrations of 0, 20, 200, and 2000 ppb atrazine in artifical pond microcosms (16 plastic wading pools; 1.22-m diameter w/ 90 L pond water) containing phytoplankton, periphyton, and the aquatic macrophyte, marshpepper knotweed (*Polygonum hydropiper*). Microcosms were covered with mesh fiber to exclue predators. Concentrations of atrazine measured in microcosms immediately following addition were consistent with those intended and showed minimal variation within treatment groups. By three weeks following addition of atrazine to the microcosms, concentrations had declined by 21%, 9%, and 16% in the 20-, 200-, and 2000-ppb treatment groups, respectively. Phytoplankton chlorophyll *a* concentrations declined slightly during the

first week following atrazine addition (in all but the 200 ppb group) and, by Day 14, rebounded above levels before exposure (in all but the 20 ppb group). Phytoplankton densities in the 200 and 2000 ppb groups increased significantly above the control during the rebound period. Over the course of study (~40 days), chlorophyll *a* was lowest in control, highest in 200 ppb, and intermediate in 20 and 2000 ppb groups. Macrophyte biomass at the end of the study was decreased, relative to controls, by 30%, 98%, and 99% in the 20, 200, and 2000 ppb groups, respectively. DO decreased to approximately 20 and 40% of pre-exposure values in the 200 and 2000 ppb treatment groups after 1 d of atrazine treatment. DO in these microcosms returned to control concentrations by 10 d after treatment, but declined again to approximately 60 to 80% of control values at 21 d after treatment and remained depressed for the remainder of the study. In the 200 and 2000 ppb groups, pH decreased similarly within 1 d of atrazine treatment and returned to control values after 16 d. The DO and pH did not differ significantly between the 0 and 20 ppb groups or the 200 and 2000 ppb groups. Frogs from the two higher treatment groups were statistically shorter (5% reduction) and had lower body weight at metamorphosis (10% reduction) than those from the control and low atrazine groups. No difference in length or body mass at metamorphosis was detectable between the 0 and 20 ppb groups or between the 200 and 2000 ppb groups. Time to metamorphosis was 5% longer in the 2000 ppb groups than in the 200 ppb group, but did not differ statistically from controls in any treatment group. No significant treatment-related differences were detected for survival rate. Given the lack of decrease in phytoplankton over time and the subsequent compensatory growth of phytoplankton following atrazine treatment, it seems unlikely that the effects on amphibian development were due to a decrease in food. However, the study author's postulate that atrazine-resistant species occurring in presence of continued atrazine exposure may be less palatable, of lower nutritive value, or toxigenic. The observed rebound of phytoplankton was likely due to elimination of macrophytes. Given the modest decline in phytoplankton biomass and the marked effects of atrazine on DO, it appears likely that the adverse effects on amphibian growth are mediated primarily by decreased oxygen availability. Other amphibian larval species have shown increased effort at gill respiration in the presence of low DO at the expense of feeding. Based on observed decreases in length and mass at metamorphosis, and decreases in pH, DO, and macrophyte biomass, the study authors suggest that these variables may lead to increased risks of predation as well as decreased fitness to anurans at $\geq$ 200 ppb atrazine. The corresponding NOAEC for this study, based on decreased length and mass, is 20 ppb.

Boone and James (2003; Ecotox Reference # 81455) studied the post-application effects of atrazine on body mass development, and survival of two anuran species (southern leopard frog, *Rana sphenocephala*, and American toad, *Bufo americanus*) and two caudate species (spotted salamander, *Ambystoma maculatum*, and small-mouthed salamander, *A. texanum*) reared in outdoor cattle tank mesocosms containing leaf litter and plankton from natural ponds. Screen-mesh lids covered each pond to exclude predators and other anurans. Animals used in the study were free-swimming larvae. Natural factors of density and pond hydroperiod were also considered. Atrazine was added as Aatrex (40.8% ai) at only one concentration of 200 ppb (mean-measured concentration at Day 1 was 197 ppb). Atrazine (at 197 ppb) reduced chlorophyll concentration of algal communities and resulted in reduced mass (for toads and leopard frogs) and lengthened larval periods (for small-mouthed salamanders). While the presence of atrazine did not cause mortality from reductions in food, it did statistically reduce

metamorph size (i.e, weight). During metamorphosis, salamander larvae lose their gills and develop lungs that enable it to breathe air. Because size at metamorphosis has been positively correlated with overwinter survival and future reproduction, atrazine may affect population dynamics when it reduces metamorph size. Atrazine also interacted with density and decreased leopard frog survival as compared to the high density (60 tadpoles/1000 L) control group. According to the study author's, this observation suggests that atrazine reduced the food supply of leopard frog tadpoles to some extent and increased the likelihood of starvation in high-density conditions where food was scarcer.

Hecker et al. (2005; Ecotox Reference # 79287) studied the effects of atrazine (97% ai) on CYP19 gene expression, aromatase activity, plasma sex steroid concentrations including testosterone (T) and 17β-estradiol (E2), and gonad size (GSI) of adult sexually mature male African clawed frogs (*Xenopus laevis*) in the lab for 36 days under static renewal conditions. Adult male frogs in 40-L aquariums (15 reps/treatment; 20 reps/control) were exposed to atrazine at nomimal concentrations of 1, 25, or 250 ppb; respective measured concentrations were 0.8, 24.6, and 259 ppb. There were no effects on any of the parameters measured, except plasma T concentrations, which were significantly less (54 % reduction) in the 259 ppb group as compared to untreated frogs. No significant increase in aromatase activity was observed; therefore, the mechanism associated with decreased testorsterone levels in adults males has not been demonstrated. The extent to which the suppression of T observed in frogs exposed to 250 ppb atrazine may affect reproductive functions in the wild is unclear. The authors concluded that aromatase enzyme activity and gene expression were at basal levels in *X. laevis* from all treatments, and that the tested concentrations of atrazine did not interfere with steroidogenesis through an aromatase-mediated mechanism of action.

Gucciardo (1999; summarized in Table A-16) exposed three frog species to technical grade atrazine at concentrations ranging from 30 to 600 ug/L from the first feeding stage through metamorphosis and evaluated potential effects on growth and development rate. Atrazine exposure to *A. crepitans* at 300 ug/L was associated with delayed development (increased time to metamorphosis) and reduced post metamorphic dry weight. No effects on the other two frog species tested (*R. sylvatica* and *R. pipiens*) were observed. This study did not produce an effect level more sensitive than than the NOAEC of 65 ug/L observed in submitted chronic fish studies.

Table A-16. Frog Toxicity Tests from Open Literature (2006 Review)					
Study type/ Test material	**Test Organism (Common and Scientific Name) and Age and/or Size**	**Test Design**	**Endpoint Concentration in ppb (significant changes as compared to control)**	**Citation (EcoRef. #)**	**Rationale for Use in Risk Assessment[1]**
Amphibian Microcosm Study (duration = 6 wks) / TGAI Atrazine (% ai NR) w/acetone solvent	- Larval gray tree frogs (*Hyla versicolor*) 15 d old and 11 d posthatch - Aquatic macrophyte marshpepper knotweed (*Polygonum hydropiper*)	- Artifical pond microcosms (16 plastic wading pools, 1.22 m diameter) w/ 90 L pond water (including phytoplankton & macrophytes) used. 5 macrophytes were added to each pool. - Treatment levels (nominal conc) = 0, 20, 200, and 2000 ppb (and solvent control) - 40 larvae/treatment; 4 reps/treatment - Microcosms covered to exclude predators. - Endpoints: Survival, mass, and length at metamorphosis; days to metamorphosis; relationshiop of amphibian endpoints to DO, pH, and estimates of phytoplankton, periphyton, and macrophyte biomass	- Survival: no effect; NOAEC = 2000 ppb - Mass: 10% reduction ($p < 0.001$) at 200 ppb (LOAEC); NOAEC = 20 ppb - Length: 5% reduction ($p < 0.001$) at 200 ppb (LOAEC); NOAEC = 20 ppb - Larval period: no effect; NOAEC = 2000 pbb	Diana et al., 2000 (59818)[2]	QUAL: - no raw data provided - pre-metamorphosis weight and length were not determined

Table A-16. Frog Toxicity Tests from Open Literature (2006 Review)

Study type/ Test material	Test Organism (Common and Scientific Name) and Age and/or Size	Test Design	Endpoint Concentration in ppb (significant changes as compared to control)	Citation (EcoRef. #)	Rationale for Use in Risk Assessment[1]
Amphibian Mesocosm Study (duration 56-58 d) / Atrazine formulation (Aatrex, 40.8%ai)	Free-swimming larvae of 2 anuran species and 2 caudate species: - Southern leopard frog (*Rana sphenocephala*) - American toad (*Bufo americanus*) - Spotted salamander (*Ambystoma maculatum*) - Small-mouthed salamander (*A. texanum*) - phytoplankton	- Mesocosm design: polyethylene cattle tank ponds (1.85 m in diameter; 1480 L volume) containing 1000 L tap water, 1 kg leaf litter from mixed deciduous forest, and plankton from natural pond (500 mL/pond at 6 times). - Mesh lids covered each pond to exclude predators and anuran colonists - Atrazine added at nomimal conc of 200 ppb and control (Day 8 pH = 7.7; temp = 13.3 °C) mean-measured concentration at Day 1 exposure = 197 ppb - 3 reps/treatment - Anuran low density = 20 tadpoles/1000 L; high density = 60 tadpoles/1000 L - Hydroperiod manipulated: constant or drying - Anuran and caudate species reared separately and together - Endpoints: body mass, developmental stage, SVL (for salamander larvae only), pond survival for all species, time to metamorphosis for toad and small-mouthed salamander, chlorophyll *a* content	<u>Leopard frog</u> - survival and developmental stage: no effect; NOAEC = 197 ppb - survival x high density: decrease relative to high density control w/no atrazine (p = 0.0235); LOAEC = 197 ppb; NOAEC = <197 ppb - mass: decreased at 197 ppb (LOAEC) (p = 0.0052); NOAEC = <197 ppb <u>American toad</u> -survival and time to met: no effect; NOAEC = 197 ppb -mass: decreased at 197 ppb (LOAEC) (p = 0.0040); NOAEC = <197 ppb <u>Spotted salamander</u> - no effect to survival, mass, SVL, and dev. stage; NOAEC = 197 ppb <u>Small-mouthed salamander</u> -survival and mass: no effect; NOAEC = 197 ppb -mass x hydroperiod: decreased during drying periods (p = 0.0202); LOAEC = 197 ppb; NOAEC = <197 ppb - time to met: increasing w/atrazine exp (p = 0.0084) and combination of atrazine exp and hydroperiod (p = 0.0093); LOAEC = 197 ppb; NOAEC = <197 ppb <u>Chlorophyll *a*:</u> reduced at 12 h at 197 ppb (p = 0.0006)	Boone and James, 2003 (81455)	QUAL: - no raw data provided - only one concentration of atrazine tested - % difference in effect of atrazine relative to control not presented - tap water used in all control and treatment test solutions; however, the chlorine content of the tap water is not specified

Table A-16. Frog Toxicity Tests from Open Literature (2006 Review)					
Study type/ Test material	**Test Organism (Common and Scientific Name) and Age and/or Size**	**Test Design**	**Endpoint Concentration in ppb (significant changes as compared to control)**	**Citation (EcoRef. #)**	**Rationale for Use in Risk Assessment[1]**
Chronic (36 d) lab study / Atrazine (97.1% ai)	African clawed frog (*Xenopus laevis*); adult sexually mature males (30-50 g)	- 40-L aquariums (10-L exposure solution). - Static renewal (50% test solution renewed every 3 days) at nominal concentrations of 0, 1, 25, and 250 ppb. Measured conc (after 36 days = ND, 0.8, 24.6, and 259 ppb) - 15 reps/treatment; 20 reps for the contol. - Temp = 19.6 ^{o}C $\pm$1.3 ^{o}C - Photoperiod: 12 h light: 12 h dark - Feeding: Nasco frog brittle 3x/wk ad libitum - Endpoints: Testicular aromatase activity, CYP19 gene expression, concentrations of plasma sex steroids testosterone (T) and 17β-estradiol (E2), and gonad size (GSI)	- T concentration: 54% decrease (p = 0.036) at 259 ppb (LOAEC); NOAEC = 24.6 ppb - Testicular aromatase activity, CYP19 gene expression, E2 concentration, and GSI: no effect; NOAEC = 259 ppb	Hecker et al., 2005 (79287)	QUAL: - no raw data provided - mechanism associated with suppression of T is unclear because aromatase activity was not increased - extent to which suppression of T may affect reproductive functions in wild is unclear
Chronic lab study / atrazine (99% pure)	Cricket frogs (A. crepitans), wood frogs (R. sylvatica), Northern leopard frogs (R. pipiens)	Tadpoles were exposed to 30, 300, or 600 ug/L atrazine from the first feeding stage through metamorphosis. Growth rate, days to metamorphosis, metaphorphic success, and juvenile weight and length were evaluated.	A statistically significant ($p<0.05$) delay in time to metamorphosis and decrease in post metamorphic dry weight was observed in *A. crepitans* at 300 ug/L and above. No effects on the other two frog species tested were observed. A Frog Embryo Teratogenesis Assay-Xenopus resulted in a 96-hr EC50 of 13.4 mg/L.	Gucciardo, 1999 (78286)	QUAL: Study did not produce the most sensitive endpoint and was not GLP; however, the study appears to be well reported and well conducted. Not all raw data were included in the report.

[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.
[2] Also reviewed as a field study. Phytoplankton density and chlorophyll a concentrations increased over the study duration (~40 days); however, macrophyte biomass was decreased, relative to controls by 30%, 98%, and 99% in the 20, 200, and 2000 ppb groups. DO decreased to 60% and 80% of control at 21 days and remained depressed for study duration. pH decreased w/in 1 day of exposure in 200 and 2000 ppb groups, but returned to control values following 16 days.
NR = Not reported.

The following four open literature frog toxicity studies were classified as invalid:

1. Sullivan and Spence, 2003 (Ecotox Reference # 68187; chronic lab study): Classified as invalid because acetone was added to all atrazine treatment groups; however, no solvent control was tested.

2. Jooste et al., 2005 (Ecotox Reference # 79286; microcosm study): Classified as invalid due to the presence of testicular oocytes in the reference control (57%) relative to the atrazine treatment groups (39-59%).
3. Coady et al., 2004 (Ecotox Reference # 78295; chronic lab study): Classified as invalid because atrazine was detected in the control sample.
4. Coady et al., 2005 (Ecotox Reference # 81457; chronic lab study): Classified as invalid because atrazine was detected in the control sample.

Salamanders (Caudates)

A total of five studies on potential sublethal effects of atrazine to salamanders were reviewed as part of the open literature. A discussion of these studies is provided below and summarized in Table A-17. One of the five studies was classified as invalid. Of the remaining four studies, one is a mesocosm study (including data for both frogs and salamanders), and the other three are chronic lab studies. All of the test species in the reviewed open literature studies were salamanders in the Ambystomatidae family or mole salamanders. Eggs of the Ambystomatidae family hatch in the water into larvae that metamorphose into terrestrial adults. During metamorphosis, the feathery external gills of the aquatic larvae are resorbed and lungs develop in the adult terrestrial form. All reviewed studies were classified as acceptable for qualitative use because they address issues of concern to the risk assessment, but are not appropriate for quantitative use due to uncertainties related to a lack of raw data and limitations in the study design. In summary, the reviewed studies contain variable results with respect to atrazine exposures and sublethal effects to salamanders. Two chronic studies on the streamside salamander (*A. barbouri*) and long-toed salamander (*A. macrodactylum*) show significant reduced mass and snout-vent length (SVL) at metamorphosis, in addition to significantly accelerated metamorphosis, relative to controls, at atrazine concentrations ranging from 184 to 400 ppb. The NOAEC values for these studies range between 18.4 and 40 ppb. In another study, the time to metamorphosis was increased in small-mouthed salamanders at the only concentration of atrazine tested (197 ppb); however, no effect in the time to metamorphosis was observed in spotted salamanders (*Ambystoma maculatum*) at the same concentration of atrazine. The interaction of atrazine and one of the iridoviruses (tiger salamander, *Ambystoma tigrinum* virus, [ATV]) was studied in long-toed salamanders. ATV is an emerging iridovirus responsible for epizootics in tiger salamanders through out western North America. Larvae exposed to both atrazine and ATV had lower levels of mortality and ATV infectivity compared to larvae exposed to virus alone, suggesting that atrazine may compromise virus efficacy or improve salamander immune competency. Behaviorial changes in locomotion (i.e., increased activity following tapping on tanks) were observed in streamside salamanders exposed to 400 ppb; however, this endpoint is not relevant to the assesssment endpoints chosen for this risk assessment. It is unclear how increased larval salamander activity due to tank tapping in the lab would translate into reduced fitness in the wild. Conversely, increased larval activity could result in an increase in predator avoidance.

The Boone and James (2003) mesocosm study, previously described and summarized in Table A-16, studied the post-application effects of one concentration of atrazine (197 ppb) on body

mass, development, and survival of two larval salamander species including the spotted and small-mouthed salamanders. There were no effects on survival, mass, SVL, and developmental stage of the spotted salamander following exposure to atrazine; however, the larval period of the small-mouthed salamander was statistically lengthened at 197 ppb atrazine as compared to the controls. According to the study authors, lengthened larval periods for salamanders may be a result of atrazine increasing energy required for growth and development, although the mechanism is not clear. Atrazine also interacted significantly with the hydroperiod treatment (i.e., constant or drying), affecting both time and mass to metamorphosis and resulting in longer larval periods in constant hydroperiods and smaller mass at metamorphosis in drying hydroperiods.

Rohr et al. (2003; Ecotox Reference # 71723) exposed streamside salamander (*A. barbouri*) embryos and larvae to atrazine (80% ai) for 37 days at nominal concentrations of 4, 40, and 400 ppb in the presence and absence of food. No effect on embryo or larval survival, hatching, or growth (i.e., mass, SVL, and limb deformities) rates were observed at any of the test concentrations. Systematically tapping of the tanks using a spring-loaded mousetrap caused greater activity (observed as movement following the disturbance) in larvae exposed to 400 ppb atrazine. The study authors attributed this startle response to a nervous system malfunction; however, the reported malfunction is not statistically documented. In addition, the locomotion behavioral endpoint is not relevant to the assessment endpoints chosen for this risk assessment. Hunger stimulated a decrease in refuge use and an increase in activity; however this response was least pronounced in the larvae exposed to atrazine at 400 ppb.

In 2004, Rohr et al. (Ecotox Reference # 81748) studied the combined effects of food limitation and drying conditions on the survival, behavior, and metamorphosis of the streamside salamander from embryo stage through metamorphosis at nominal atrazine concentrations of 4, 40 and 400 ppb. In general, food and atrazine levels did not interact statistically. Exposure to 400 ppb atrazine decreased embryo survival to Day 16 and increased time to hatching. However, most embryo mortality was associated with a white film covering the embryo, suggesting the presence of a fungal pathogen. It is unknown whether the fungi caused or simply followed mortality. Delayed hatching could prolong time in streams and result in mortality from stream drying or from aquatic predation. Drying conditions and food limitation decreased larval survival, while 400 ppb atrazine only reduced larval survival in one of the two years tested. The study author attributes the difference between the years in atrazine-related mortality to possible condition-dependent mortality. Sublethal effects included elevated activity and reduced shelter use associated with increasing atrazine conc (400 pbb) and food limitation. Although atrazine-induced reduction in refuge use and increase in activity did not appear to strongly influence feeding rates, they may elevate predation risk by increasing conspicuosness and encounters with predators. Larval period was lengthened by food limitation and shortened by 400 ppb atrazine. Earlier metamorphosis may provide a benefit to atrazine-exposed animals by reducing exposure; however, their smaller size at metamorph could result in lower terrestrial survival, lower reproduction and compromised immune function. Drying conditions accelerated metamorphosis for larvae exposed to 0 and 4 ppb atrazine, but did not affect metamorphosis timing for the 40 or 400 ppb groups. Therefore, combined effects of stream drying and atrazine exposure may not pose a greater threat to salamander larvae than either factor alone. Food limitation, drying

conditions, and 400 ppb of atrazine reduced size at metamorphosis without affecting body condition (relationship between mass and length), even though feeding rates did not differ significantly among atrazine concs at any time during development. The authors suggest that food limitations, drying conditions and atrazine exposure (at 400 ppb) have the potential to contribute to decreased amphibian populations in impacted systems because atrazine levels of 400 ppb may result in increased larval energy expenditures, and reduced the feeding duration due to a shortened larval period. The authors also suggest that because smaller size at metamorphosis may result in lower terrestrial survival and lifetime reproduction,

Recent studies suggest that agricultural contaminants, such as atrazine, may have suppressive effects on the amphibian immune system, thereby increasing susceptibility to parasites and pathogens such as iridoviruses in the genus *Ranavirus* and the chytrid fungus (*Batrachochytrium dendrobatidis*). A study by Forson and Storfer (2006; Ecotox Reference # 82033) tested the interaction of emerging infectious diseases and atrazine (86.5% ai) in long-toed salamanders (*A. macrodactylum*). 6-week old long-toed salamanders were exposed to *Ambystoma tigrinum* virus (ATV; 0 or $10^{3.5}$ plaque-forming units/ml) and sublethal concentrations of atrazine (0, 1.84, 18.4, and 184 ppb) in a 4x2 factorial design for 30 days. The effects of atrazine and the virus were tested on weight and snout-vent length (SVL) at metamorphosis and length of larval period as well as on rates of mortality and viral infectivity. ATV transmission was confirmed, although infection rates were lower than expected, consistent with the theory predicting lower pathogen transmission to nonnative hosts. Larvae exposed to both atrazine and ATV had lower levels of mortality and ATV infectivity (13.3% across all 3 atrazine concentrations) compared to larvae exposed to virus alone (25%), suggesting atrazine may compromise virus efficacy or improve salamander immune competency. The highest atrazine level (184 ppb) accelerated metamorphosis and reduced mass and SVL at metamorphosis relative to controls. The authors suggest that the mechanism for this effect may be an alteration of the neuroendocrine stress pathway involving the thyroid hormones and corticoid hormones. Exposure to ATV also significantly reduced SVL at metamorphosis. Atrazine alone had no significant effect on mortality. The study suggests moderate concentrations of atrazine may ameliorate ATV effects on long-toed salamanders, whereas higher concentrations initiate metamorphosis at a smaller size, with potential negative consequences to fitness. Larger size at metamorphosis is correlated with higher survival to maturity and reduced time to maturity, thereby increasing fitness relative to smaller individuals. The study authors suggest that smaller size at metamorphosis may be a fitness cost resulting from high-level atrazine exposure. Lighter, smaller animals may have reduced terrestrial locomotor performance and, therefore, reduced ability to avoid predators or capture prey. Smaller, newly metamorphosed adults also tend to have weakened immune systems, which could make them more susceptible to disease.

Table A-17. Salamander Toxicity Tests from Open Literature (2006 Review)					
Study type/ Test material	**Test Organism (Common and Scientific Name) and Age and/or Size**	**Test Design**	**Endpoint Concentration in ppb (significant changes as compared to control)**	**Citation (EcoRef. #)**	**Rationale for Use in Risk Assessment[(1)]**
Chronic (37 d) lab study / Atrazine (80% ai)	Streamside salamander (*Ambystoma barbouri*) embyos tracked through larval development	- Static renewal (50% test solution renewed every other day) - Tested in 3.7 L glass bowls containing submerged, translucent, gray semicircular glass refuge plate - Treatment levels (nominal conc) = 4, 40, and 400 ppb including DMSO solvent (and solvent control containing DMSO and acetone) - 10 embryos/bowl; 4 reps/ treatment level - Temperatue = 15 ^{o}C - Photoperiod = 12:12 h light:dark - Feeding: larvae fed live blackworms (*Lumbriculus variegates*) ad libitum - Endpoints: Larval behavior in presence and absence of food, growth (mass and snout-vent length [SVL]), and development (limb deformities); hatching; and survival	- Survival: no effect; NOAEC = 400 ppb - Growth (mass and SVL): No effect; NOAEC = 400 ppb - Hatching: no effect; NOAEC = 400 ppb - Behavior: Systematic tapping of tanks caused greater activity ($p < 0.05$) in larvae exposed to 400 ppb (LOAEC); NOAEC = 40 ppb	Rohr et al., 2003 (71723)	QUAL: - no raw data provided - solvent control contained both DMSO and acetone, whereas the atrazine treatment groups contained DMSO only. - DMSO is not an acceptable solvent because it accelerates movement of a chemical across cell membranes; therefore, it represents a worst case scenario - the locomotion behavior endpoint is not relevant to the chosen assessment endpoints for this risk assessment

Table A-17. Salamander Toxicity Tests from Open Literature (2006 Review)					
Study type/ Test material	**Test Organism (Common and Scientific Name) and Age and/or Size**	**Test Design**	**Endpoint Concentration in ppb (significant changes as compared to control)**	**Citation (EcoRef. #)**	**Rationale for Use in Risk Assessment[1]**
Chronc (~117 d) lab study / Atrazine (80% ai)	Streamside salamander (*Ambystoma barbouri*) Embryos through metamorphosis	- Static renewal (50% test solution renewed every other day) - Tested in aquaria (37 L) wrapped in black plastic, containing refuge plates and a strip of refuge above the water line - Treatment levels (nominal conc) = 4, 40, and 400 ppb w/DMSO solvent (included DMSO solvent, but no negative control) - 31-40 embryos/aquaria; 6 reps/treatment - Temperatue = 15 ºC - Photoperiod = 12:12 h light:dark - Feeding: 50% larvae fed live blackworms ad libitum (high food); 50% rationed 2.24 g 2x/wk (low food) - Hydroperiods: constant or lowered water level -Endpoints: embryo hatching and survival to Day 16, larval survival, larval activity and refuge use, and metamorphosis (mass, SVL, and time to met)	- Embryo hatching and survival: both reduced at 400 ppb ($p < 0.001$); LOAEC = 400 ppb; NOAEC = 40 ppb - Larval survival: no effect in 2002; in 2003, survival was reduced at 400 ppb ($p = 0.003$); LOAEC = 400 ppb; NOAEC = 40 ppb -Larval refuge use: lower at 400 ppb ($p < 0.034$); LOAEC = 400 ppb; NOAEC = 40 ppb -Larval activity: higher at 400 ppb ($p = 0.007$); LOAEC = 400 ppb; NOAEC = 40 ppb -Mass at met: reduced at 400 ppb (ppb ($p = 0.022$); LOAEC = 400 ppb; NOAEC = 40 ppb - Time to met: shortened at 400 ppb (ppb ($p = 0.006$); LOAEC = 400 ppb; NOAEC = 40 ppb -SVL at met: reduced at 400 ppb (ppb ($p = 0.022$); LOAEC = 400 ppb; NOAEC = 40 ppb	Rohr et al., 2004 (81748)	QUAL: -There is uncertainty associated with the effect on embryos and hatched larvae because of the presence of a white film covering the embryo, suggesting a fungal pathogen, which may have decreased survival and increased time to hatching - Effects on larval survival were different for 2002 (no effect) and 2003 (significant effect for 400 ppb treatment compared to control) - Metamorphic parameters for 2003 included outliers and were not included in the analyses - Duration of study not specified - No raw data provided - DMSO is not an acceptable solvent because it accelerates movement of a chemical across cell membranes - No negative control tested

Table A-17. Salamander Toxicity Tests from Open Literature (2006 Review)					
Study type/ Test material	**Test Organism (Common and Scientific Name) and Age and/or Size**	**Test Design**	**Endpoint Concentration in ppb (significant changes as compared to control)**	**Citation (EcoRef. #)**	**Rationale for Use in Risk Assessment[1]**
Chronic (30 day) lab study / Atrazine 90DF (86.5% ai)	Long-toed salamander (*Ambystoma macrodactylum*) 6-weeks old	- Static renewal (water changed every 3 days) -Tested in round, polyethylene containers (12.7 x 7.62 cm) containing 500 ml artesian spring water - Treatment levels (nominal conc = 0, 2, 20, and 200 ppb); measured conc = 0, 1.84, 18.4, and 184 ppb - Also exposed to *Ambystoma tigrinum* virus (ATV; 0 or $10^{3.5}$ plaque-forming units/ml) - Factorial 4x2 design - Temperatue = 20 $\pm$ 1 ºC - Photoperiod = 15:9 h light:dark to mimic natural conditions - Feeding: larvae fed live blackworms 2x/wk ad libitum - Endpoints:mass and SVL at metamorphosis, larval period, mortality, and viral infectivity	- Larval period accelerated ($p = 0.046$); mass ($p = 0.002$) and SVL ($p < 0.001$) at met. reduced at 184 ppb; LOAEC = 184 ppb; NOAEC = 18.4 ppb - Mortality: no effect; NOAEC = 184 ppb - Mortality and ATV infectivity: lower in larvae exposed to both atrazine and ATV (13.3% across all 3 atrazine conc) as compared to larvae exposed to virus alone (25%)	Forson and Storfer, 2006 (82033)	QUAL: - No raw data provided

(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.

The salamander open literature toxicity study by Larson et al., 1998 (Ecotox Reference # 60632; chronic lab study) was classified as invalid because atrazine was detected in the control sample.

A.2.5 Freshwater Invertebrates, Acute

A freshwater aquatic invertebrate toxicity test using the TGAI is required to establish the toxicity of atrazine to aquatic invertebrates. The preferred test species is *Daphnia magna*. Results of this test and others are summarized below in Table A-18.

Table A-18. Freshwater Invertebrate Acute Toxicity

Surrogate Species/ Static or Flow-through	**% ai**	**96-hour LC_{50}/EC_{50} µg/L (ppb) (measured/nominal)**	**Toxicity Category**	**MRID No. Author/Year**	**Study Classification**
Midge (*Chironomus tentans*) Static test	94	**720** (nominal)	highly toxic	000243-77 Macek *et al*. 1976	Supplemental (48-hour LC50 & raw data are missing)
Midge (*Chironomus riparius*)	85.5	1,000 (unknown)	highly toxic	450874-13 Johnson 1986	Supplemental (raw data are missing)
Waterflea (*Daphnia magna*)	85.5	3,500 (unknown)	moderately toxic	450874-13 Johnson 1986	Supplemental (raw data are missing)

Table A-18. Freshwater Invertebrate Acute Toxicity

Waterflea < 24-hours old (*Daphnia magna*) 26-Hour static test	??	3,600 (unknown)	at least moderately toxic	000028-75 Frear & Boyd 1967	Supplemental (unknown ai, 26-hour test & no raw data)
Waterflea (*Ceriodaphnia dubia*) 48-Hour static test	97	> 4,900 (measured) Slope - no mortality	unknown	452083-09 Jop 1991	Supplemental (EC50 value not determined)
Scud (*Gammarus fasciatus*) Static test	94	5,700 (nominal)	moderately toxic	000243-77 Macek *et al*. 1976	Supplemental (48-hour LC50 & raw data are missing)
Stonefly (nymph) (*Acroneuria* sp.) Flow-through test 67.4 mg/L $CaCO_3$	98.5	6,700 (measured)	moderately toxic	Brooke 1990	Supplemental (study not seen; OW in draft WQC)
Waterflea (*Daphnia magna*) Static test	94	6,900 (nominal)	moderately toxic	000243-77 Macek *et al*. 1976	Supplemental (raw data are missing)
Scud juvenile (*Hyalella azteca*) Flow-through test 67.4 mg/L Ca CO_3	98.5	14,700 (measured)	slightly toxic	Brooke 1990	Supplemental (no study; cited by OW in draft WQC)
Scud juvenile (*Gammarus pulex*) Static-renewal - daily	??	14,900 (measured) 4.4 @ 10 days	slightly toxic	452029-17 Taylor, Maund & Pascoe 1991	Supplemental (raw data are missing)
Leech (*Glossiphonia complanata*) Static-renewal weekly	99.2	> 16,000 (measured) 6,300 µg/L @ 28 days	slightly toxic	452029-16 Streit & Peter 1978	Supplemental (raw data are missing)
Leech (*Helobdella stagnalis*) Static-renewal weekly	99.2	> 16,000 (measured) 9,900 µg/L @ 27 days	slightly toxic	452029-16 Streit & Peter 1978	Supplemental (raw data are missing)
Snail (*Ancylus fluviatilis*) Static-renewal weekly	99.2	>16,000 (measured) > 16, 000 µg/L @ 40 days (35 % mortality)	slightly toxic	452083-05 Oris, Winner & Moore 1991	Supplemental (raw data are missing)
Waterflea <12 hr old (*Ceriodaphnia dubia*) Static 48-hour test 57 mg/L $CaCO_3$	> 99	> 30,000 (measured) Slope - no data	slightly toxic	452029-17 Taylor, Maund & Pascoe 1991	Supplemental (raw data are missing)
Midge (*Chironomus riparius*) Static-renewal - daily 10-Day test	??	> 33,000 (measured) 18,900 µg/L @ 10 days	slightly toxic	000272-04 Drake 1976	Supplemental (raw data are missing) (EC_{50} 115 ppm exceeds water solubility (33 ppm)
Midge (*Chironomus tentans*) Flow-through 10-Day test; water-spiked exposure	98.5	Mortality: LC_{50} > 24,000 (measured) (37% mortality) NOAEC = 16,000 LOAEC = 24,000 Growth (dry weight): EC_{50} = 8,300 (measured) NOAEC <3,200 LOAEC = 3,200	slightly toxic	459040-01 Putt, 2002	Supplemental (does not fulfill any currently-approved U.S. EPA SEP guideline)

Table A-18. Freshwater Invertebrate Acute Toxicity

Midge (*Chironomus temtans*) Static-renewal – to maintain water quality 10-Day test; sediment-spiked exposures	98.5	Mortality (measured conc): SED NOAEC = 130,000 SED LOAEC = 270,000 Pore Water (PW) NOAEC = 26,000 PW LOAEC = 29,000 (14% mortality) PW LC_{50} >30,000 Growth: Dry Weight (measured conc): SED NOAEC = 24,000 SED LOAEC = 60,000 PW NOAEC = 4,000 PW LOAEC = 21,500	slightly toxic	459040-02 Putt, 2003	Supplemental (does not fulfill any currently-approved U.S. EPA SEP guideline)
Formulations	**% ai Product**				
Waterflea (*Daphnia magna*) Flow-through test	79.6 80 WP	49,000 (higher concs. than 31,000 υg/L were cloudy) (measured) slope 2.433	slightly toxic	420414-01 Putt 1991	Supplemental for formulation (EC_{50} was not identified due to insolubility)
Waterflea (*Daphnia pulex*) Static test; 15EC 282 mg/L hardness With & without sediment	40.8 4 L	36,500 (nominal) 46,500 (with sediment)	slightly toxic	452277-12 Hartman & Martin 1985	Supplemental for formulation (EC50 exceeds water solublity and low temp.)

Since the lowest LC_{50}/EC_{50} is in the range of 0.1 to 1 ppm, atrazine is categorized as highly toxic to aquatic invertebrates on an acute basis. The freshwater invertebrate LC_{50} value of 720 ppb is based on an acute 48-hour static toxicity test for the midge, *Chironomus tentans* (MRID # 000243-77). The preferred test species, *Daphnia magna*, was not particularly sensitive to atrazine; therefore, acute toxicity data from the midge (*Chironomus tentans*) was chosen as the most sensitive endoint. The formulated end products were less toxic to aquatic invertebrates than theTGAI.

Degradates: Acute aquatic invertebrate testing with *Daphnia magna* (72-2) was completed to address degradate concerns. Table A-19 presents freshwater invertebrate toxicity data for hydroxyatrazine.

Table A-19. Freshwater Invertebrate Acute Toxicity (Hydroxyatrazine)

Surrogate Species/ Flow-through or Static	**% ai formul.**	**48-hour EC_{50} (ppb) (measured/nominal)**	**Toxicity Category**	**MRID No. Author/Year**	**Study Classification**
Waterflea (*Daphnia magna*); 1st instar (6-24 h old) Static test	98	>4,100 (measured dissolved)	moderately toxic*	465000-01 Peither, 2005c	Acceptable

* Biological results for the study were based on the mean-measured concentration of dissolved Hydroxyatrazine, which remained constant at the limit of its water solubility throughout the duration of the test. Therefore, hydroxyatrazine is not acutely toxic to *Daphnia magna* at the limit of its water solubility.

Although the freshwater invertebrate EC_{50} value (>4,100 ppb) for the degradate, hydroxyatrazine, is within the range classifying it as moderately toxic, the biological results for the study were based on dissolved (filtered) mean-measured concentrations of hydroxyatrazine, which remained constant at the limit of its water solubility (3-4 ppm ai) throughout the duration of the test (MRID 465000-01). Therefore, the potential toxicity of hydroxyatrazine appears to be limited by its solubility.

A.2.6 Freshwater Invertebrate, Chronic

A freshwater aquatic invertebrate life-cycle test using the TGAI is required for atrazine since the end-use product 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; an aquatic acute LC50 is less than 1 mg/L; 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-20.

Table A-20. Freshwater Aquatic Invertebrate Life-Cycle Toxicity

Surrogate Species/ Study Duration/ Flow-through or Static Renewal	% ai	NOAEC/LOAEC µg/L (ppb) (measured or nominal)	Statistically sign. (p=0.05) Endpoints Affected	MRID No. Author/Year	Study Classification
Scud (*Gammarus fasciatus*) 30 days / flow-through	94	**NOAEC 60** LOAEC 140 (measured)	25 % red. in development of F_1 to seventh instar.	000243-77 Macek *et al.* 1976	Acceptable
Midge (*Chironomus tentans*) 38 days / flow-through	94	NOAEC 110 LOAEC 230 (measured)	25 % red. in F_0 pupation 29 % red. in F_0 adult emergence 18 % red. in F_1 pupation 28 % red. in F_1 adult emergence	000243-77 Macek *et al.* 1976	Acceptable
Waterflea (*Daphnia magna*) 21 days / flow-through	94	NOAEC 140 LOAEC 250 (measured)	54 % red. in F_0 young/female	000243-77 Macek *et al.* 1976	Acceptable
Waterflea (*Daphnia pulex*) 28-Day static-renewal	99.2	NOAEC 1,000 LOAEC 2,000 (nominal)	16 % sign. red. in young/adult	452029-15 Schober & Lampert 1977	Supplemental (no raw data for statistical analyses)
70-Day static-renewal test			31 % red. in young/adult		
Waterflea - 6 generations (*Daphnia magna*) Static-renewal test	NR	Cups: NOAEC 200 LOAEC 2,000 (unknown) 4 L aquarium: NOAEC ?? LOAEC ?? (water from treated corrals)	66 % reduction in # of young in generations 4, 5, & 6. 72% reduction in # of young	Kaushik, Solomon, Stephenson and Day 1985	Supplemental (methods and raw data are not reported)
Leech (*Helobdella stagnalis*) 40 Days Static-Renewal weekly	99.2	NOAEC <1,000 LOAEC 1,000 (measured)	65% red. in percent hatch	452029-16 Streit & Peter 1978	Supplemental (no raw data for statistical analyses)

Table A-20. Freshwater Aquatic Invertebrate Life-Cycle Toxicity

Waterflea < 12 hr. old (*Ceriodaphnia dubia*) Two 7-Day static-renewal tests; Renewed M, W, & F 57 $CaCO_3$; Temp. 25EC	> 99	NOAEC 2,500 LOAEC 5,000 NOAEC 2,500 LOAEC 5,000 (measured)	sign. red. in mean total number of young per living female (3 broods)	452083-05 Oris, Winner and Moore 1991	Supplemental (no raw data for analyses)
Green hydra (normal) (*Chlorohydra viridissima*) 21-Day Static test	NR	NOAEC <5,000 LOAEC 5,000 (nominal)	sign. red. in budding rates	452029-01 Benson & Boush 1983	Supplemental (no raw data for analyses)
Waterflea 3-day old adult (*Ceriodaphnia dubia*) Two 4-Day static-renewal tests; Renewed M & W 57 $CaCO_3$; Temp. 25EC	> 99	NOAEC 5,000 LOAEC 10,000 NOAEC 10,000 LOAEC 20,000 (measured)	sign. red. in mean total number of young per living female (3 broods)	452083-05 Oris, Winner and Moore 1991	Supplemental (no raw data for analyses)
Freshwater Snail (*Ancylus fluviatilis*) 40 Days Static-Renewal weekly	99.2	1,000 4,000 16,000 (measured)	38-39% red. in egg capsules & eggs in April/May 56-57% red. in eggs in April/May 15-16% red. in eggs in July/Aug. 68-73% red. in eggs in April/May 65-71% red. in eggs in July/Aug.	452029-16 Streit & Peter 1978	Supplemental (no raw data for statistical analyses)
Leech (*Glossiphonia complanata*) 27-Days Static-Renewal weekly	99.2	1,000 4,000 16,000 (measured)	no reduction in egg production 17 % higher mortality 33 % higher mortality 67 % higher mortality	452029-16 Streit & Peter 1978	Supplemental (no raw data for statistical analyses)

Growth stages and/or number of young are reduced by atrazine exposures for insects and crustaceans. The most sensitive chronic endpoint for freshwater invertebrates is based on a 30-day flow-through study on the scud (*Gammarus fasciatus*), which showed a 25% reduction in the development of F_1 to the seventh instar at atrazine concentrations of 140 ppb; the corresponding NOAEC is 60 ppb (MRID 000243-77).

Daphnia pulicaria was tested in a 12-day partial life cycle study to determine whether atrazine has an effect on the sex ratio (Madsen, 2000). No male *Daphnia* young were found at measured test concentrations 0, 0.93, 4.1, 8.7, 44, and 87 μg/L (MRID # 452995-04).

A.2.7 Freshwater Invertebrates, Acute Open Literature Data

Johnson et al. (1993) tested juvenile and mature freshwater mussels *Anodonta imbecilis* under static conditions in a 48-hour acute study (summarized in Table A-21). These results suggest that 48-hour exposures at atrazine concentrations up to 60 mg/L do not affect survival of *A. imbecilis.*

Table A-21. Acute Aquatic Invertebrate Toxicity Tests from Open Literature (2006 Review)

Study type/ Test material	Test Organism (Common and Scientific Name) and Age and/or Size	Test Design	Endpoint Concentration in ppb	Citation (EcoRef. #)	Rationale for Use in Risk Assessment[1]

Table A-21. Acute Aquatic Invertebrate Toxicity Tests from Open Literature (2006 Review)					
Study type/ Test material	**Test Organism (Common and Scientific Name) and Age and/or Size**	**Test Design**	**Endpoint Concentration in ppb**	**Citation (EcoRef. #)**	**Rationale for Use in Risk Assessment[1]**
Acute toxicity study in freshwater snails / 97% pure	Freshwater snail *A. imbecillis* juvenile and mature organisms	Anodonta imbecillis (20/group) were exposed to atrazine for 24-48 hours under static conditions and evaluated for survival. LC50 values were estimated.	LC_{50} was >60 mg/L in both juvenile and mature *A. imbecillis.*	Johnson et al. 1993 (50679)	Qual: Study suggests that *A. imbecillis* is less sensitive than other invertebrates tested; however, freshwater snails are underrepresentated taxa.

(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.2.8a Freshwater Microcosm/Field Studies (2003 IRED Data)

A summary of all the freshwater aquatic microcosm, mesocosm, and field studies that were summarized as part of the 2003 IRED is included in Tables A-22 through A-24. Freshwater microcosm data are presented in Table A-22. Summaries of mesocosm and limnocorral studies for freshwater ponds, lakes, reservoirs are included in Table A-23 and natural and artificial stream mesocosm data are summarized in Table A-24.

Walker (1964) treated Missouri ponds and plastic-lined limnocorrals with atrazine for aquatic weed control at levels of 500 to 2,000 µg/L and quantitatively examined effects on bottom organisms. Among the most sensitive organisms were mayflies (*Ephemeroptera*), caddis flies (*Tricoptera*), leeches (*Hirudinea*) and gastropods (*Musculium*). The most significant reduction in bottom fauna was observed during the period immediately following the application of atrazine. Six to eight weeks after treatment, nine out of fourteen taxonomic groups had not recovered. The total number of bottom organisms per square foot was 52 percent lower than in the controls. In addition, three categories of invertebrates (water bugs, mosquitoes, and leeches) were no longer present. (MRID # 452029-19).

Streit and Peter (1978) reviewed Walker's findings and investigated long-term atrazine effects on three benthic freshwater invertebrates: *Ancylus fluviatilis* (Gastropoda - Basommatophora), *Glossiphonia complanata* and *Helobdella stagnalis* (both: Annelida - Hirudinea) in the laboratory (see Chronic Invertebrate toxicity table). Ingestion rates for *G. complanata* were determined over a 27-day period at atrazine concentrations of 1,000, 4,000 and 16,000 ppb. The total ingestion per individual was measured daily (except between Day 23 and 27). Two significant results were: (1) Contaminated leeches ate significantly more limpets than the controls (300, 345 and 405% of control ingestion rates for 1,000, 4,000 and 16,000 µg/L atrazine exposures, respectively). (2) There was a constant feeding intensity from immediately after the beginning of the exposure period. The same phenomenon was seen for snails, *A. fluviatilis*, but the intensity of feeding was much less (i.e., 120, 130 and 140% of control ingestion rates at 1,000, 4,000 and 16,000 µg/L, respectively). Other observations included: (1) Leeches were found sometimes lying on their backs suggesting that they may have difficulty staying firmly attached to the substrate. (2) With increasing atrazine concentrations, an increasing percentage

of snails could be detected that not wholly eaten. Similar effects were observed with the snails which suggest that leech and snail behavior might be affected in some way. Compared to controls, *Ancylus* egg production was significantly reduced after 40 days exposure to atrazine at 16,000 µg/L in March/April, April/May (68% fewer egg capsules and 73% fewer eggs) and July/August (65% fewer egg capsules and 71% fewer eggs). Lower *Ancylus* reproduction was also found at 4,000 µg/L in April/May (56-57 percent) and July/August (15-16 percent). At 1,000 υg/L, fewer capsules and eggs were found only in April/May (38 and 39 percent, respectively). The average number of eggs per brood in the leech, *Glossiphonia complanata* was not affected by 27-days of atrazine exposure. Atrazine treatment did not affect the number of live-born young of *Helobdella stagnalis*. At 1,000 and 4,000 µg/L only a part of the egg masses developed. Only about 10 percent of the young in the 16,000 µg/L treatment hatched. Atrazine did not affect the time for normal development (5-6 days). (MRID # 452029-16).

Kettle *et al*. (1987) monitored effects of atrazine (40.8%) on diet and reproductive success of bluegill in experimental, Kansas ponds. The 0.045-hectare, 2.1-meter deep ponds were each stocked with adult fish (50 bluegills, 20 channel catfish and 7 gizzard shad). On July 24, atrazine was applied to two ponds at 20 µg/L, and to another two ponds at 500 µg/L and two controls. Atrazine concentrations were measured during the study and 70% of the original concentration was detected at the end of the 136-day study. Bluegills were the only species to spawn during the study. Atrazine had no significant effect on mortality of the original stocked fish, but the number of young bluegills retrieved were significantly ($p \leq 0.01$) reduced compared to control ponds (i.e., 95.7 % fewer in 20 µg/L-treated ponds and 96.1 % fewer in 500 µg/L-treated ponds). Stomach analyses of adult bluegills indicate that the bluegill controls had significantly ($p \leq 0.001$) higher numbers of food items per fish stomach and higher numbers of prey taxa per fish stomach. The number of food items per stomach were reduced 85 and 78 percent in 20 and 500 µg/L -treated ponds, respectively. Reductions in taxa per stomach were 57 and 52 percent in 20 and 500 µg/L-treated ponds, respectively. Stomachs of bluegills from treated ponds had fewer numbers of Ephemeroptera ($p \leq 0.001$), Odonata ($p \leq 0.001$), Coleoptera ($p \leq 0.01$) and Diptera (not significant, $p > 0.05$) than the controls. The macrophyte community in treated ponds was noticeably reduced, relative to controls, throughout the summer. Visual estimates of the macrophyte communities in the ponds showed roughly a 60 percent decline in the 20 µg/L ponds and a 90 percent decline in the 500 µg/L ponds two months after atrazine addition. These estimates were verified by rake hauls which produced these same relative differences. The following May, 10 months after treatment, when macrophytes are normally well established in Kansas ponds, the ponds were drained. Relative to control ponds, 20 µg/L ponds had a 90 percent reduction in macrophyte coverage and the 500 µg/L ponds had a >95 percent reduction in macrophyte coverage. Differences were noted in the macrophyte species present. Control ponds contained *Potamogeton pusillus* and *P. nodosus, Najas quadalupensis*, and small amounts of *Chara globularis*, whereas the treated ponds contained mostly *C. globularis.* (MRID # 452029-12).

Table A-22. Freshwater Microcosm Tests

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o percent difference from controls	Narrative of Study Trends	MRID No. Author/Year
Freshwater microcosm: Measured close to nominal throughout the testing period: concentrations of 0.5, 5, 50, 100, 500, and 5000 ppb	0.5 and 5 ppb o no reduction in net oxygen loss 50 ppb o 25-30% reduction in net oxygen loss 100 ppb o 40-50% reduction in net oxygen loss 500 ppb o 90% reduction in net oxygen loss 5,000 ppb o 100% reduction to negative net oxygen production	*Spirogyra, Oedogonium, Microcystis, Apthanothece, and Scenedesmus* sp. in mixed culture. Microcosms inoculated with algae demonstrated effects at concentrations ≥50 ppb. Physical appearance of the microcosms was altered at 5,000 ppb. Observations and reculture demonstrated that the effects were algistatic.	450874-07 Brockway *et al*., 1984
Freshwater Microcosm: (Duration 7 weeks exposure) Mean measured concentrations of 5.08 ± 0.03 µg/L; range: 4.2 - 6.0 µg/L	NOEC: 5 ppb o slight non-sign. shifts in water parameters: o DO decreased from means of 9.4 - 9.9 mg/L (controls) differing weekly by 0.2 - 0.6 mg/L o pH decreased from means of 8.4 - 9.0 (controls) differing weekly by 0.0 - 0.4 units o conductivity increased from 159.3 - 189.3 µS/cm (controls) differing by 0.2 - 10.0 µS/cm o alkalinity increased from means of 1.4 - 2.2 mg/L (controls) differing by 0.0 - 0.3 mg/L o no significant adverse effects on phyto- & zooplankton, or 15 macro-invertebrate species o Cyclopoida sign. increased in week 3	Laboratory microcosms (4 replicates) were tested with 0 and 5 µg/L atrazine for 7 weeks. The plankton and macro-invertebrates were introduced together with 2-cm layer of natural sediments into glass aquaria with a 50 cm water column with a 14-hour photoperiod. Water was circulated through the microcosms at a flow rate of 3.5 L/min. during an acclimation period for biota of 3 months. This test was part of a study of pesticide interaction between atrazine and chlorpyrifos to determine the adequacy of chronic safety factors.	450874-17 van den Brink *et al*. 1995 Supplemntal (raw data unavailable)
Freshwater Microcosm: Mean measured concentrations of 3.2, 10, 32, 110, and 337 ppb	NOEC: 10 ppb; LOEC: 32 ppb o dissolved oxygen, magnesium, and calcium; NOEC: 110 ppb; LOEC: 337 ppb o potassium, chlorophyll-a, protein, and species equilibrium number	Laboratory microcosms were inoculated with foam blocks taken from a pond. The effect to protozoans from atrazine exposure was examined by measuring structure (species number, biomass), and function (colonization rate, oxygen production, chlorophyll concentration) of the community as well as ion concentrations of the biomass after 21 days.	450874-16 Pratt *et al*. 1988 Supplemental (raw data unavailable)
Freshwater Microcosm: (6 weeks) Meas. peak 20 ppb on day 1, mean measured concentration of approximately 10 ppb	10 ppb (6 weeks) o sign. (0.05) reduced dissolved oxygen (DO), but was recovering by test termination	Laboratory microcosms were treated with a stock solution of atrazine and soil to which atrazine was bound. At the end of the study, no significant effects on plant biomass or daphnid/midge survival were noted, but DO was affected.	452051-02 Huckins *et al*. 1986 Supplemental (raw data unavailable)

Table A-22. Freshwater Microcosm Tests

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o percent difference from controls	Narrative of Study Trends	MRID No. Author/Year
Freshwater microcosm: (30 days): Macrophytes, algae, zooplankton and benthic invertebrates; Nominal conc. of 10, 100 and 1,000 ppb as a soil slurry	10 ppb (Day 2) o 23% red. in gross primary productivity (GPP); recovery by Day 7 and similar to controls at Day 30 100 ppb (Day 2) o 32% red. in GPP; recovery by Day 7 and similar to controls at Day 30 1,000 ppb (Day 2) o 91% red. in GPP; no recovery, 70% red. throughout test 1,000 ppb (Day 30) o 48% red. (sign. P≤0.05 level) macrophyte biomass o 36% red. (sign., P≤0.05) *Selenastrum* dry weight 1,000 ppb (30-day aged microcosm water) o 76% red. (sign. P≤0.05) *Selenastrum* dry weight 1,000 ppb (Day 30) o reduced O_2, community respiration, pH o 20% increase in conductivity o 120% increase in alkalinity o no effect on soil microbial activity	4-L microcosms were established in the laboratory and treated with a soil slurry of atrazine. The endpoints examined over the 30-day experiment included effects to zoo- and phytoplankton as well as macrophytes (i.e., *Lemna* sp., *Ceratophyllum* sp., and *Elodea* sp.). Static acute and chronic assays were conducted with *Daphnia magna* and *Chironomus riparius* using treated water that had come from the microcosm after 30 days or from a vessel that contained the treated water for 30 days (i.e., aged treated water). The author concluded that microcosm itself ameliorated the phytotoxic effect at 1,000 ppb. No effect on invertebrates up to 1,000 ppb and effects to phytoplankton at 10 and 100 ppb were not observed by test termination (30 days). Conductivity, pH, and alkalinity were also affected at 1,000 ppb.	450874-13 Johnson, 1986 Supplemental (raw data unavailable)
Freshwater Microcosm: Emergent vascular plants; Nominal water conc. of 10, 50, 100, 500, and 1,500 ppb; measured water conc. in the 50 and 500 ppb treatments of 1.3 and 1.6 ppb, respectively, after 16 weeks	500 ppb (6 weeks) o sign. (0.05 level) red. shoot length of *Scirpus acutus* 1,500 ppb (6 weeks) o sign. red. shoot length of *Scirpus acutus* and *Typha latifolia*	Greenhouse microcosms were made by placing rhizome sections in tubs which were filled with treated water to 1 cm above the soil surface. The plants were allowed to grow for 16 weeks and shoot height of hardstem bulrush and broad-leaved cattail was monitored bi-weekly. Also non-sign. effects of chlorosis and reduced growth noted at 50 and 100 ppb. A second test demonstrated resiliency of both plants at 500 ppb.	450874-15 Langan and Hoagland, 1996 Supplemental (raw data unavailable)
Freshwater Microcosm: (14 days) Measured atrazine concentrations approximately 75% of nominal (15 and 153 ppb) for first application and 150% of nominal (385 and 2,167 ppb) for the second application	Sign. (0.1 level) reduction in turbidity and chlorophyll (7 days), and increase in phosphorous (day 14) and nitrogen (days 7 and 14) after the 1st application. Copepod and rotifer densities were also sign. reduced on days 7 and 14. Sign. reductions in productivity, chlorophyll, green algal colonies, rotifers, and *Bosmina* sp. (zooplankton) after 2nd application. Phosphorous, nitrogen, and pH were also sig. affected.	A 3x3 factorial design with three conc. of atrazine (0, 15, and 153 ppb) and three conc. of bifenthrin (0, 0.039, and 0.287 ppb) applied as soil slurry in May, then again one month later but with atrazine conc. of 0, 385, and 2,167 ppb and bifenthrin conc. of 0, 0.125, and 3.15 ppb. Atrazine alone caused dose-responsive reductions in chlorophyll, turbidity, primary production, increases in nitrogen and phosphorous, and reduced levels of chlorophytes, cladocerans, copepod nauplii, and rotifers. General recovery after 14 days for atrazine alone in the first phase, but recovery not complete at sampling termination after second phase (14 days). No synergistic or antagonistic effects were noted.	450200-14 Hoagland *et al*., 1993 Supplemental (raw data unavailable)

Table A-22. Freshwater Microcosm Tests

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o percent difference from controls	Narrative of Study Trends	MRID No. Author/Year
Freshwater microcosm: (2 months; measured) Nominal concentrations of 0, 60, 100, 200, 500, 1,000 and 5,000 ppb. Measurements made three times during the two month study.	60 ppb (nominal) o 14-carbon uptake decreased immediately after treatment; recovery began after 10 days; o stimulated production of chlorophyll a; 100 ppb (nominal) o 14-carbon uptake decreased immediately after treatment; recovery began after 10 days; o stimulated production of chlorophyll a; 200 ppb (nominal) o 14-carbon uptake decreased immediately after treatment; slight recovery 2 months after treatment; o stimulated production of chlorophyll a; o inhibited increases in dissolved oxygen during light phase and decreases in DO during dark phase 500 ppb (nominal) o 14-carbon uptake decreased immediately after treatment; no recovery; o minimal inhibition of chlorophyll a production; 1,000 and 5,000 ppb (nominal) o 14-carbon uptake decreased immediately after treatment; recovery began after 10 days. EC50s for Days 0-10, 53-60, & Mean (mean measured conc.) Time period; 14C uptake; DO (light); DO (dark) Days 0-10 : 103 ppb 126 ppb 106 ppb Days 53-60: 159 ppb 154 ppb 164 ppb Days 1-60: 131 ppb 165 ppb 142 ppb	Results of single species assays, microcosm, and pond studies were compared. 14-Carbon fixation was used as the end-point for all three study types. Laboratory results with eight algal species ranged from 37 to 308 ppb for carbon uptake inhibition EC_{50} values. Microcosm EC_{50} values ranged from 103 to 159 ppb. The mean pond EC_{50} was 100 ppb for carbon uptake and 82 ppb for chlorophyll-a inhibition. The authors stated that multiple laboratory studies or a microcosm study represent(s) entire ecosystem functional effects.	450200-15 Larsen *et al*., 1986 and 450874-19 Stay *et al*. 1985 Supplemental (raw data unavailable)
Freshwater microcosm: (60 days; measured) Nominal concentrations of 60, 100, 200, 500, 1,000, and 5,000 ppb. Concentrations measured on Days 7, 28, 53, 60.	NOEC < 60 ppb; 60 ppb (1 - 20 days) o sign. (0.05) red. 14-carbon uptake for first 20 days ≥ 100 ppb (2 weeks) o sign. (0.05 level) red. primary productivity; o sign. red. in productivity/ dark respiration ratio; o pH sign. less than control values ≥ 500 ppb (6 weeks) o all endpoints declined immediately after treatment and never recovered during the experiment.	Taub microcosms were 3-L jars inoculated with 10 algal species on Day 0, *Daphnia magna* and 4 other animal species on Day 4. On Day 7, 27 microcosms were treated with atrazine; no other atrazine treatments um from four different aquatic systems. Community metabolism was measured for primary productivity and light and dark respiration. At the high treatment levels (500, 1000 and 5000 ug/L), all process variables declined immediately after atrazine treatment and did not recover during the experiment. At the low treatment levels (60, 100 and 200 ug/L), the magnitude of the responses to atrazine was not constant, but with 3 phases; an autotrophic phase, daphnid bloom and an equilibrium phase.	450874-19 Stay *et al*., 1989 Supplemental (raw data unavailable)

Table A-22. Freshwater Microcosm Tests

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o percent difference from controls	Narrative of Study Trends	MRID No. Author/Year
Freshwater microcosm: (6 weeks; measured) Single dose; Nominal conc. 20, 100, 200, 500, 1,000 and 5,000 ppb. Concentrations were measured on Days 0 and 42. On Day 42, atrazine levels averaged 69 to 80% of the initial concentrations.	NOEC = 20 ppb LOEC = 100 ppb in 3 out of 4 natural plankton communities and 200 ppb for the fourth community. ≥ 100 ppb (2 weeks) o sign. (0.05 level) red. primary productivity o sign. red. in productivity/dark respiration ratio o pH sign. less than control values	Leffler microcosms were constructed with inoculum from four different aquatic systems from natural communities and contains organisms representing several trophic levels. The vessels were dosed after 6 weeks of seeding and monitoring for 6 more weeks. The LOEC for 3 of the systems was reported to be 100 ppb, while the LOEC for the fourth was 200 ppb.	450874-18 Stay *et al*. 1989 Supplemental (raw data unavailable)

Table A-23. Freshwater Ponds, Lakes, and Reservoirs (including Mesocosms and Limnocorrals)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Freshwater Lake: Plankton (Duration 18 days) Measured = ≥90% of nominal over the test period (18 days): nominal concentrations of 0.1, 1, 10, and 100 ppb	NOEC = < 0.1 ppb o transient effects on water chemistry 1 ppb (1 week) o decreased primary production; o increased bacterial numbers o decreased in zooplankton numbers (cladocerans affected greater than copepods) 10 ppb (3 weeks) o 65% sign. ($p < 0.01$) red. in daphnid population growth (combined effect of water & algae) o 59% sign. ($p < 0.05$) red. in daphnid growth (algae) 100 ppb (3 weeks) o 92% sign. ($p < 0.01$) red. in daphnid growth (combined) o 69% sign. ($p < 0.01$) red. daphnid growth (algae)	*In situ* enclosures in a German lake were treated and monitored over 18 days. Dose-responsive reductions in chlorophyll-a and oxygen and increases in particulate organic carbon were observed at 1, 10, and 100 ppb. Within 1 week at 1 ppb, primary production decreases and bacterial number increases were observed. Zooplankton numbers then decreased, with cladocerans affected more than copepods. Additional studies at 0.1 ppb also demonstrated transient effects on water chemistry and biological parameters. Most of the parameters were recovered or were recovering within 42 days of application.	450874-14 Lampert *et al*., 1989 Supplemental (raw data unavailable)

Table A-23. Freshwater Ponds, Lakes, and Reservoirs (including Mesocosms and Limnocorrals)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Freshwater Pond: Plankton Treated 3 times on 7/31, 8/28 (29 days later), and 9/21/1990 (24 days later) at 5, 10, 25, 75, 200, and 360 ppb. Weekly conc. relatively constant; mean measured conc. over two months are 5, 10, 22, 68, 182, and 318 ppb (63 days; measured)	NOEC: 5 ppb (63 days) compared to controls 10, 22 and 68 ppb o up to 40% red. dissolved oxygen (Days 7-46) o up to 10% incr. pH (Days 18-63) o up to 10% red. conductivity (Days 7-53) 68 ppb o up to 78% red. copepod nauplii and no increase in nauplii at 182 & 318 ppb o diatoms appear to become the dominant phytoplankton 182 ppb o strong red. in dissolved oxygen and conductivity and strong increase in pH levels (same for 318 ppb) o up to 98% red. Cryptophyceae, *Cryptomonas marsonii* and *S. erosa/ovatata* (Days 21 to tests end) o up to 10% red. conductivity (Days 7-53) o up to 98% red. seasonal blooms of *Cryptomonas marsonii* & *S. erosa/ovatata* (Days 21 to tests end) o prevented *Mallomonas* sp. seasnal bloom (318 ppb too) o prevented the seasonal bloom of *Planktosphaeria* sp. (Chlorophyceae) after Day 30 (same at 318 ppb) o lower numbers & early seasonal decline of rotifers, *Synchaeta* sp. (same at 318 ppb) 318 ppb o up to 80% red. phytoplankton cell density (throughout test, except on Day 35) o up to 98% red. Cryptophyceae, *Cryptomonas marsonii* and *S. erosa/ovatata* (first appeared on Day 10 - Days 21 to tests end) o up to 9% incr. pH (Days 18-63) o up to 10% red. conductivity (Days 7-53) o strong red. in cell numbers of *Planktosphaeria* sp. (Chlorophyceae) after Day 30 o delays in reaching and lower peak daphnid egg ratio, and delayed peaks for numbers of young and adults	Mesocosms (1,000 L cylinders) in southern Bavaria were treated with atrazine 3 times (29 and 24 day intervals) over 63 summer days. Strongly dose-response reductions in dissolved O_2, pH, and conductivity were noted at concentrations greater than 5 ppb. Changes in oxygen concentrations at $\geq$ 10 ppb and some zooplankton populations at 68, 182, and 318 ppb reflect indirect functional links as a result of altered primary production. At 68 ppb, up to a 78% reduction in copepod nauplii was found and no increase in the number of nauplii was found at 182 and 318 ppb. At 182 ppb, threshold concentrations for direct effects by atrazine were exceeded in several phytoplankton species. Diatoms appeared to become the dominant phytoplankton at 182 and 318 ppb. One rotifer species decreased at 182 ppb and another at 318 ppb and was virtually absent from Day 18 to the end of the study. Daphnid reproduction and populations decreased at 318 ppb.	45020022 Juttner *et al*. 1995 Supplemental (raw data unavailable)

Table A-23. Freshwater Ponds, Lakes, and Reservoirs (including Mesocosms and Limnocorrals)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Artificial freshwater ponds in Kansas treated with atrazine to achieve concentrations of 20 and 500 µg/L Atrazine levels measured in the water column four times during the first two months of the study: 100% of nominal at time zero (163 days; measured).	Laboratory data shows results for atrazine sensitivity tests for treated field samples: 1 ppb o sign. (0.05) 4% increase in fluorescence 5 ppb o sign. (0.05) 9% increase in fluorescence o sign. (0.05) 8% decrease in C-14 uptake 20 ppb o sign. (0.05) 30% increase in fluorescence o sign. (0.05) 12% decrease in C-14 uptake 500 ppb o sign. (0.05) 136% increase in fluorescence o sign. (0.05) 88% decrease in C-14 uptake o Field pond study results: 20 ppb o sign. (0.05) 51% red. C-14 uptake (4 hr.) (Days 2-7) o sign. 42% red. phytoplankton biomass (Days 2-7) o 3% red. growth & 28% red. daphnid reproduction o *Simocephalus serrulatus* correlated with food levels 500 ppb o pH red. 0.3 units lower than controls for a few weeks o dissolved O_2 generally red. 1-3 mg/L (a few weeks) o sign. 94% red. C-14 uptake (4 hr.) (Days 2-163) o usually sign. red. phytoplankton biomass (Days 2-136) o rapid, nearly complete red. in abundant *Peridinium inconspicuum*, a small dinoflagellate and rapid red. in 7+ other dominate phytoplankton sp. after 7 days o incr. in several flagellate species; mainly *Mallomonas pseudocoronata*, *Cryptomonas marssonii* & *C. erosa* o zooplankton dominance shifted to rotifers, mainly *Keratella cochlearis* after Day 31 o >50% red. in the copepod, *Tropocyclops prasinus mexicanus* by Day 14	Single treatment of two 0.045 hectare ponds each with either 20 or 500 ppb atrazine produced dose responsive changes in pH, DO and daily carbon uptake. Phytoplankton growth was reduced; population shifts were apparent at 20 and 500 ppb. Effects on phytoplankton were immediate, within 2 days, for daily carbon-14 uptake and biomass declines at both treatment levels, which is consistent with other researchers in laboratory tests. Atrazine concentrations down to 1 ppb affected photosynthesis in lab tests with phytoplankton samples from the pond. While atrazine produced direct toxic effects on just certain members of the aquatic community, their responses also affected other members of the community. At 500 ppb, one species of herbivorous zooplankton declined by more than 75% within 14 days of treatment. Subsequent laboratory tests demonstrated some atrazine resistance in phytoplankton and showed zooplankton population effects were due to loss of food (algae). Further evidence of resistance was indicated by a dominant phytoplankton species which showed less toxic responses than the same species in the control pond.	450200-11 DeNoyelles *et al*. 1982 Supplemental (raw data unavailable)

Table A-23. Freshwater Ponds, Lakes, and Reservoirs (including Mesocosms and Limnocorrals)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Artificial freshwater ponds in Kansas treated with atrazine to achieve concentrations of 20 and 500 µg/L	NOAEC < 20 µg/L 20 µg/L - 29% increase in turbidity. - initial depressed phytoplankton, followed by an increase in standing crop and numerical dominance of resistant species. - red. production of *Naajas* sp. and Potamogeton spp. in areas excluding carp. - increase in *Chara* - 82% reduction in total insect emergence. - 89% red. in non-predator insect emergence. - 90% red. *Labrundinia pilosella* emergence. - 50% red. in total insect species richness. - 57% red. in non-predator insect species richness. 100 µg/L - 62% increase in turbidity. - absence of periphyton on walkway supports. increase in *Chara* sp. 83% reduction in total insect emergence. - 95% red. in non-predator insect emergence. - 96% red. *Labrundinia pilosella* emergence. 71% red. in total insect species richness. - 85% red. in non-predator insect species - richness. 5% red. in insect species evenness. 500 µg/L - 65% increase in turbidity. - absence of periphyton on vascular plants. - absence of *Chara* sp. 70% reduction in total insect emergence. - 85% red. in non-predator insect emergence. - 90% red. *Labrundinia pilosella* emergence. - 59% red. in total insect species richness. - 66% red. in non-predator insect species richness. - 15% red. in insect species evenness.	Two artificial Kansas ponds each (0.045 ha. and 2.1 m. deep) were treated with technical atrazine at 20 µg/L and 100 µg/L and with a 41% ai CO-OP liquid atrazine at 20 µg/L in 1981; two ponds served as controls. The ponds were treated again on 30 May 1982, but the 41% ai ponds were converted to 500 µg/L with technical atrazine. The macrophyte community in treated ponds was noticeably reduced, relative to controls, throughout the summer. For 16 sampling dates between 8 May and 28 September 1982 insect emergence was monitored in each pond with 4 emergence traps for 48 hour periods. No significant differences between ponds were found in water level, temperature or oxygen levels. Mean turbidity varied significantly among treatments (ANOVA), increasing with increasing atrazine levels up to 100 µg/L. The phytoplankton community responses to atrazine during the present study corroborate results from the 1979 study by deNoyelles *et al*. (1979). Macrophyte response also paralleled the 1979 study. The presence of live plants of the primary emergent vegetation, *Typha* spp., gradually decreased, as in previous studies, with increasing atrazine concentration both within and outside carp exclusion areas (Carney 1983, deNoyelles and Kettle 1983).	452277-06 Dewey 1986

Table A-23. Freshwater Ponds, Lakes, and Reservoirs (including Mesocosms and Limnocorrals)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Artificial freshwater ponds in Kansas treated with atrazine to achieve concentrations of 20 and 500 µg/L	NOAEC < 20 µg/L 20 µg/L - 60% sign. ($p < 0.05$) reduction in macrophtye vegetation at summer's end including elimination of *Potamogeton pusillus, P. nodosus*, & *Najas quadalupensis*; - 95% sign. ($p < 0.05$) red. macrophyte coverage in May, 10 months after treatment; - 96% sign. ($p < 0.01$) reduction in the number of young bluegill; - 85% sign. ($p < 0.001$) red. in the number of food items/ fish stomach; - 57% sign. ($p < 0.001$) red. in the number of prey taxa/ fish stomach. 500 µg/L - 90% sign. ($p < 0.05$) reduction in macrophtye vegetation at summer's end including elimination of *Potamogeton pusillus, P. nodosus*, & *Najas quadalupensis*; - >95% sign. ($p < 0.05$) red. macrophyte coverage in May, 10 months after treatment; - 96% sign. ($p < 0.01$) reduction in the number of young bluegill; - 78% sign. ($p < 0.001$) red. in the number of food items/ fish stomach; - 52% sign. ($p < 0.001$) red. in the number of prey taxa/ fish stomach.	Two artificial Kansas ponds each (0.045 ha. and 2.1 m. deep) were treated with 20 µg/L and 500 µg/L on 24 July and two ponds served as controls. The macrophyte community in treated ponds was noticeably reduced, relative to controls, throughout the summer. Visual estimates of the macrophyte communities in the ponds showed roughly a 60 percent decline in the 20 µg/L ponds and a 90 percent decline in the 500 µg/L ponds two months after atrazine addition. These estimates were verified by rake hauls which produced these same relative differences. The following May, 10 months after treatment, when macrophytes are normally well established in Kansas ponds, the ponds were drained. Relative to control ponds, 20 µg/L ponds had a 90 percent reduction in macrophyte coverage and the 500 µg/L ponds had a >95 percent reduction in macrophyte coverage. Differences were noted in the macrophyte species present. Control ponds contained *Potamogeton pusillus* and *P. nodosus, Najas quadalupensis*, and small amounts of *Chara globularis*, whereas the treated ponds contained mostly *C. globularis*. Significant indirect effects were found on bluegill diet and reproduction.	452029-12 Kettle, de Noyelles, Jr., Heacock and Kadoum 1987 Supplemental (raw data are not available for analyses)

Table A-23. Freshwater Ponds, Lakes, and Reservoirs (including Mesocosms and Limnocorrals)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Freshwater limnocorrals: (3 controls and 3 treated at nominal concentrations of 100 ppb on June 1 & July 6, 1983) Measured conc. range: 80-140 ppb after the first application, 120-165 ppb after the second application (329 days; measured)	Effects on periphyton and environmental parameters: first application: 80 - 140 ppb o no sign. effects on DO, temperature, Secchi depth, dissolved inorganic carbon (DIS), NO_3-NO_2-N), total nitrogen, and total phosphorus o periphyton dry wt. lower than controls after Day 14 at most depths; sign. (0.05) red. at a depth of 0.5 m on Day 34 and thereafter o sign. 94% red. C-14 uptake (4 hr.) (Days 2-163) o usually sign. red. phytoplankton biomass (Days 2-136) o rapid, nearly complete red. in the abundant *Peridinium inconspicuum*, a small dinoflagellate and rapid red. in 7+ other dominate phytoplankton sp. after 7 days o incr. in several flagellate species; mainly *Mallomonas pseudocoronata*, *Cryptomonas marssonii* & *C. erosa* o zooplankton dominance shifted to rotifers, mainly *Keratella cochlearis* after Day 31 o >50% red. in the copepod, *Tropocyclops prasinus mexicanus* by Day 14 second application 120 - 165 ppb o sign. (0.05) 20% red. dissolved oxygen (Days 37-137) o sign. (0.05) 33% increase in Secchi depth o sign. (0.05) 62% increase dissolved inorganic carbon o sign. (0.05) 103% increase in NO_3-NO_2-N o sign. (0.05) red. periphyton dry weight at depths of 0.5 and 1.5 m on most sampling days o sign. (0.05) red. decr. chlorophyll (19 days after second appl. (Day 54 & on some days thereafter) o zooplankton dominance shifted to rotifers, mainly *Keratella cochlearis* after Day 31 o >50% red. in the copepod, *Tropocyclops prasinus mexicanus* by Day 14	Elaboration of the 80 ppb treatment from Hamilton *et al*., 1987. After the first application (pulse), blue-green algae were eliminated and organic matter was significantly reduced. After the second pulse, organic matter, chlorophyll, biomass, and carbon assimilation were reduced by between 36 and 67%, along with certain species of green algae. Diatom numbers were greater in treatment limnocorrals than in the control limnocorrals for nine weeks after the second pulse.	450200-12 Herman *et al*., 1986 Supplemental (raw data unavailable)

Table A-23. Freshwater Ponds, Lakes, and Reservoirs (including Mesocosms and Limnocorrals)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Texas Lake Mesocosm: Measured atrazine concentrations approximately 75% of nominal (15 and 153 ppb) for first application and 150% of nominal (385 and 2,167 ppb) for the second application	Phyto- and zooplankton	A 3x3 factorial design with three conc. of atrazine (0, 15, and 153 ppb) and three conc. of bifenthrin (0, 0.039, and 0.287 ppb) applied as soil slurry in May, then again one month later but with atrazine conc. of 0, 385, and 2,167 ppb and bifenthrin conc. of 0, 0.125, and 3.15 ppb. Atrazine alone caused dose-responsive reductions in chlorophyll, turbidity, primary production, increases in nitrogen and phosphorous, and reduced levels of chlorophytes, cladocerans, copepod nauplii, and rotifers. General recovery after 14 days for atrazine alone in the first phase, but recovery not complete at sampling termination after second phase (14 days). No synergistic or antagonistic effects noted.	45020-14 Hoagland *et al*., 1993 Supplemental (raw data unavailable) Duplicate check & delete this
Artificial ponds: (measured) Mean measured concentrations of 18.4, 91.5 or 114 ppb (two years data), and 314 ppb	Aquatic plants, phyto- and zooplankton	Nominal applications of either 20, 100, or 300 ppb atrazine were monitored for effect 8 weeks after June application and in the next summer. Conductivity and oxygen concentration were affected at the 100 and 300 ppb levels. Reductions in aquatic plant numbers were observed at ≥100 ppb in the summer after application, but no effects on microflora or fauna were observed. The year after treatment (with 10 to 30% of atrazine still in the water column), *Chara* sp. replaced *Myriophyllum spicatum* and *Potamogeton natans* at levels ≥100 ppb. Phytoplankton became dominated with cyanophytes and then cryptophytes as the concentration of atrazine increased. Zooplankton numbers at 100 and 300 ppb were also reduced the following year.	450200-17 Neugebaur *et al*., 1990 Supplemental (raw data unavailable)
Measured = nominal (50 ppb) at time zero; declined to 40% of nominal after 8 weeks	Aquatic plants and fish	Atrazine and esfenvalerate were applied together in mesocosms to examine possible synergism (reduction of macrophytes leading to extension of insecticide residues and increased fish mortality). Combinations of 50 ppb atrazine and esfenvalerate at 0.25 to 1.71 ppb did not result in synergism. However, *Chara* sp. totally replaced the co-dominant *Naja* sp. six weeks after application.	Fairchild et al., 1994

Table A-23. Freshwater Ponds, Lakes, and Reservoirs (including Mesocosms and Limnocorrals)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Day 1 measured concentrations of 80, 140, or 1560 ppb	Periphyton	Applications were made to *in situ* limnocorrals in June (140 and 1,560 ppb) or June & July (80 ppb) and colonized periphyton slides were submersed in August and monitored for either 56 days (140 and 1,560 ppb) or 210 days (80 ppb). Trends from both years included a shift from a chlorophyte to a diatom community, and a development of some atrazine "resistant" colonies. Community production was reduced by 21% and 82% at the 140 and 1,560 ppb levels, respectively, and certain algae were reduced up to 93%. All biotic measures indicated reduced growth, with cell densities lagging productivity. All parameters except species richness returned to control levels prior to 56 days after first or second applications.	450200-20 Hamilton et al., 1987 Supplemental (raw data unavailable)
Day 1 measured concentration of 80 ppb (two applications of 100 ppb made 35-days apart)	Phyto- and zooplankton	Elaboration of the 80 ppb treatment from Hamilton *et al*., 1987. Two weeks after first application, significant declines in multiple species of green algae were observed, whereas crypto- and dinoflagellates either increased or stayed the same. Low population densities persisted for 114 days after the second application. Average of ~25% fewer species in atrazine limnocorrals. Control and treated values equilibrated within one year of treatment. Only two zooplankters were affected (after the second application). A MATC was suggested to be between 100 and 200 ppb.	Hamilton et al., 1988
Measured after a single dose at 1100 ppb – Day 1: 200 ppb, 55 days later: 60 ppb	Phytoplankton	Treatment related reductions in oxygen, and pH, and increases in conductivity were noted after atrazine treatment, with oxygen and pH returning to control values within 30-40 days. At 26 days after dosing, 78 algal cells/mL were present in the control and no cells were present in the treated enclosures. Diversity was also reduced the month after application.	450200-16 Lay et al., 1984 Supplemental (raw data unavailable)
Not assayed, nominal concentrations of 50000, 100000, and 150000 ppb	Autotrophs	Primary production and respiration was monitored in a freshwater ecosystem in India. Net productivity in water samples was reduced by 23% and 73%, respectively, at 50,000 and 100,000 ppb, in comparison to control values, and was negative in the 150,000 ppb treatment group.	Piska and Waghray, 1990

Table A-24. Freshwater Natural and Aritfical Streams

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Small Canadian first-order stream adjacent to a tiled-corn field. Atrazine of unspecified purity was applied at 4 liters per hectare on 6 June 1989. The Canadian Water Quality Guidelines (CCREM, 1987) specify a guideline of 2.0 - µg/L to protect freshwater life.	Non-statistical pair-wise comparison of Total Phytoplankton counts vs sta 9, the control indicates reductions at all downstream stations with effects generally decreasing with time and distance. Downstream station 11 (2.5 km from atrazine source -sta. 5): 0.047 µg/L (range 0.004-0.2µg/L) atrazine conc. o all samples with reduced total phytoplankton counts o mean reduction of 63 % (range 6 - 97 %) o highest red. (97 %) on June 9, first sampling day o reduced 70 % in final sample on 16 Nov. Downstream station 10 (50 to 75 m from sta. 5) 0.366 µg/L (range 0.1 - 1.7 µg/L) atrazine conc. o 2 out of 11 samples exceed count at sta. 9 o mean reduction of 45 % (range +55 - 92 %) o highest red. (92 %) on June 9 o reduced 47 % in final sample on 16 Nov. Downstream stations 6 & 7 (a few meters from sta. 5) 0.81 (0.17 - 1.89) and 0.05 (0.001-0.224) µg/L, resp. o 1 out of 9 samples at sta. 6 exceeds count at sta. 9 o mean reduction sta. 6 of 53 % (range +68 - 99) o mean reduction sta. 7 of 66 % (range 3 - 95) o highest red. (99 and 93 %, resp.) on July 21 o red. 45 & 27 %, resp. in final sample on 16 Nov. Ditch (station 5) receiving waters from the 4 tile outlets: 2.62 µg/L (range 0.211 - 13.9 µg/L) atrazine conc. o mean reduction of 79 % (range 46 - 99 %) o highest red. (92 %) on 3 dates, June 23 - July 21 o reduced 51 % in final sample on 16 Nov.	Atrazine concentrations up to 20.39 µg/L (sta. 4) in field tile water, 13.9 µg/L (sta. 5) in receiving ditch and 1.89 µg/L in a small stream (sta. 6) were measured in New Brunswick, Canada in a rural headwater basin of the Petitcodiac River. The fist-order stream flowed parallel to an 8-hectare sub-surface tile-drained field of silage corn. The field was divided into 4 plots and each drained separately into a small canal and into the stream. Water, phytoplankton and zooplankton were sampled at 15-day intervals at 11 sampling sites during the growing season. Total phytoplankton numbers in downstream samples were consistently much less than those from upstream (control) samples during the period of low flow and higher atrazine levels (during the summer). Diatoms dominated the phytoplankton community. Occurrence of other algal species were erratic between stations and over time. Zooplankton numbers were too low to discern trends, but downstream samples were consistently lower in individuals than control samples.	450200-08 Lakshinarayana, O'Neill, Johnnavithula, Leger and Milburn 1992 Supplemental (Replication of samples and statistical analyses were not made)

Table A-24. Freshwater Natural and Aritfical Streams

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Artificial stream test: (14 day; measured) Simulated pulsed-exposures; 5 µg/l atrazine on Day 1 and gradually diluted until only about 1 µg/L on Day 7	5 µg/L to about 1 µg/L on Day 7 o atrazine concentrations: Day 1: Mean conc. 4.74 Day 5: 3.56 Day 10: 1.20 Day 14: 1.19 Possible atrazine effect: o 58 to 126 fold increase sign. ($p<0.05$) in number of emergent insects on Days 3, 5 and 7; treatment numbers were equal to or greater than controls in all samples No statistical effects found in atrazine treatments on: o periphyton growth measured as chlorophyll a levels; chlorophyll a levels decreased gradually in all samples (treatments & controls) over time, "may have masked an effect of atrazine" o indirect effects on function or taxonomic composition of benthic community structure	A community of benthic, stream invertebrates from the Patrick Brook in Hinesburg, Vermont, located in the LaPlatte River watershed. Microbial community growth was incubated for 2 weeks this substrate was placed in 10 x 10 x 7 cm polyethylene boxes and placed in the stream for invertebrate colonization for 3 weeks in July 1993. During the same 3-week period glass slides were placed in the stream for algal settling and growth. Four benthic invertebrate boxes and 9 periphyton slides were randomly placed in each of six replicate tanks. The flow rate was calculated as 20.8 L/min. throughout the test. After a 24-hour equilibration period, treatment at 5 µg/L atrazine was introduced to 3 replicates and 3 controls. On Day 3, about 15 percent of the water was replaced; on Days 6 and 7 water replacements were 50 percent each day; about 15 % was replaced on Day 11 during the 14-day test. "Dewey (1986) also observed herbivorous insects emerging earlier from artificial ponds treated with 20 µg/L atrazine compared to controls. Dewey suggested that the changes she saw were the indirect effect of atrazine exposure, which had reduced the amount of food available to herbivorous insects."	450874-11 Gruessner and Watzin 1996 Supplemental (raw data unavailable for statistical analyses)
Artificial stream tests: (14 day; measured) One dose and recirculation; two atrazine levels (40.8% ai): 15.2 $\pm$ 1.4 and 155.4 $\pm$ 1.4 µg/l atrazine on Day 1; 17.5 $\pm$ 1.2 and 135.0 $\pm$ 4.5 µg/L on Day 28 Interaction test with alachlor discussed under the section on pesticide interactions.	15.2 µg/L (initial atrazine concentration): o 45% red. in benthic algal biovolume after 1 week sign. ($p \leq 0.05$); o 35% red. in benthic algal biovolume after 2 weeks non. sign. ($p \leq 0.05$); o 45% red. in benthic algal biovolume after 4 weeks sign. ($p \leq 0.05$). 155.6 µg/L (initial atrazine concentration): o 45% red. in benthic algal biovolume after 1 week sign. ($p \leq 0.05$) o 50% red. in benthic algal biovolume after 2 weeks sign. ($p \leq 0.05$); o 57% red. in benthic algal biovolume after 4 weeks sign. ($p \leq 0.05$). Time-dependent analyses showed sign. ($p = 0.0083$) reduction in algal biovolume treated with both 15.2 and 155.6 µg/L atrazine throughout the test, but no sign. ($p = 0.3629$) difference between 15.2 and 155.6 µg/L levels.	A benthic mud community of epipelic algae were collected from various locations of Wahoo Creek and acclimated for 6 weeks prior to atrazine treatments. Stream water came from Wahoo Creek on March 25, 1993. Wahoo Creek is a third-order, sediment-dominated Nebraska stream draining primarily agricultural land and subject to major runoff events. Each model stream was constructed from a 114-L oval-shaped plastic tub and lined with two-layers of 4-mil clear plastic. Stream velocities ranged from 0.05 to 0.1 m/sec. in the sending segment and 0.01 to 0.05 m/sec. in the returning segment. Lighting was 12 hour/12 hour light/dark cycle. To replace evaporated water, stream water from the transport tank was mixed for 24 hours prior addition to each stream. Epipelic algae were sampled immediately before herbicide atrazine addition, 24 hours after addition, and after 1, 2 and 4 weeks. Algal samples were analyzed for cell density, cell biovolume and the relative abundance of 6 dominant taxa.	450200-02 Carder & Hoagland 1998

Table A-24. Freshwater Natural and Aritfical Streams

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Natural Tasmanian stream: (2 weeks to 7 months: measured concentrations) Forests aerially sprayed once at either 3 or 6 liters ai per hectare of Gesaprim: peak of 22 ppb; median conc. of 2.5 ppb for the 2 weeks after application	Atrazine levels in 24 Tasmanian streams averaged 2.85 µg/L (range< 0.01-53 mg/L). In forestry areas, the mean stream conc. was 2.00 (<0.01-8.9) µg/L with 35% below the detection limit of 1.0 µg/L. Spray drift into the stream appeared the same as in the treated forest as estimated by spray-droplet deposits on wood. 22 µg/L: o sign. increase ($p < 0.01$) in daytime invertebrate drift at site 2, 12 hours after treatment o site 3 also showed an increase in daytime invertebrate drift on day of treatment, but not statistically sign. ($p > 0.05$) o sign. ($p<0.001$) increase in night drift in number of hydroptylid larvae on days 1, 2, 4, and 9 o sign. ($p<0.001$) increase in night drift in number of hydropsychid larvae on days 2, 4, and 9 The effects of invertebrate drift at site 2 were associated with increased spray drift, during the 12 hours immediately following application. Poor habitat and limited taxa at site 2 precluded drift analyses on specific taxa. o no sign. affect on mean densities of benthic invertebrates, number of taxa or taxa proportions o 71% sign. ($p<0.01$) increase in trout population at site 2 sustained over four months o no sign. effect on fish mortality or physiology	Tasmanian stream, Big Creek, with a catchment area of 36 km^2 was studied for atrazine aerially sprayed on two forest areas of 20 and 66 hectares, at rates of 3 and 6 kg ai/ha on 13 and 14 October 1987, respectively. Three sampling sites were picked: Site 1 above the 2 plantations, sites 2 and 3 were just below each plantation. Each site consisted of an upstream riffle for invertebrate samples and an area 100 m downstream for sampling brown trout (*Salmo trutta*). Atrazine levels in 174 water samples from 44 sites from 24 streams averaged 2.85 µg/L (range< 0.01-53 mg/L). Only 9.6% of samples were below detection limit (0.1µg/L) and only 24 % were below 1.0 µg/L. In forestry areas, the mean stream conc. was 2.00 µg/L (range <0.01-8.9 µg/L) with 35% below the detection limit of 1.0 µg/L. The initial measured concentration in Big creek was 22 µg/L, 2 weeks later atrazine averaged 2.5 (range 1.2-4.6) µg/L, and over the following 2 months ranged from 0.01 to 0.09 µg/L. Atrazine levels in a small seepage draining the 2 plantations range 0.8- 68 µg/L over the next 2 months. Site 2 sediments ranged from 1.6 to 22 µg/kg wet weight two weeks after spraying. No fish mortality or behavioral changes were recorded during applications. However, brown trout movement within the application area was significantly different (increased) than the upstream control movement. No changes in trout physiology were observed.	450200-03 Davies *et al*., 1994 (Species are not native to North America; Raw data unavailable for statistical analyses)

Table A-24. Freshwater Natural and Aritfical Streams

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Artificial stream in laboratory Technical Atrazine: 98.2% Experiment 1: Constant 12-day exposures at 0, 24 & 134 µg/L atrazine Experiment 2 involved pulsed exposures of 4 herbicides mixed together at nominal concentrations of: Atrazine at 135 µg/L; Alachlor at 90 µg/L; Metolachlor at 200 µg/L; Metribuzin at 20 µg/L. Full concentrations on Days 8 & 9, halved on Days 10 & 11, and discontinued on Day 12.	Constant 12-day exposure tests (Days 8-17) 10 and 25EC: o 24 µg/L: - 24% red. sign. (p<.001) in ash-free dry wt. at 25EC - 30% red. sign. (p<.01) in chlorophyll a at 25EC o 134 ug/L: - 47% red. sign. (p<.001) in ash-free dry wt. at 10EC - 31% red. sign. (p<.001) in ash-free dry wt. at 25EC - 44% red. s ign. (P<.001) in chlorophyll a at 25EC - 30% red. s ign. (P<.01) in chlorophyll a at 10EC Nutrient uptake was affected more by the 15EC difference, than the atrazine concentrations. Raw data were absent and statistically analyses could not be assessed. As cited: - 35% red. N uptake at 134 µg/L at 10EC; not sign. - 25% red. N uptake at 134 µg/L at 25EC; not sign. - 31% red. silica uptake at 134 µg/L at 10EC; not sign. - 58% red. silica uptake at 134 µg/L at 25EC; not sign. - 14% red. P uptake at 134 µg/L at 10EC; not sign. - 8 % red. P uptake at 134 µg/L at 25EC; not sign.	Six artificial streams consisting of a 7.5 cm OD x 123 cm long Pyrex glass tube were tested concurrently for pesticide effects on *aufwuchs* productivity and nutrient uptake (NO_2, NO_3, phosphorus PO_4 and silica were tested after an 7-day colonization period with natural waters from a third order stream in the Sandusky Basin, Ohio. Two experimental designs (continuous and pulsed exposures) were tested under constant lighting, flow rates of 7.8 mL/min. natural creek water and 1.0 mL/min. nutrient water for 20-day periods. Experiment 1. Two "streams" were exposed to continuous nominal atrazine concentrations of 0, 50 and 200 µg/L at 25EC and then repeated at 10EC on Days 8-17. Experiment 2. Three streams were treated to pulsed exposures of a mixture of four herbicides. These results are not relevant to the risk assessment for atrazine.	450200-07 Krieger, Baker and Kramer 1988 Supplemental (The solvent methanol 0.00057% v/v was not added to controls; raw data unavailable for statistical analyses)

Table A-24. Freshwater Natural and Aritfical Streams

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Two artificial model streams in laboratory continuously exposed for 30 days with 60-day recovery period and repeated 4 times in one year. Nominal concentration of 25 µg/L technical grade atrazine dissolved in DMSO; atrazine concentrations in streams were not measured.	25 µg/L Atrazine: After one year of 4 treatment and recovery cycles, it was reported that the treatment did not have any significant or lasting effect on macroinvertebrate population structure, periphyton standing biomass or rates of primary production and community respiration. Two out of 200 statistical tests showed significant effects for atrazine treatment: equitability ($p < 0.029$) during Winter , month 3, and taxa/sample ($P < 0.001$) during the Spring, month 3. Macroinvertebrate drift in streams increased abruptly upon injection in both controls and treatments which was attributed to the solvent rather than to atrazine. Initial drift samples were collected only in the autumn and summer. Drift in the summer samples were "substantially higher" in the atrazine-treated streams than in the DMSO-treated control. Pulses in the number of drifting organisms following toxicant/solvent injection were primarily due to *Baetis* mayflies.	Continuous-flow stream treatment for 30 days at 25 ppb, followed by 60 days of no treatment, and repeated 4 times for one year in artificial, 3.96 m.-long concrete-lined streams inside a laboratory. Invertebrate populations were introduced by colonization from incoming drift with water flowing from a natural creek over a one year period before treatment. Atrazine was injected into the flowing water for periods as described above. Benthic invertebrate populations as follows: two samples (10.2-cm diameter cores) during pretreatment were collected at 45-day intervals for 1 year. Three post-treatment samples were made every 30 days. 24-Hour invertebrate drift samples were collected were collected on days 1, 5, 10, 20, and 29 during treatment and on days 14, 42 and 60 during recovery periods. Dry and ash weights of periphyton standing crop on four 25 x 75 mm glass slides were sampled at 4-day intervals for 28 days before and after each treatment. 24-Hour gross primary production and community respiration rates (O_2 levels) were measured during the autumn on days 2, 4, 8, 15, 24 and 29 after treatment and on days 20, 42, 54 and 60 during the recovery period.	450200-09 Lynch *et al*., 1985 Supplemental DMSO is not an acceptable solvent, because it accelerates the movement of chemicals across cell membranes. As such it represents a worst case exposure. Raw data were not available for statistical analyses. Three or four samples are considered inadequate for field samples to show anything short of severe effects.
Artificial model streams in laboratory: (7 days; nominal) Single applications to spring water; Brazos, Texas. Nominal test concentrations: 0, 100, 1000 and 10,000 µg/L	o statistically significant reductions (*) in net stream community productivity compared to controls: Day 1 / Day 3 / Day 7 100 µg/L: 736 %* / 117 %* / 34 % 1000 µg/L: 1367 %* / 227 %* / 119 %* 10,000 µg/L: 1716 %* / 264 %* / 135 o sign. ($p<0.02$) increase in *Nitzschia* cell numbers o no significant effect on other dominant algal groups o no significant effect on community respiration rates o no significant effect on conductivity or alkalinity	Four replicate recirculating artificial streams per treatment. Each stream (2.43 m long, 12.5 cm wide and 6 cm deep) was lined with polyethylene plastic and a single layer of gravel. Water from Minter Spring is a nearly anoxic and has a constant temperature (21EC). The flow rate was about 5 cm/sec. The principal algae genera were *Anabaena, Nitzschia, Rhopalodia* and *Navicula*. Five weeks for colonization of benthic algae on glass slides. Each stream received a single treatment which was recirculated. Nominal conc. were 0, 0.1, 1.0 and 10 µg/L. Endpoints were net community productivity, respiration rate, cell numbers of dominant species, conductivity and alkalinity.	450200-10 Moorhead and Kosinski 1986 Supplemental (raw data unavailable)
Not assayed, nominal conc. of 5, 25, and 125 ppb	Snail (*Lymnaea palustris*)	Snails exposed to one time dosing in mesocosm of either 5, 25, or 125 ppb and monitored for 12 weeks, no affect on growth, fecundity, or saccharide metabolism.	450200-13 Baturo *et al* ., 1995

Table A-24. Freshwater Natural and Aritfical Streams

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Mean concentrations over two months of 5, 10, 22, 68, 182, and 318 ppb	Phyto- and zooplankton	Mesocosms in Bavaria were treated with atrazine 3 times over 3 summer months. Dose responsive reductions in dissolved oxygen and pH were noted at concentrations greater than 5 ppb. Substantial biological effects were generally noted at concentrations ≥182 ppb. Some effects on copepod nauplii were noted at 68 ppb. Diatoms appeared to become the dominant phytoplankton.	450200-22 Jüttner *et al*., 1995 Supplemental (raw data unavailable)
Nominal concentrations of 20, 100, 200, and 500 ppb. Measurements bi-weekly or monthly but results based on nominal concentration	Phytoplankton	Results of single species assays, microcosm, and pond studies were compared. Carbon fixation was used as the end-point for all three study types. Laboratory results with eight algal species ranged from 37 to 308 ppb for carbon uptake inhibition EC_{50} values. Microcosm EC_{50} values ranged from 103 to 159 ppb. The mean pond EC_{50} was 100 ppb for carbon uptake and 82 ppb for chlorophyll-a inhibition. Authors stated that multiple laboratory studies or a microcosm study represent(s) entire ecosystem functional effects.	450200-15 Larsen *et al*., 1986 Supplemental (raw data unavailable)

A.2.8b Freshwater Field Studies (New Open Literature Data)

Based on the results of the 2003 IRED for atrazine, potential adverse effects on sensitive aquatic plants and non-target aquatic organisms including their populations and communities, are likely to be greatest when atrazine concentrations in water equal or exceed approximately 10 to 20 µg/L on a recurrent basis or over a prolonged period of time. Given the large amount of microcosm/mesocosm and field data for atrazine, only effects data that are less than or more conservative than the 10 µg/L aquatic-community effect level were considered. In addition, data for taxa that are directly relavent to the endangered species evaluated as part of this assessment were also considered. Field study data for amphibians, including frogs and salamanders are included in Section D.2.3. Based on the selection criteria for review of new open literature, all of the available studies show effects levels to freshwater fish and invertebrates at concentrations greater than 10 µg/L.

One open literature artifical stream mesocosm study was reviewed because it provides data on freshwater snails, which may be used as surrogate for the endangered dwarf mussel. The results of this study, which are summarized as part of Table A-25, show potential indirect effects to grazing behavior (i.e., increased searching velocity and movement patterns) at 15 µg/L atrazine, due to a decrease in periphyton biomass (Roses et al., 1999; Ecotox Reference # 60860). No significant effects were observed in rates of snail mortality and biomass. An increase in snail activity may represent a change in resource quantity, resulting in increased searching speed when the biomass of periphyton decreases.

Table A-25. Freshwater Mesocosm Study from Open Literature (2006 Review)

Study type/ Test material	Test Organism (Common and Scientific Name) and Age and/or Size	Test Design	Endpoint Concentration in ppm	Citation (EcoRef. #)	Rationale for Use in Risk Assessment[1]
Artifical stream 18 day exposure Atrazine (% ai NR)	Freshwater snails (*Physa acuta* and *Ancylus fluviatilis*)	- U-shaped artifical streams (170 cm L x 20 cm W x 20 cm deep); water velocity = 1 cm/sec; depth = 1.9 – 2.2 cm; photoperiod: 8:16 h light/dark; channel bottoms contained surfaces for algae attachment. - Atrazine injected continuously at 15 ppb in 3 ponds, 3 ponds = control - Endpoints: snail mortality, biomass, and activity; chlorophyll *a* concentration	LOAEC = 15 ppb Sign. changes in grazer behavior, increased searching velocity, and different movement patterns at 15 ppb. No sign. effects on snail mortality or biomass	Roses, et al., 1999 (60860)	QUAL: - no raw data provided - only one atrazine concentration tested - relevance of increased searching velocity in snails to survival, growth and reproductive success is uncertain

[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.3 Toxicity to Estuarine and Marine Animals

A.3.1 Estuarine and Marine Fish, Acute

Acute toxicity testing with estuarine/marine fish using the TGAI is required for atrazine because the end-use product is expected to reach this environment due to its use in coastal counties. The preferred test species is sheepshead minnow. Results of these tests are summarized in Table A-26

Table A-26. Estuarine/Marine Fish Acute Toxicity

Surrogate Species/ Static or Flow-through/ Salinity & Temperature	% ai	96-hour LC50 (ppb) (measured/nominal) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification
Sheepshead Minnow larvae < 24-hours old (*Cyprinodon variegatus*) Static test, T - 20EC Salinity 25, 15, 5 g/L;	97.1	Sal. 25 g/L **2,000** Sal. 15 g/L 2,300 Sal. 5 g/L 16,200 (measured) Slope - no data	moderately toxic	452083-03 & 452277-11 Hall, Jr., Ziegenfuss, Anderson, Spittler & Leichtweis 1994	Supplemental (no raw data on mortalities)
Spot (*Leiostomus xanthurus*) Static test Salinity - 12 g/L; T - 22±1EC	97.4	8,500 (nominal) Slope - no data	moderately toxic	452029-20 Ward & Ballantine 1985	Supplemental (no raw data)
Sheepshead minnow (*Cyprinodon variegatus*) Flow-through test Salinity - 31 g/L; T - 22-23EC	97.1	13,400 (measured) Slope 4.377	slightly toxic	433449-01 Machado 1994	Acceptable
Spot (juvenile) (*Leiostomus xanthurus*) Flow-through test Salinity - 29 g/L; T - 28EC	99.7	> 1,000 (nominal) Slope - none	unknown	402284-01 Mayer 1986	Supplement (48-hour test)
Sheepshead minnow (*Cyprinodon variegatus*) Flow-through test	97.4	> 16,000 (30 % mortlity) (measured) Slope - none	unknown	452029-20 Ward & Ballantine 1985	Supplemental (no raw data)

Since the LC_{50} values are in the range of 1,000 – 10,000 ppb, atrazine is categorized as moderately toxic to estuarine/marine fish on an acute exposure basis. Toxicity data on sheepshead minnow, *Cyprinodon variegatus*, indicates that atrazine toxicity increases with increasing salinity levels. The acute effects endpoint for estuarine/marine fish is based on the LC_{50} value of 2,000 ppb for sheepshead minnow at a salinity of 25 $^{o}/_{oo}$ (MRID 452083-03 and 452277-11).

A.3.2 Estuarine and Marine Fish, Acute (Open Literature 2006 Review)

A.3.3. Acute Marine/Estuarine Toxicity Data - Degradates

A special acute estuarine fish test (72-3) is required to address concerns for the toxicity of atrazine degradates to estuarine fish (preferably sheepshead minnow). Table A-27 presents estuarine/marine fish toxicity data for hydroxyatrazine.

Table A-27. Marine/Estuarine Invertebrate Acute Toxicity (Hydroxyatrazine)

Surrogate Species/ Flow-through or Static	% ai formul.	96-hour LEC_{50} (ppb) (measured/nominal)	Toxicity Category	MRID No. Author/Year	Study Classification
Sheepshead minnow (*Cyprinodon variegatus*) Static test; T = 21-24 °C Salinity = 32‰	97.1	>1,900 (no mortality) (measured)	moderately toxic*	465000-06 Sayers, 2005a	Acceptable

* Biological results for the study were based on the mean-measured concentrations of Hydroxyatrazine, which remained constant at the limit of its water solubility throughout the duration of the tests. Therefore, hydroxyatrazine is not acutely toxic to estuarine/marine fish at the limit of its water solubility.

Although the estuarine/marine fish LC_{50} value (>1,900 ppb) for the degradate, hydroxyatrazine, is within the range classifying it as moderately toxic, the biological results for the study were based on mean-measured concentrations of hydroxyatrazine, which remained constant (≥90% recovery of nominal concentrations) at the limit of its water solubility (~1 ppm ai) throughout the duration of the test (MRID 465000-06). Therefore, the solubility of hydroxyatrazine may limit its toxicity to marine and estuarine invertebrates.

A.3.2 Estuarine and Marine Fish, Chronic

An estuarine/marine fish early life-stage toxicity test using the TGAI is required for atrazine because the end-use product may be applied directly to the estuarine/marine environment or is expected to be transported to this environment 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; an aquatic acute LC_{50} or EC_{50} is less than 1 mg/L; and the pesticide is persistent in water (*i.e.*, half-life greater than 4 days). The preferred test species is sheepshead minnow. Results of this test are summarized below in Table A-28.

Table A-28. Estuarine/Marine Fish Early Life-Stage Toxicity Under Flow-through Conditions

Surrogate Species/ Study Duration/ Flow-through or Static Salinity & Temperature	% ai	NOAEC/LOAEC µg/L (ppb) (measured or nominal)	Statistically sign. (p=0.05) Endpoints Affected	MRID No. Author/Year	Study Classification
Sheepshead Minnow (*Cyprinodon variegatus)* Study duration - unknown Flow-through test Salinity -13g/L; T 30±1EC	97.4	NOAEC 1,900 LOAEC 3,400 (measured)	89 % red. in juvenile survival	452029-20 Ward & Ballantine 1985	Supplemental (no raw data for statistical analyses)
Sheepshead Minnow (*Cyprinodon variegatus)* Study duration – 28 days PH Flow-through test Salinity = 29 – 31 °/oo T = 24 – 27 °C	97.1	NOAEC = **1,100** LOAEC = 2,200 (measured	17% reduction in mean length; 46% reduction in mean wet weight	466482-03 Cafarella, 2005a	Supplemental (only 2 replicate aquaria were tested [4 reps are required], time to hatch endpoint was not assessed, and study was terminated at 28 day PH [32 days are required])

In the 2003 atrazine IRED, chronic estuarine/marine fish data from Ward and Ballentine (1985; MRID # 452029-20) were used to evaluate chronic risks to estuarine/marine fish, based on 89% reduction in juvenile survival of sheepshead minnow (*Cyprinodon variegates*). However, the results of more recent chronic estuarine/marine fish data from Caferalla, 2005a (MRID # 466482-03) show that juvenile growth may be a more sensitive endpoint than survival. Although no effect on pre- or post-hatch survival was observed at atrazine concentrations ranging from 1,500 to 2,200 ppb, juvenile length and wet weights were significantly decreased at the 2,200 ppb treatment level, relative to the control. The NOAEC and LOAEC values, based on growth (i.e., larval length and wet weight) are 1,100 and 2,200 ppb, respectively. Because juvenile growth appears to be the more sensitive endpoint, chronic risks associated with estuarine/marine fish exposure to atrazine are based on respective NOAEC and LOAEC values of 1,100 and 2,200 ppb (MRID # 466482-03).

A.3.3a Sublethal Effects: Estuarine/Marine Fish (2003 IRED Data)

Biagianti-Risbourg and Bastide (1995) exposed juvenile gray mullets (*Liza ramada*) to 170 µg/L atrazine for 9, 20, and 29 days in static tests and for 11 days followed by 18 days of decontamination; and then measured the sublethal effects on the liver. At 170 µg/L, 10, 25 and 60 percent mortality occurred following 9-, 20- and 29-day exposures, respectively; control mortality was a constant 10 percent throughout the test. Treated mullets showed normal behavior until Day 20 after which they stopped feeding and rapidly died; which is in contrast to the 90 percent survival of the treated fish that were transferred to clean water after 11 days of exposure. After 3-days exposure, a number of abnormalities were found in the liver (i.e., hepatic parenchyma with a few cytologically detectable perturbations and hepatocytes had particularly large lipofuscin granules (MRID # 452049-02).

A.3.3b Sublethal Effects: Estuarine/Marine Fish (New Open Literature Data)

Studies identified in the open literature on potential effects to salmon from exposure to atrazine were presented in the freshwater fish discussion. In summary, these studies demonstrated an associated between atrazine exposure and effects on gill physiology (Waring and Moore, 2004) and olfactory function (Moore and Lower, 2001). These effects occurred at or below 1 ug/L. Together, these data suggest that atrazine exposure may cause treatment related effects in salmon; however, the relevancy of these measurement endpoints to assessment endpoints is unclear. The olfaction-related effects are not clearly associated with decreased survival or reproduction in the species considered in this assessment and their relevancy under field conditions is questionable.

In addition, Alvarez (2005; ECOTOX No. 81672) reported results from a chronic study in red drum (*Sciaenops ocellatus*) larvae. However, the study was classified as invalid because a negative control was not used.

A.3.4 Estuarine and Marine Invertebrates, Acute

Acute toxicity testing with estuarine/marine invertebrates using the TGAI is required for atrazine because the end-use product is expected to reach this environment due to its use in coastal counties. The preferred test species are mysid shrimp (*Americamysis bahia*) and eastern oyster (*Crassostrea virginica*). Results of these tests for the TGAI and formulations of atrazine are provided below in Tables A-30 and A-31.

Table A-30. Estuarine/Marine Invertebrate Acute Toxicity

Surrogate Species/ Static or Flow-through/ Salinity & Temperature	% ai.	96-hour LC_{50}/EC_{50} µg/L (ppb) (measured/nominal) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification
Copepod (*Acartia tonsa*) Static-renewal - daily Salinity - 31 g/L; T 22°C	70 Tech.	88 (measured) Slope 0.947	very highly toxic	452029-18 Thursby *et al*. 1990 memo	Supplemental (12% control mortality)
Copepod (*Acartia tonsa*) Static test Salinity - 20 g/L; T 20±1 °C	97.4	**94** (nominal) Slope - none	very highly toxic	452029-20 Ward & Ballantine 1985	Supplemental (no raw data)
Copepod (*Acartia tonsa*) Static-renewal - daily Salinity - 31-32 g/L; T 22 °C	70 Tech.	139 (measured) Slope 0.543	highly toxic	452029-18 Thursby *et al*. 1990 memo	Supplemental (20% control mortality)
Copepod nauplii < 24 hours old (*Eurytemora affinis*) Static test; T – 20 °C Salinity - 5, 15 & 25g/L	97.1	Sal. 5 g/L 500 Sal. 15 g/L 2,600 Sal. 25 g/L 13,300 (measured) Slope - no data	highly toxic to slightly toxic	452083-03 & 452277-11 Hall, Ziegenfuss, Anderson, Spittler & Leichtweis 1994	Supplemental (no raw data on mortality)
Mysid Shrimp (*Americamysis bahia*) Flow-through test Salinity 26 g/L; T 22±1 °C	97.4	1,000 (Measured) Slope - none	highly toxic	452029-20 Ward & Ballantine 1985	Supplemental (no raw data)

Table A-30. Estuarine/Marine Invertebrate Acute Toxicity

Surrogate Species/ Static or Flow-through/ Salinity & Temperature	% ai.	96-hour LC_{50}/EC_{50} µg/L (ppb) (measured/nominal) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification
Brown Shrimp (juvenile) (*Penaeus aztecus*) Flow-through test Salinity - 30 g/L; T 27 °C	99.7	1,000 (nominal) Slope - none	at least highly toxic	402284-01 Mayer 1986	Supplemental (48-hr LC_{50} & no raw data)
Copepod - 17 days old (*Acartia tonsa*) Flow-through test Salinity - 31-33 /L, T – 20 °C	97.1	4,300 (measured) Slope - 2.467	moderately toxic	452083-08 McNamara 1991	Supplemental (cloudy with no 0.45 µm filter of undissolved test material)
Mysid Shrimp (*Americamysis bahia*) Flow-through test Salinity -32 g/L; T 25-26 °C	97.1	5,400 (measured) Slope 4.513	moderately toxic	433449-02 Machado 1994	Acceptable
Pink Shrimp (*Penaeus duorarum*) Static test Salinity 26 g/L; T 22±1 °C	97.4	6,900 (nominal) Slope - none	moderately toxic	452029-20 Ward & Ballantine 1985	Supplemental (no raw data)
Copepod (*Acartia clausii*) Static-renewal - daily Salinity - 31 g/L; T 6-6.2 °C	70 Tech.	7,900 (nominal) Slope 0.958	moderately toxic	452029-18 Thursby *et al*. 1990 memo	Acceptable
Grass Shrimp (*Palaemonetes pugio*) Static test Salinity - 26 g/L; T 22±1 °C	97.4	9,000 (nominal) Slope - none	moderately toxic	452029-20 Ward & Ballantine 1985	Supplemental (no raw data)
Eastern oyster (juvenile) (*Crassostrea virginica*) (Shell deposition) Flow-through test Salinity - 28 g/L; T – 28 °C	99.7	> 1,000 no effect (nominal) Slope - none	unknown	40228-01 Mayer 1986	Supplemental (EC_{50} has not been identified & no raw data)
Eastern oyster (juvenile) (*Crassostrea virginica*) (Shell deposition) Flow-through test Salinity 31-32 g/L; T =20-21 °C	97.1	> 1,7 00 no effect (measured) Slope - none	unknown	466482-01 Caferalla, 2005b	Acceptable
Mud Crab (*Neopanope texana*) Static test Salinity & T - unknown	Tech.	> 1,000 (nominal) Slope - none	slightly toxic	000247-19 Bentley & Macek 1973	Supplemental (LC_{50} exceeds water solubility)

Since the lowest acute LC_{50}/EC_{50} value is 94 ppb (i.e., < 0.1 ppm), atrazine is categorized as very highly toxic to estuarine/marine invertebrates on an acute exposure basis. The estuarine/marine LC_{50} value of 94 ppb is based on an acute static toxicity test for the copepod, *Acartia tonsa* (MRID # 452029-20).

Toxicity data for a different copepod, *Eurytemora affinis*, indicates that atrazine toxicity decreases with increasing salinity levels. The pattern of decreasing toxicity for estuarine/marine invertebrates is opposite to the atrazine toxicity data pattern for estuarine/marine fish,

sheepshead minnows (*C. variegates*) where toxicity increased with increasing salinity. The acute toxicity shows that estuarine/marine mollusks, including the Eastern oyster (*Crassostrea virginica*) are less sensitive to atrazine with shell deposition EC_{50} values >1,700 ppb (MRID # 466482-01).

Table A-31. Estuarine/Marine Invertebrate Acute Toxicity - Formulations

Surrogate Species/ Static or Flow-through	% ai. Product	96-hour LC50/EC50 µg/L (ppb) (measured/nominal) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification
Eastern Oyster (*Crassostrea virginica*) (Shell deposition) Flow-through test Salinity -11.8 mg/L; T 21EC	79.6 80 WP	> 800 no effect (nominal) Slope - none	unknown	000247-20 Wright & Beliles 1966	Supplemental (EC_{50} has not been identified)
Pacific Oyster (*Crassostrea gigas*) 24-Hour Static-Renewal	??	> 100 (nominal) 0.1 - 50% dead at 22 days 0.2 - 50% dead at 18 days	unknown	452277-22 Moraga & Tanguy 2000	Supplemental (no 96-hour LC50 value)
European Brown Shrimp (*Crangon crangon*) Static test; 15EC	?? WP	10,000 - 33,000 (nominal) no slope	slightly toxic	452277-28 Portmann 1972	Supplemental (only 48 hours & no raw data)
European Cockle (*Cardium edule*) Static test; 15EC	?? WP	> 100,000 (nominal) no slope	practically non-toxic	452277-28 Portmann 1972	Supplemental (only 48 hours; LC_{50} exceeds water solubility & no raw data)
Fiddler Crab (*Uca pugilator*) Static test Salinity - 30 g/L; T 19EC	79.6 80 WP	198,000 (nominal) Slope - none	unknown	000243-95 Union Carbide Corp. 1975	Supplemental (LC_{50} exceeds water solubility)
Fiddler Crab (*Uca pugilator*) Static test Salinity - 30 g/L; T 19EC	Unknown 4-1-3-1 WDL	239,000 (nominal) Slope - none	unknown	000243-95 Union Carbide Corp. 1975	Supplemental (LC_{50} exceeds water solubility)

The toxicity of formulated atrazine products to marine/estuarine invertebrates are uncertain, because the EC/LC_{50} values are not definitive.

Degradates: Estuarine invertebrate acute tests (72-3) are required to address concerns for the toxicity of atrazine degradates to estuarine invertebrates (preferably *Americamysis bahia*). Table A-32 presents estuarine/marine invertebrate toxicity data for hydroxyatrazine.

Table A-32. Estuarine/Marine Invertebrate Acute Toxicity (Hydroxyatrazine)

Surrogate Species/ Flow-through or Static	% ai formul.	96-hour LEC_{50} (ppb) (measured/nominal)	Toxicity Category	MRID No. Author/Year	Study Classification

Table A-32. Estuarine/Marine Invertebrate Acute Toxicity (Hydroxyatrazine)

Surrogate Species/ Flow-through or Static	% ai formul.	96-hour LEC_{50} (ppb) (measured/nominal)	Toxicity Category	MRID No. Author/Year	Study Classification
Mysid Shrimp (*Americamysis bahia*) Static test Salinity +22-25 g/L; T 25-26 °C	97.1	>2,000 (5% mortality (measured)	moderately toxic*	465000-03 Sayers, 2005b	Acceptable

* The highest concentration tested in this study approximated the functional water solubility of hydroxyatrazine in natural seawater; therefore, hydroxyatrazine is not toxic to mysids on an acute basis at the limit of its water solubility.

Although the estuarine/marine invertebrate LC_{50} value (>2,000 ppb) for the degradate, hydroxyatrazine, is within the range classifying it as moderately toxic, the highest concentration tested in this study approximated the functional water solubility of hydroxyatrazine in natural seawater; therefore, hydroxyatrazine is not likely to be acutely toxic to estuarine/marine invertebrates at the limit of its water solubility. During the 96-hour test, mortality was 5% in the control and mean-measured 500 and 2000 ppb a.i. treatment groups and 0% in the mean-measured 62, 130, 250, and 1000 ppb a.i. treatment groups (MRID # 465000-03). No sub-lethal effects were observed during the exposure period.

A.3.5 Estuarine and Marine Invertebrate, Chronic

An estuarine/marine invertebrate life-cycle toxicity test using the TGAI is required for atrazine because the end-use product may be applied directly to the estuarine/marine environment or is expected to be transported to this environment 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 recurrent; an aquatic acute EC_{50} is less than 1 mg/L; and the pesticide is persistent in water (*e.g.*, half-life greater than 4 days). The preferred test species is mysid shrimp. Results of this test are summarized below in Table A-33.

Table A-33. Estuarine/Marine Invertebrate Life-Cycle Toxicity

Species/ Duration/ Flow-through/ Static-renewal	**% ai**	**NOAEC/LOAEC µg/L (ppb) (measured/noml)**	**Statistically sign. (P=0.05) Endpoints Affected**	**MRID No. Author/Year**	**Study Classification**
Mysid *(Americamysis bahia)* Duration of test - unknown Flow-though test Salinity 20 g/L; T 25±1 °C	97.4	**NOAEC 80** LOAEC 190 (measured)	37 % red. in adult survival	452029-20 Ward & Ballantine 1985	Supplemental (no raw data for statistical analyses)
Mysid *(Americamysis bahia)* Study Duration = 28 days Flow-though test Salinity 19-21 g/L; T 26±2 °C	97.1	NOAEC 260 LOAEC 500 (measured)	9.8% red. in male length 11% red. in male dry weight 8.5% red. in female dry weight	466482-02 Cafarella, 2005c	Supplemental (no raw data for statistical analyses)

The chronic endpoint for estuarine/marine invertebrates is based on a 37% reduction in adult mysid survival at a concentration of 190 ppb, with a corresponding NOAEC of 80 ppb (MRID 452029-20).

A.3.6 Sublethal Effects: Estuarine/Marine Invertebrates (New Open Literature Data)

Two studies in the marine invertebrate copepod were located; one study was considered invalid because a negative control was not used. The remaining study is summarized in Table A-34. Forget-Leray et al. (2004) reported results from a 96-hour, a 10-day, and a 30-day exposure study. An acute 96-hour LC_{50} of 125 ug/L in the copepod *E. affinis* nauplii. In a 10-day study reported in the same study report, a NOAEC of 25 ug/L (LOAEC of 49 ug/L) was reported for mortality. Delayed maturity was also observed at 25 ug/L in a 30-day exposure study. These studies, however, were limited because DMSO was used as a solvent. DMSO is not an acceptable solvent, because it accelerates the movement of chemicals across cell membranes. As such it represents a worst case exposure. For this reason, this study was not used to quantify potential risks to marine/estuarine invertebrates, but was used to qualitatively characterize such risks. In addition, the relationship between delayed maturity and survival and reproductive success is uncertain.

Table A-34. Estuarine/Marine Invertebrates Sublethal Effects Toxicity Tests from Open Literature (2006 Review)

Study type/ Test material	Test Design	Test Organism (Common and Scientific Name) and Age and/or Size	Endpoint Concentration in ppb	Citation (EcoRef. #)	Rationale for Use in Risk Assessment[1]
Acute and chronic studies / Atrazine unspecified purity	**Study duration:** 4 – 30 days **Atz Concs:** not reported (acute); 25 ug/L (10-day study) **Exposure:** Static (acute); semi-static (10-day study) **Endpoints:** Survival, development **Temp**: 18 Deg C. **Solvent:** DMSO	Copepods (*Eurytemora affinis*) from the Seine river estuary (France).	An acute 96-hour LC50 was estimated for the copepod *E. affinis* nauplii of 125 ug/L for atrazine. A 10-day study was conducted using *E. affinis* (nauplius stage) that produced a NOAEC for survival of 25 ug/L and a LOAEC of 49 ug/L. Delayed maturity was also observed at 25 ug/L in the 30-day exposure study.	Forget-Leray et al., 2004 (80951)	Qualitative. No chronic value was previously available in copepods. However, reporting limitations and use of DMSO as a solvent preclude its use to calculate RQs. Reporting limitations included number and identification of test concentrations, % mortality at the LOAEC, and control responses.

Bejarano and Chandler (2003; ECOTOX No. 73333) reported results from a 2.5 generation reproduction study in copepods. This study was considered invalid because a negative control group was not used.

A.3.7a Estuarine and Marine Field Studies (2003 IRED Data)

A summary of all the estuarine/marine aquatic microcosm and mesocosm field studies that were summarized as part of the 2003 IRED is included in Tables A-35 and A-36, respectively.

Table A-35. Marine/Estuarine Microcosm Tests

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Estuarine microcosm: Wild celery *Vallisneria Americana* 1 treatment Nominal concentrations of 4, 8,16, 32, and 64 ppb	NOAEC < 4 ppb 4 ppb (reproductive season) o sign. 16% reduction in tuber formation o 55% reduction in biomass 8 ppb (reproductive season) o 21% reduction in tuber formation 16 ppb (mid season and reproductive season) o 60% reduction in tuber formation o 27% reduction in tuber weight o sign. reduction in leaf growth, biomass, and female flowers 64 ppb (reproductive season) o 75% reduction in tubers o reduction in female flowers	Laboratory microcosms were used to grow *Vallisneria americana* through entire seasons (divided into three periods: early-, mid-, and reproductive). The aquaria were dosed one time at nominal concentrations after a 14-day acclimation period. With respect to leaf growth, atrazine caused the plants to be shorter and more fragile. With respect to flowering and rhizome production, effects were generally first noted at the 16 to 32 ppb range. Tuber formation appeared to be the most sensitive endpoint, with production in terms of numbers significantly reduced at the 4 ppb level.	450200-01 Cohn, 1985 Supplemental (raw data unavailable)

Table A-35. Marine/Estuarine Microcosm Tests

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Estuarine lab microcosm: 7-day exposure Nominal concentrations of 22, 220, and 2200 ppb Estuarine field microcosm 108-days duration Single exposure Nominal applications of 0.4, 1.4, 4.5, and 45 lb ai/A	"NOAEC" = 10 ppb (based on author's use of a 10-fold safety factor from the I_1 level = 100 ppb) 200 ppb (1 week) o significant (0.05 level) reduction in cell # of *Thalassiosira fluviatilis* o significant reduction in photosynthesis of *T. fluviatilis* and *Nitzschia sigma* 2200 ppb (1 week) o significant reduction in cell #, photosysnthesis, and chlorophyll content for both algae 1.4 lb ai/A (effect up to 5 days) o significant reduction in surface chlorophyll and primary production (85-89%) 1.4 lb ai/A (effect up to 8 and 17 days) o significant reduction in carbon fixation (52-73%) 0.4/4.5 lb ai/A (effect at 16 days, but not 26 days) o significant reduction in carbon fixation 45 lb ai/A (42 days) o significant reduction in carbon fixation No statistical effects found in atrazine treatments on: o periphyton growth measured as chlorophyll a levels; chlorophyll a levels decreased gradually in all samples (treatments & controls) over time, "may have masked an effect of atrazine" o indirect effects on function or taxonomic composition of benthic community structure	Laboratory studies were conducted with the salt marsh edaphic diatoms *Thalassiosira fluviatilis* and *Nitzschia sigma*. The I_{50} for both species combined was reported to be 939 ppb. The I_1 was reported to be 100 ppb, and by applying a 10-fold safety factor, the acceptable level (NOAEC) was reported to be 10 ppb. Subsequently, studies were conducted in greenhouse microcosms (1.4 lb ai/A) and in two field studies (1.4 lb ai/A or 0.4, 4.5, and 45 lb ai/A) on the beach wherein enclosures were sunk into the sand and exposed to a tidal action. Atrazine treatment also appeared to cause a shift to a *Navicula* sp. Dominated system. Field results with higher rates of atrazine were expected, with carbon fixation reduced for up to 16 days at the 2 lower rates and up to 42 days at the highest rate.	450874-06 Plumley and Davis, 1980 Supplemental (raw data unavailable)

Table A-35. Marine/Estuarine Microcosm Tests

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Estuarine microcosm: 5 weeks 3 weekly applications followed by 2 weeks observation. Mean-measured concentration at approx. mid-point of *Spartina* test were 30, 280, and 3100 ppb and in the *Juncus* test were 30, 250, and 3800 ppb	30, 280, and 3100 ppb (5 weeks): o sign. (0.05 level) increase in peroxidase activity in *Spartina alterniflora* 30, 250 and 3100 ppb (5 weeks) o sign. (0.05 level) reduction in chlorophyll a (Chl-a) and Chl- a/Chl-b ration in *Juncus roemerianus* 250 and 3800 ppb (5 weeks) o sign. red. in Chl-b in *J. roemerianus* 3100 ppb (1 week) o sign. red. in growth *of S. alterniflora* 3800 ppb (1 week) o sign. red. in growth of *J. roemerianus* o sign. increase in oxidized lipids in *J. roemerianus* 250 ppb (1 year) o partial recovery in *J. roemerianus* 3800 ppb (1 year) o practically no survival of *J. roemerianus*	Two aquatic estuarine plants were exposed to atrazine in greenhouse microcosms. The plants were exposed to atrazine by placing treated sand on the surface of the pots three times (once a week for the first 3 weeks of the study) followed by 2 more weeks for a total of 5 weeks. The water samples were taken after the third application. The pots were also tidally-exposed (i.e., low tide during the day and high tide at night). *S. alterniflora* plants demonstrated a dose-response increase in peroxidase activity. In contrast, *J. roemerianus* plants demonstrated a dose-responsive reduction in chlorophyll and increase in the amount of oxidized lipids. The authors state that atrazine "should pose no significant adverse effects on *S. alternaiflora*. In contrast, if chronic levels of atrazine persist in the range of 250 ppb or greater, *J. roemerianus* most likely will exhibit die off or decline that may lead to loss of this species within the habitat.	450874-05 Lytle and Lytle, 1998 Supplemental (raw data unavailable)
Estuarine microcosm: Duration not reported Nominal concentrations of 0, 50, and 100 ppb	Both *Nannochloris oculata* and *Phaeodactylum tricornutum* were significantly (mostly at the 0.01 level) affected by changes in light, temperature, and atrazine concentration	A 3x3x3 factorial design examined the effect of temperature, light, and atrazine concentration on two species of estuarine algae. *N oculata* was significantly affected by all variables, and the three two-way and one three-way interactions wer also significant. *P. tricornutum* was affected by the main variables and the only significant interaction was light by atrazine	Mayasich et al., 1986
Estuarine microcosm Duration not reported Nominal concentrations of 0, 15, 30, and 50 ppb	The above mentioned algae were tested together and this variable also caused a significant (0.01 level) effect on *N. oculata* growth rate.	An extension of the above described study. In addition to separate culture, the two estuarine algae were cultured together. The end result was that *P. tricornutum* dominated the cultures due to the stress of atrazine *N. oculata* under optimum growth conditions.	Mayasich et al., 1987

Table A-35. Marine/Estuarine Microcosm Tests

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Estuarine microcosm: 4 weeks Mean-measured concentrations in water were 130 ppb for the "low" treatment and 1200 ppb for the "high" treatment over a 4 week period	130 ppb (Week 1): o no photosynthesis 130 ppb (Weeks 2-4) o sign. reduction in plant total biomass, no change in biomass for 3 weeks 130 ppb (Weeks 1-4) o sign.; averaged 50% reduction in photosysnthesis of *Potamogeton perfoliatus* during the test; steady recovery after first week, but not fully recovered 1200 ppb (Weeks 1-4) o sign. 100% red. in photosynthesis throughout the test 1200 ppb (Weeks 2-4) o sign. plant biomass steadily reduced 1200 ppb (Weeks 3-4) o sign. 80% reduction in shoot density	Aquatic plants were planted and atrazine-treated sediments were added to 700-L microcosms. On Day 1.5, 93.4% of the total atrazine was dissolved in water. In addition to photosynthesis, it was demonstrated that shoot growth was relatively unaffected at 130 ppb, but total biomass was sign. reduced after 2-4 weeks. Plant biomass reductions followed a 1 week lage after photosynthesis reduction. At 1200 ppb, plant biomass had been virtually eliminated by the end of the test. Mean shoot length in the controls declined after week 1 and after week 3 for 1200 ppb.	450874-03 Cunningham et al, 1984 Supplemental (raw data unavailable)
Estuarine microcosm: 22-23 days Single dose Day 0: 30000 ppb – nominal; measured only Day 22-23: 16400-17000 ppb	30,000 ppb (Day 5-22): o sign. ($p \leq 0.05$) red. average ratio of # or ramets (branches): initial # or ramets 30,000 ppb (Day 22 or 23): o sign. ($p \leq 0.05$) 46-58% reduction in total above-ground biomass o sign. ($p \leq 0.05$) 18% reduction in average dry weight per ramet	Experiments were conducted with seagrass Halodule wrightii, examining the effect of atrazine and any interactions of salinity (15, 25, 35 ppt), light intensity (115, 140, 173 $uEm^{-2}s^{-1}$), and cropping (either cut at 4-cm or 6-cm). None of these environmental factors affected the response of the grass to atrazine.	452051-01 Mitchell, 1987 Supplemental (raw data unavailable)

Table A-36. Marine/Estuarine Mesocosm Tests

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Marine Mesocosm: Open Ocean: Phytoplankton: (15 days; measured conc.) Measured = nominal at time zero, concentrations of 0.12, 0.56, and 5.8 ppb	0.12 ppb (differences compared to controls) o sign. lower pH levels (Days 5-14); indicative of reduced photosynthesis o higher dissolved organic nitrogen (DON) (Days 6-11) o up to 50% red. primary production (Days 3-11) o up to 60% red. particulate carbohydrates (Days 5-15) o up to 70% red. chlorophyll (Days 4-15) 0.56 ppb o sign. lower pH levels (Days 5-13) o incr. total dis. organic phosphate (DOP) (Days 3-14) o higher DON (Days 5-15) o up to 50 % red. primary production (Day 3-13) o up to 85% red. particulate carbohydrate (Days 5-15) o up to 80% red. chlorophyll (Days 4-15) 5.8 ppb o sign. lower pH levels (Days 5-11) o up to 200% increase in total DOP (Days 3-14) o up to 200 % increase in total DON (Days 2-15) o up to 50% red. in primary productivity (Days 3-7) o up to 60% red. in partic. carbohydrates (Days 5-15) o up to 30% red. in chlorophyll conc. (Days 4-15)	Mesocosms (2 m^2) inoculated with the diatoms *Thalassiosira punctigera*, *T. rotula*, *Nitzschia pungens* and *Skeletonema costatum* and a prymnesiophtye, *Phaeocystis globosa*. evidenced a dose-responsive elevation in dissolved nitrogen and phosphorous and reduction in primary production at 0.12, 0.56, and 5.8 ppb. The NOEL was reported to be <0.12 ppb. Atrazine at concentrations at 0.12, 0.56 and 5.8 ppb, adversely effects primary production of unicellar algal species at certain growth phases and causes increases in "excretions" of dissolved organic nitrogen and phosphorus. "Excretions" may be caused by atrazine stress on cells or lysis of cells.	450200-21 Bester *et al*., 1995 Supplemental (raw data unavailable)
Nominal applications of 0.4, 4.5, or 45 lb ai/A	Salt marsh edaphic alage	Elaboration of Plumley *et al*., concerning the carbon uptake for algae in the top 0.5 cm of enclosure sediment. Carbon fixation was significantly reduced at the 0.45 and 4.5 lb ai/A treatment levels for 16 days and at the 45 lb ai/A treatment level for 42 days.	450874-06 Plumley and Davis, 1980

A.3.7b Estuarine and Marine Field Studies (New Open Literature Data)

As previously discussed, the 2003 IRED identified 10-20 µg/L as the range of atrazine concentrations in freshwater that are likely to have adverse effects on sensitive aquatic plants and non-target aquatic organisms including their populations and communities. As such, estuarine/marine field data from the open literature were considered only when the relevant endpoints were less than or more sensitive than the 10 µg/L aquatic-community effect level. In addition, data for taxa that are directly relavent to the endangered species evaluated as part of this assessment were also considered. Based on the selection criteria for review of new open literature, all of the available studies show effects levels to estuarine/marine fish, invertebrates, and plants at concentrations greater than 10 µg/L.

One estuarine/marine field study on saltwater eelgrass (*Zostera capricorni*) was reviewed as part of the open literature because it provides data on seagrass, a potential food item and source of habitat for sea turtles (Macinnis-Ng, 2003; Ecotox Reference # 72996). The results of this study, which are summarized as part of Table A-37, show that atrazine is unlikely to affect the chlorophyll *a* concentration of estuarine/marine sea grasses at exposure concentrations ranging from 10 to 100 ppb.

Table A-37. Estuarine/Marine Field Study from Open Literature (2006 Review)

Study type/ Test material	Test Organism (Common and Scientific Name) and Age and/or Size	Test Design	Endpoint Concentration in ppm	Citation (EcoRef. #)	Rationale for Use in Risk Assessment[1]
Field study 10 h exposure Atrazine (% ai NR)	Seagrass (*Zostera capricorni*)	- open-bottom cylindrical containers enclosed grasses within a seagrass meadow; salinity = 35 ppt; temp = 25 ± 1 ºC - Atrazine doses = 0, 10, and 100 ppb at one application - Endpoints: total chlorophyll *a* concentration, effective quantum yield via fluorescence measurements	NOAEC = 100 ppb No difference in total chlorophyll a concentration between treatments and control. Reduction in effective quantum yield (via fluorescence measurements) at both treatments relative to the control, but recovery to control values by end of 10 hour exposure period.	Macinnis-Ng and Ralph, 2003 (72996)	QUAL: - no raw data provided - low number of replicates (2) - relevance of fluorescence endpoints is of limited use in risk assessment.

[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.4 Toxicity to Plants

A.4.1 Terrestrial Plants

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 volatilization (vapor pressure >1.0 x 10^{-5}mm Hg at 25^{o}C) or drift (aerial or irrigation) and/or that may have endangered or threatened plant 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 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 herbicides and any pesticide showing a negative response equal to or greater than 25% in Tier I tests. Tier II tests measure the response of plants, relative to a control, and five or more test concentrations at a test level that is equal to the highest use rate (expressed as lbs ai/A). Results of Tier II seedling emergence and vegetative vigor toxicity testing on the technical material are summarized below in Tables A-38 and A-39.

Based on the results of the tests, it appears that emerged seedlings are more sensitive to atrazine via soil/root uptake exposure than emerged plants via foliar routes of exposure. However, all tested plants, with the exception of corn in the seedling emergence and vegetative vigor tests and ryegrass in the vegetative vigor test, exhibited adverse effects following exposure to atrazine.

For Tier II seedling emergence, the most sensitive dicot is the carrot and the most sensitive monocots are oats. EC_{25} values for oats and carrots, which are based on a reduction in dry weight, are 0.003 and 0.004 lb ai/A, respectively; NOAEC values for both species are 0.0025 lb ai/A.

For Tier II vegetative vigor studies, the most sensitive dicot is cucumber and the most sensitive monocot is onion. In general, dicots appear to be more sensitive than monocots via foliar routes of exposure with all tested monocot species showing a significant reduction in dry weight at EC_{25} values ranging from 0.008 to 0.72 lb ai/A. In contrast, two of the four tested monocots showed no effect to atrazine (corn and ryegrass), while EC_{25} values for oats and onion were 0.61 and 2.4 lb ai/A, respectively.

Table A-38. Nontarget Terrestrial Plant Seedling Emergence Toxicity (Tier II)

Surrogate Species	% ai	EC_{25} / NOAEC (lbs ai/A) Probit Slope	Endpoint Affected	MRID No. Author/Year	Study Classification
Monocot - Corn (*Zea mays*)	97.7	> 4.0 / > 4.0	No effect	420414-03 Chetram 1989	Acceptable
Monocot - Oat (*Avena sativa*)	97.7	**0.004 / 0.0025**	red. in dry weight	420414-03 Chetram 1989	Acceptable

Table A-38. Nontarget Terrestrial Plant Seedling Emergence Toxicity (Tier II)

Surrogate Species	% ai	EC_{25} / NOAEC (lbs ai/A) Probit Slope	Endpoint Affected	MRID No. Author/Year	Study Classification
Monocot - Onion (*Allium cepa*)	97.7	0.009 / 0.005	red. in dry weight	420414-03 Chetram 1989	Acceptable
Monocot - Ryegrass (*Lolium perenne*)	97.7	0.004 / 0.005	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Root Crop - Carrot (*Daucus carota*)	97.7	**0.003 / 0.0025**	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Soybean (*Glycine max*)	97.7	0.19 / 0.025	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Lettuce (*Lactuca sativa*)	97.7	0.005 / 0.005	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Cabbage (*Brassica oleracea alba*)	97.7	0.014 / 0.01	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Tomato (*Lycopersicon esculentum*)	97.7	0.034 / 0.01	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Cucumber (*Cucumis sativus*)	97.7	0.013 / 0.005	red. in dry weight	420414-03 Chetram 1989	Acceptable

Table A-39. Nontarget Terrestrial Plant Vegetative Vigor Toxicity (Tier II)

Surrogate Species	% ai	EC25 / NOAEC (lbs ai/A)	Endpoint Affected	MRID No. Author/Year	Study Classification
Monocot - Corn (*Zea mays*)	97.7	> 4.0 / > 4.0	No effect	420414-03 Chetram 1989	Acceptable
Monocot - Oat (*Avena sativa*)	97.7	2.4 / 2.0	red. in dry weight	420414-03 Chetram 1989	Acceptable
Monocot - Onion (*Allium cepa*)	97.7	**0.61 / 0.5**	red. in dry weight	420414-03 Chetram 1989	Acceptable
Monocot - Ryegrass (*Lolium perenne*)	97.7	> 4.0 / > 4.0	No effect	420414-03 Chetram 1989	Acceptable
Dicot - Root Crop - Carrot (*Daucus carota*)	97.7	1.7 / 2.0	red. in plant height	420414-03 Chetram 1989	Acceptable
Dicot - Soybean (*Glycine max*)	97.7	0.026 / 0.02	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Lettuce (*Lactuca sativa*)	97.7	0.33 / 0.25	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Cabbage (*Brassica oleracea alba*)	97.7	0.014 / 0.005	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Tomato (*Lycopersicon esculentum*)	97.7	0.72 / 0.5	red. in plant height	420414-03 Chetram 1989	Acceptable
Dicot - Cucumber (*Cucumis sativus*)	97.7	**0.008 / 0.005**	red. in dry weight	420414-03 Chetram 1989	Acceptable

A summary of safety studies evaluating phytoxicity of atrazine to woody plants (target species) was submitted to the Agency in 2006 (Wall, 2006). A total of 35 species were tested in 13

separate trials at application rates of 1.5 to 4.0 lbs a.i./Acre. Signs of phytotoxicity were summarized and reported. These data are summarized in Table A-39b below.

Table A-39b. Summary of woody plant safety study (Hall, 2006).

Species	Application Rate (lbs a.i./Acre)	Phytotoxicity (%)
Abies balsamea	2	0
	4	3% General
Azalea	2	5% General
	2	5% General
Barberry	2	50% General[b]
Black pine	2	Chlorosis (IS-1)[a]
Boxwood	2	3.8% General
Chitalpa	2	0%
Common Lilac	2	22% chlorosis 25% necrosis
Conifer shrubs and trees	2	0%
Crabapples	2	0%
Crape-Myrtle	2	Chlorosis (IS-1) [a]
Creeping juniper	2	7.5% General
Cupressocyparis leylandii	2	0 – 1% General
Cypruss leylandii	2	Chlorosis (IS-1) [a]
Ginko	2	0%
Gleditsia triacanthos	2	7.5% General
Hydrangea	2	4 – 16% General
Juniperus	1.5	0%
Ligustrum	2	0%
Locust	2	0%
Macadamia nuts	2	0%
Maple	2	0%
Oak	2	0%
Pears	2	0%
Pinus palustris	1.5	70% General[c]
Pinus strobus	2	5% General
Pinus virginiana	1.5	90% General[c]
Pseudotsuga menziesii	4.3	0%
Purpleleaf plum	2	Chlorosis (IS-1) [a]
Raywood ash	2	Chlorosis (IS-1) [a]
Redbud, Eastern	2	2.5% chlorosis 0.3% necrosis 10% general
Rhododendron, catawba	2	5
Shrubby althaea	2	100% chlorosis 40% necrosis
Spiraea	2	16[b]

Table A-39b. Summary of woody plant safety study (Hall, 2006).		
Species	**Application Rate (lbs a.i./Acre)**	**Phytotoxicity (%)**
Spruce	2	0
Tilia	2	0
a IS rating grades chlorosis severity (normal to excessive color) and ranges from 1 to 5 b Phytotoxicity in controls was up to 45%; other pesticides were included in trial, and sprayer may not have been adequately cleaned. c Effect was noted as being atypical for conifers, and the effect may not be related to atrazine treatment		

A.4.2 Aquatic Plants

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. The following species should be tested at Tier II: *Kirchneria subcapitata*, *Lemna gibba*, *Skeletonema costatum*, *Anabaena flos-aquae*, and a freshwater diatom. Aquatic plant testing is required for atrazine because it is applied on crops outdoors and appears to be mobile with a water solubility value of 33 ppm.

Results of Tier II toxicity testing on technical grade and typical end-use products (TEP) are tabulated below. The data are presented in four toxicity tables separating the freshwater data from the marine data and the short, 7-day or less tests from the longer tests. Tables A-40 and A-41 summarize freshwater plant toxicity for short-term (i.e., $\leq$ 7 days exposure) and longer-term tests. Tables A-42 and A-43 summarize short-term ($\leq$ 10 days exposure) estuarine/marine plant toxicity for technical grade and formulations of atrazine, respectively. Toxicity data for longer-term exposure of atrazine to estuarine/marine plants are provided in Table A-44.

Field studies involving atrazine toxicity to freshwater and estuarine/marine aquatic plants are summarized as part of Sections A.2.8 and A.3.7, respectively.

Table A-40. Nontarget Freshwater Plant Toxicity: short-term ($\leq$ 7 days) (Tier II)

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Vascular Plants:					
Duckweed (*Lemna gibba*) 5-Day test; Static-Renewal	97	170 (nominal) Slope 3.93	50% red. in growth	410652-03d Hughes 1986	Supplemental (5 days, not 14 days)
Duckweed (*Lemna gibba*) 7-Day test; Static-Renewal	97	170 (measured) Slope 2.2	50% red. in growth	420414-04 Hoberg 1991	Supplemental (7 days, not 14 days)
Non-Vascular Plants:					

Table A-40. Nontarget Freshwater Plant Toxicity: short-term (≤ 7 days) (Tier II)

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Cyanophyceae *Oscillatoria lutea* (1week; nominal)	76 80 W	< 1 1,000	93% red. chlorophyll production 100% red. chlorophyll prod.	Torres and O'Flaherty 1976	Supplemental (raw data unavailable)
Chlorophyceae *Stigeoclonium tenue* (1 week; nominal)	76 80 W	< 1 1,000	67% red. chlorophyll production 90% red. chlorophyll production	Torres and O'Flaherty 1976	Supplemental (raw data unavailable)
Green Algae - Chlorophyceae *Chlorella vulgaris* (1 week; nominal)	76 80 W	**1** 1,000	50% red. chlorophyll production 80-87% red. chlorophyll production	Torres and O'Flaherty 1976	Supplemental (raw data unavailable)
Xanthophyceae *Tribonema* sp. (1 week; nominal)	76 80 W	**1** 1,000	42% red. chlorophyll production 75% red. chlorophyll production	Torres and O'Flaherty 1976	Supplemental (raw data unavailable)
Xanthophyceae *Vaucheria geminata* (1 week; nominal)	76 80 W	**1** 1,000	41% red. chlorophyll production 100% red. chlorophyll production	Torres and O'Flaherty 1976	Supplemental (raw data unavailable)
Chlorophyceae *Chlamydomonas reinhardi* (24 hour; nominal)	Unk.	19 44 48	50% red. carbon uptake; media: Taub & Dollar (TD)	450200-15 Larsen *et al*. 1986	Supplemental (raw data unavailable)
Chlorophyceae *Kirchneria subcapitata* =*Selenastrum capricornutum* (96 hours; nominal)	Tech.	26 26	50% red. cell growth 50% red. floresence	Caux, Menard, and Kent 1996	Supplemental (NOAEC and raw data unavailable)
Chlorophyceae *Kirchneria subcapitata* = *Selenastrum capricornutum* (24 hours; nominal)	Unk.	34 42 53	50% red. 14-carbon uptake; media: Taub & Dollar (TD); algal assay & TD, respect.	450200-15 Larsen *et al*. 1986	Supplemental (raw data unavailable)
Cyanophyceae *Anabaena cylindrica* (?? hours; nominal)	97	37	50% red. in photosynthesis	Stratton & Corke 1981	Supplemental (no raw data)
Chlorophyceae *Scenedesmus obliquus* (24 hour; nominal)	Unk.	38 49 57	50% red. 14-carbon uptake; media: Taub & Dollar (TD)	450200-15 Larsen *et al*. 1986	Supplemental (raw data unavailable)
Chlorophyceae *Kirchneria subcapitata* =*Selenastrum capricornutum* (120 hours; measured)	97.1	49 NOAEC 16 Slope 4.002	50% red. cell growth	430748-02 Hoberg 1993	Acceptable
Cyanophyceae *Anabaena inaequalis* (?? hours; nominal)	97	50	50% red. in photosynthesis	Stratton & Corke 1981	Supplemental (no raw data)
Chlorophyceae *Kirchneria subcapitata* = *Selenastrum capricornutum* (120 hours; nominal)	97.4	53 NOAEC <32 LOAEC 32 Slope 4.127	50% red. growth 17% red. growth	410652-04 Parrish 1978	Supplemental (NOAEC, method & raw data unavailable)

Table A-40. Nontarget Freshwater Plant Toxicity: short-term (≤ 7 days) (Tier II)

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Bacillariophyceae Navicula pelliculosa (120 hours; nominal)	97.1	60 NOAEC <10 LOAEC 10 Slope 2.31	50% red. growth	410652-03a Hughes 1986	Acceptable (EC50 extrapolated; and NOAEC was not determined)
Chlorophyceae *Ankistrodesmus* sp. (24 hours; nominal)	Unk.	61 72 219	50% red. 14-carbon uptake; media: Taub & Dollar (TD), TD & algal assay, respect.	450200-15 Larsen *et al*. 1986	Supplemental (raw data unavailable)
Ulothrix subconstricta Tentative species identification (24 hours; nominal)	Unk.	88	50% red. 14-carbon uptake; medium: Taub & Dollar (TD)	450200-15 Larsen *et al*. 1986	Supplemental (raw data unavailable)
Cyanophyceae *Anabaena variabilis* (?? hours; Nominal)	97	100	50% red. in photosnythesis	Stratton & Corke 1981	Supplemental (no raw data)
Stigeoclonium tenue Tentative species Identification (24 hours; nominal)	Unk.	127 224	50% red. 14-carbon uptake; media: Taub & Dollar (TD)	450200-15 Larsen *et al*. 1986	Supplemental (raw data unavailable)
Chlorophyceae *Kirchneria subcapitata* *=Selenastrum capricornutum* (96 hours; measured)	97	130 NOAEC 76 Slope 6.628	50% red. cell growth	420607-01 Hoberg 1991	Supplemental (higher light intensity than recommended)
Cyanophyceae *Anabaena cylindrica* (24 hour; nominal)	Unk.	178 182 253	50% red. 14-carbon uptake; media: Taub & Dollar (TD), algal assay, & TD, respect.	450200-15 Larsen *et al*. 1986	Supplemental (raw data unavailable)
Cyanophyceae *Anabaena flos-aquae* (120 hours; nominal)	97	230 NOAEC <100 LOAEC 100 Slope 1.95	50% red. growth 22% red. growth	410652-03a Hughes 1986	Acceptable (NOAEC was not determined)
Chlorophyceae *Chlorella pyrenoidosa* (120 hours; nominal)	97.4	282 NOAEC 130 Slope 4.216	50% red. growth 7% red. growth	410652-04 Parrish 1978	Supplemental (NOAEC, method & raw data unavailable)
Chlorophyceae *Chlorella vulgaris* (24 hours; nominal)	Unk.	293 305 325	50% red. 14-carbon uptake; media: Algal assay, Taub & Dollar (TD), & TD, respect.	450200-15 Larsen *et al*. 1986	Supplemental (raw data unavailable)

Table A-41. Longer Term, Nontarget Freshwater Plant Toxicity

Surrogate Species/ Duration/ Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Vascular Plants:					
Broad Waterweed *Elodea canadensis* (20 days; measured)	????	NOAEC 2 LOAEC 10	200% incr. dark respiration 33% incr. net photosynthesis	452277-14 Hofmann and Winkler 1990	Supplemental (raw data unavailable)

Table A-41. Longer Term, Nontarget Freshwater Plant Toxicity

Surrogate Species/ Duration/ Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Pondweed *Potamogeton perfoliatus* (4 weeks; initial conc. nominal, terminal conc. measured)	???	30 Week 3: LOAEC 5 NOAEC < 5 4 Weeks: LOAEC 50 NOAEC 5	50% red. O_2 product. sign. red. O_2 product. sign. red. O_2 product.	Kemp *et al*. 1985	Supplemental (raw data unavailable)
Duckweed *Lemna gibba* (14 days; measured)	97.1	**37** LOAEC 3.4 NOAEC < 3.4 Slope 1.716	50% red. growth 19% red. growth (frond production)	430748-04 Hoberg 1993	Supplemental (NOAEC was not determined)
Duckweed - *Lemna gibba* (14 days; measured)	97.4	43 NOAEC 10 Slope 1.995	50% red. growth (frond production)	430748-03 Hoberg 1993	Acceptable
Duckweed *Lemna gibba* (14 days; measured) Includes recovery phase	98.5	64 67 NOAEC = 18 Slope 3.96 $\pm$ 0.316	50% red biomass 50% red frond count	461509-01 Desjardins et al., 2003	Acceptable
Broad Waterweed *Elodea canadensis* (3 weeks; nominal)	???	80	50% red. shoot length	450874-10 Forney and Davis 1981	Supplemental (raw data unavailable)
Eurasian Water-Milfoil *Myriophyllum spicatum* (4 weeks; initial conc. nominal, terminal conc. measured)	????	91 NOAEC 5 LOAEC 50	50% red, O_2 product. Sign. red. O_2 product.	Kemp *et al*. 1985	Supplemental (raw data unavailable)
Non-Vascular Plants:					
36 freshwater algal strains (2 weeks; nominal)	99.0	10 1,000	growth <than control strong growth red.	Butler *et al*. 1975	Supplemental (raw data unavailable)
Chlorophyceae *Chlorella vulgaris* (11 days; nominal)	99.9	25	50% red. cell growth	452277-03 Burrell *et al. 1985*	Supplemental (raw data unavailable)
Cyanophyceae *Anabaena inaequalis* (12-14 days[1]; nominal)	>95	30 100 300	50% red. cell count 50% red. growth rate 50% red. photosynthesis	450874-01 Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Chlorophyceae *Ankistrodesmus braunii* (11 days; nominal)	99.9	60	50% red. cell growth	452277-03 Burrell *et al*. 1985	Supplemental (raw data unavailable)
Chlorophyceae *Scenedesmus quadricauda* (12-14 days[1]; nominal)	> 95	100 200 300	50% red. cell count 50% red. growth rate 50% red. photosynthesis	450874-01 Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Chlorophyceae *Chlorella pyrenoidosa* (12-14 days[1]; nominal)	> 95	300 1,000 500	50% red. cell count 50% red. growth rate 50% red. photosynthesis	Stratton 1984	Supplemental (NOAEC and raw data unavailable)

Table A-41. Longer Term, Nontarget Freshwater Plant Toxicity

Surrogate Species/ Duration/ Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Cyanophyceae *Anabaena cylindrica* (12-14 days; nominal)	> 95	1,200 3,600 500	50% red. cell count 50% red. growth rate 50% red. photosynthesis	Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Cyanophyceae *Anabaena variabilis* (12-14 days; nominal)	> 95	4,000 5,000 100	50% red. cell count 50% red. growth rate 50% red. photosynthesis	Stratton 1984	Supplemental (NOAEC and raw data unavailable)

Table A-42. Nontarget Marine/Estuarine Plant Toxicity (Tier II)

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Vascular Plants:					
Fontinalis sp. (24 hours; measured)	NR	NOAEC 2 LOAEC 10	red. net O_2 production		Supplemental (raw data unavailable)
Pondweed (Estuarine) *Potamogeton perfoliatus* (2 hours; nominal)	NR	77	50% red. O_2 evolution	452277-18 Jones and Winchell 1984	Supplemental (Insufficient duration; raw data unavailable)
Pondweed *Potamogeton perfoliatus* (2 hours; nominal)	NR	80 650	50% red. O_2 product. 87% red. O_2 product..	452277-18 Jones *et al*. 1986	Supplemental (Insufficient duration; raw data unavailable)
Zannichellia palustris (2 hours; nominal)	NR	91	50% red. O_2 evolution	452277-19 Jones and Winchell 1984	Supplemental (Insufficient duration; raw data unavailable)
Pondweed (Estuarine) *Potamogeton perfoliatus* (2 hours; nominal)	NR	100	52 to 69% red. in photosynthesis	450874-04 Jones & Estes 1984	Supplemental (raw data unavailable)
Widgeon-Grass (Estuarine) Ruppia maritima (2 hours; nominal	NR	102	50% red. O_2 evolution	452277-19 Jones and Winchell 1984	Supplemental (Insufficient duration; raw data unavailable)
Non-Vascular Plants:					
Blue-green - Cyanophyceae *Oscillatoria lutea* (1week; nominal)	76 80 W	1 1,000	93% red. chlorophyll production 100% red. chlorophyll prod.	000235-44 Torres and O'Flaherty 1976	Supplemental (raw data unavailable)
Green Algae - Chlorophyceae *Stigeoclonium tenue* (1 week; nominal)	76 80 W	1 1,000	67% red. chlorophyll production 90% red. chlorophyll production	000235-44 Torres and O'Flaherty 1976	Supplemental (raw data unavailable)
Green Algae - Chlorophyceae *Chlorella vulgaris* (1 week; nominal)	76 80 W	1 1,000	50% red. chlorophyll production 80-87% red. chlorophyll prod.	000235-44 Torres and O'Flaherty 1976	Supplemental (raw data unavailable)

Table A-42. Nontarget Marine/Estuarine Plant Toxicity (Tier II)

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Xanthophyceae *Tribonema* sp. (1 week; nominal)	76 80 W	1 1,000	42% red. chlorophyll production 75% red. chlorophyll production	000235-44 Torres and O'Flaherty 1976	Supplemental (raw data unavailable)
Xanthophyceae *Vaucheria geminata* (1 week; nominal)	76 80 W	1 1,000	41% red. chlorophyll production 100% red. chlorophyll prod.	000235-44 Torres and O'Flaherty 1976	Supplemental (raw data unavailable)
Chrysophyceae *Isochrysis galbana* (120 hours; nominal)	97.4	**22** NOAEC < 13 LOAEC 13 Slope 3.065	50% red. growth 30% red. growth	410652-04 Parrish 1978	Supplemental (NOAEC, method & raw data unavailable)
Marine Diatom *Skeletonema costatum* (120 hours; nominal)	97.4	24 NOAEC < 13 LOAEC 13 Slope 3.343	50% red. growth 14% red. growth	410652-04 Parrish 1978	Supplemental (NOAEC, method & raw data unavailable)
Marine Diatom Skeletonema costatum (120 hours; measured)	97.1	53 NOAEC 14 Slope 2.798	50% red. cell growth	430748-01 Hoberg 1993	Core
Marine Green - Chlorophyceae *Chlamydomonas* sp. (72 hours; nominal); Salinity 30 g/L	99.7	60	50% red. O_2 production	402284-01 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Yellow - Chrysophyceae *Monochrysis lutheri* (72 hours; nominal); Salinity 30 g/L	99.7	77	50% red. O_2 production	402284-01 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Red - Rhodophyceae *Porphyridium cruentum* (72 hours; nominal); Salinity 30 g/L	99.7	79	50% red. in O_2 production	402284-01 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Green - Chlorophyceae *Neochloris* sp. (72 hours; nominal); Salinity 30 g/L	99.7	82	50% red. in O_2 production	402284-01 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Bacillariophyceae Cyclotella nana (72 hours; nominal); Salinity 30 g/L	99.7	84	50% red. in O_2 production	402284-01 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Bacillariophyceae *Achnanthes brevipes* (72 hours; nominal); Salinity 30 g/L	99.7	93	50% red. in O_2 production	402284-01 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Yellow - Chrysophyceae *Isochrysis galbana* (240 hours; nominal); Salinity 30 g/L	99.7	100	50% red. cell growth	402284-01 Mayer 1986	Supplemental (NOAEC unavailable)

Table A-42. Nontarget Marine/Estuarine Plant Toxicity (Tier II)

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Marine Green - Chlorophyceae *Chlorococcum* sp. (240 hours; nominal); Salinity 30 g/L	99.7	100	50% red. cell growth	402284-01 Mayer 1986	Supplemental (NOAEC unavailable)
Marine Green - Chlorophyceae *Platymonas* sp. (72 hours; nominal); Salinity 30 g/L	99.7	100	50% red. O_2 production	402284-01 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Bacillariophyceae *Thalassiosira fluviatilis* (72 hours; nominal); Salinity 30 g/L	99.7	110	50% red. O_2 production	402284-01 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Bacillariophyceae *Stauroneis amphoroides* (72 hours; nominal); Salinity 30 g/L	99.7	110	50% red. O_2 production	402284-01 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Algae *Microcystis aeruginosa* (120 hours - nominal)	97.4	129 NOAEC 65 Slope 3.162	50% red. growth 7% red. growth	410652-04 Parrish 1978	Supplemental (NOAEC, method & raw data unavailable)
Marine Green - Chlorophyceae *Chlorella* sp. (72 hours; nominal); Salinity 30 g/L	99.7	140	50% red. O_2 production	402284-01 Mayer 1986	Supplemental (NOAEC unavailable
Blue-green - Cyanophyceae *Anabaena cylindrica* (24 hour; nominal)	Unk.	178 182 253	50% red. 14-carbon uptake; media: Taub & Dollar (TD), algal assay, & TD, respect.	450200-15 Larsen *et al*. 1986	Supplemental (raw data unavailable)
Marine green - Chlorophyceae *Dunaliella tertiolecta* (120 hours; nominal)	97	180 NOAEC < 100 LOAEC 100 Slope 1.95	50% red. growth 34% red. growth	410652-03 Hughes 1986	Supplemental (NOAEC unavailable)
Marine Yellow - Chrysophyceae Phaeodactylum tricornutum (240 hours; nominal); Salinity 30 g/L	99.7	200	50% red. cell growth	402284-01 Mayer 1986	Supplemental (NOAEC unavailable)
Marine Bacillariophyceae *Nitzschia closterium* (72 hours; nominal); Salinity 30 g/L	99.7	290	50% red. O_2 production	402284-01 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Bacillariophyceae *Amphora exigua* (72 hours; nominal); Salinity 30 g/L	99.7	300	50% red. O_2 production	402284-01 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Green - Chlorophyceae *Dunaliella tertiolecta* (240 hours; nominal); Salinity 30 g/L	99.7	300	50% red. cell growth	402284-01 Mayer 1986	Supplemental (NOAEC unavailable)

Table A-42. Nontarget Marine/Estuarine Plant Toxicity (Tier II)

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Marine Red - Rhodophyceae *Porphyridium cruentum* (120 hours)	97.4	308 NOAEC <130 LOAEC 130 Slope 2.449	50% red. growth 16% red. growth	410652-04 Parrish 1978	Supplemental (NOAEC, method & raw data unavailable)
Marine Bacillariophyceae *Nitzschia* (Ind. 684) (72 hours; nominal); Salinity 30 g/L	99.7	430	50% red. O_2 production	402284-01 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Green -Chlorophyceae *Kirchneria subcapitata* (120 hours; nominal)	97.4	431 NOAEC 200 Slope 4.217	5% red. in growth 4% red. in growth	410652-04 Parrish 1978	Supplemental (NOAEC, method & raw data unavailable)
Marine Bacillariophyceae *Navicula inserta* (72 hours; nominal); Salinity 30 g/L	99.7	460	50% red. in O_2 production	402284-01 Mayer 1986	Supplemental (72 hrs & endpoint)

Table A-43. Formulation Nontarget Marine/Estuarine Algal Toxicity (Tier II)

Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit slope	% Response	MRID No. Author/Year	Study Classification
Mar. Yellow - Chrysophyceae *Isochrysis galbana* (nominal); Salinity 30 g/L	76 80 WP	100 (240 hrs) 200 (2 hrs)	50% red. cell growth 50% red. O_2 production	402284-01 Mayer 1986	Supplemental (NOAEC unavailable)
Mar. Yellow Chlorophyceae *Chlorococcum* sp. (nominal); Salinity 30 g/L	76 80 WP	100 (240 hrs) 400 (2 hrs)	50% red. cell growth 50% red. O_2 production	402284-01 Mayer 1986	Supplemental (NOAEC unavailable)
Mar. Yellow - Chrysophyceae Phaeodactylum tricornutum (nominal); Salinity 30 g/L	76 80 WP	200 (240 hrs) 200 (2 hrs)	50% red. cell growth 50% red. O_2 production	402284-01 Mayer 1986	Supplemental (NOAEC unavailable)
Mar. Green - Chlorophyceae *Dunaliella tertiolecta* (nominal); Salinity 30 g/L	76 80 WP	400 (240 hrs) 600 (2 hrs)	50% red. cell growth 50% red. O_2 production	402284-01 Mayer 1986	Supplemental (NOAEC unavailable)

Table A-44. Longer-term (≥ 10 days exposure) Nontarget Marine/Estuarine Plant Toxicity

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Vascular Plants:					
Sago Pondweed (Estuarine) *Potamogeton pectinatus* (28 days; measured/nominal)	NR	Salinity 12 ppt: **NOAEC 7.5** LOAEC 14.3 Salinity 1 & 6 ppt: NOAEC 14.3 LOAEC 30	 sign. red. dry weight sign. red. dry weight	450882--31 Chesapeake Bay Program 1998	Supplemental (raw data unavailable)

Table A-44. Longer-term (≥ 10 days exposure) Nontarget Marine/Estuarine Plant Toxicity

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Estuarine rush *Juncus roemerianus* (5 weeks - 1 year; measured	97.1	LOAEC 30 NOAEC 30 NOAEC < 30 250 ppb 3, 800 ppb	sign. red. chlorophyll a in 5 weeks (1 year) partial recovery (1 yr) practically no survival	450874-05 Lytle & Lytle 1998	Supplemental (raw data unavailable)
Pondweed *Potamogeton perfoliatus* (4 weeks; initial conc. nominal, terminal conc. measured)	NR	30 Week 3: LOAEC 5 NOAEC < 5 4 weeks: LOAEC 50 NOAEC 5	50% red. O_2 product. sign. red. O_2 product. sign. red. O_2 product.	452277-20 Kemp *et al*. 1985	Supplemental (raw data unavailable)
Pondweed (Estuarine) *Potamogeton perfoliatus* (3 weeks; nominal)	NR	53	50% red. ????	450874-10 Forney and Davis 1981	Supplemental (raw data unavailable)
Eelgrass (Estuarine) *Zostera marina* (10 days; measured)	NR	est. 69 50 80	50% red. leaf growth 25% red. leaf growth 62% red. leaf growth	452277-29 Schwarzschild *et al*. 1994	Supplemental (raw data unavailable)
Estuarine Eelgrass *Zostera marina* (21 days; nominal)	NR	100 NOAEC 10	21-day LC50 red. production	452277-05 Delistraty and Hershner 1984	Supplemental (raw data unavailable)
Wild Celery (Estuarine) *Vallisneria americana* (6 weeks; nominal)	NR	163	50% red. shoot length no difference at 0, 3, or 6 parts/thousand	450874-10 Forney and Davis 1981	Supplemental (raw data unavailable)
Seagrass (Estuarine) *Halodule wrightii* (22 - 23 days; measured)	Atrazine 4L	30,000	46-58% red. total above-ground biomass	452051-01 Mitchell 1987	Supplemental (raw data unavailable)
Non-Vascular Plants:					
Marine Brown macroalgae *Laminaria hyperborea* (18 days; nominal)	NR	NOAEC < 10 LOAEC 10 50 & 100	sign. red. growth rate delayed sporophyte formation	???? Hopkin &Kain 1978	Supplemental (raw data unavailable)
Marine Yellow - Chrysophyceae *Isochrysis galbana* (240 hours; nominal); Salinity 30 g/L	99.7	100	50% red. cell growth	402284-01 Mayer 1986	Supplemental (NOAEC unavailable)
Marine Green - Chlorophyceae *Chlorococcum* sp. (240 hours; nominal); Salinity 30 g/L	99.7	100	50% red. cell growth	402284-01 Mayer 1986	Supplemental (NOAEC unavailable)
Marine Yellow - Chrysophyceae Phaeodactylum tricornutum (240 hours; nominal); Salinity 30 g/L	99.7	200	50% red. cell growth	402284-01 Mayer 1986	Supplemental (NOAEC unavailable)

Table A-44. Longer-term (≥ 10 days exposure) Nontarget Marine/Estuarine Plant Toxicity

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Marine Green - Chlorophyceae *Dunaliella tertiolecta* (240 hours; nominal); Salinity 30 g/L	99.7	300	50% red. cell growth	402284-01 Mayer 1986	Supplemental (NOAEC unavailable)

The Tier II results for freshwater aquatic plants indicate that atrazine causes a 41 to 98% reduction in chlorophyll production of freshwater algae; the corresponding EC_{50} value for four different species of freshwater algae is 1 ppb, based on data from a 7-day acute study (MRID # 000235-44). Non-vascular plants are less sensitive to atrazine than their freshwater vascular counterparts with an EC_{50} value of 37 ppb, based on reduction in duckweed growth (MRID # 430748-04).

The Tier II results indicate that the marine algae *Isochrysis galbana* is the most sensitive nonvascular aquatic plant (EC_{50} = 22 ppb; MRID # 410652-04), and the most sensitive vascular aquatic plant is Sago pondweed (7.5 ppb; MRID # 450882-31).

Comparison of atrazine toxicity levels for three different endpoints suggests that the endpoints in decreasing order of sensitivity are cell count, growth rate and oxygen production (Stratton 1984). Walsh (1983) exposed *Skeletonema costatum* to atrazine and concluded that atrazine is only slightly algicidal at relatively high concentrations (i.e., 500 & 1,000 ppb). Caux *et al*. (1996) compared the cell count IC_{50} and fluorescence LC_{50} and concluded that atrazine is algicidal at concentrations which effect cell counts. Abou-Waly *et al*. (1991) measured growth rates on days 3, 5, and 7 for two algal species. The pattern of atrazine effects on growth rates differ sharply between the two species. Atrazine had a strong early effect on *Anabaena flos-aquae* followed by rapid recovery in clean water (i.e., EC_{50} values for days 3, 5, and 7 are 58, 469, and 766 ppb, respectively). The EC_{50} values for *Selenastrum capricornutum* continued to decline from Day 3 through 7 (i.e., 283, 218, and 214 ppb, respectively. Based on theses results, it appears that the timing of peak effects for atrazine may differ depending on the test species.

Degradates: Special tests are required for algal and vascular plant species (123-2) to address concerns for the toxicity of atrazine degradates to aquatic plants. A summary of the degradate aquatic plant toxicity data for deethylatrazine, deisopropylatrazine, diamino-atraine, and hydroxyatrazine is provided in Tables A-45 through A-48, respectively.

Table A-45. Degradate Deethylatrazine 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	> 95	1,000	50% red. cell count	Stratton 1984	Supplemental
Anabaena inaequalis		4,000	50% red. growth rate		(NOAEC and raw data
(12-14 days[1]; nominal)		2,500	50% red. photosynthesis		unavailable)

Table A-45. Degradate Deethylatrazine Nontarget Aquatic Plant Toxicity (Tier II)

Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit slope	% Response	MRID No. Author/Year	Study Classification
Freshwater Green - Chlorophyceae	> 95	1,200	50% red. cell count	Stratton 1984	Supplemental
Scenedesmus quadricauda		2,000	50% red. Growth rate		(NOAEC and raw data
(12-14 days; nominal)		1,800	50% red. photosynthesis		unavailable)
Freshwater Green - Chlorophyceae	> 95	3,200	50% red. cell count	Stratton 1984	Supplemental
Chlorella pyrenoidosa		7,200	50% red. growth rate		(NOAEC and raw data
(12-14 days[1]; nominal)		1,800	50% red. photosynthesis		unavailable)
Fresh. Blue-Green - Cyanophyceae	> 95	3,500	50% red. cell count		Supplemental
Anabaena variabilis		7,500	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		700	50 % red. photosynthesis		unavailable)
Fresh. Blue-Green - Cyanophyceae	> 95	8,500	50% red. cell count		Supplemental
Anabaena cylindrica		5.500	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		4,800	50% red. photosynthesis		unavailable)

Table A-46. Degradate Deisopropylatrazine 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	> 95	2,500	50% red. cell count		Supplemental
Anabaena inaequalis		7,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days[1]; nominal)		9,000	50% red. photosynthesis		unavailable)
Freshwater Green - Chlorophyceae	> 95	6,900	50% red. cell count		Supplemental
Scenedesmus quadricauda		6.500	50% red. Growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		4,000	50% red. photosynthesis		unavailable)
Freshwater Green - Chlorophyceae	> 95	> 10,000	50% red. cell count		Supplemental
Chlorella pyrenoidosa		> 10,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days[1]; nominal)		3,600	50% red. photosynthesis		unavailable)
Fresh. Blue-Green - Cyanophyceae	> 95	5,500	50% red. cell count		Supplemental
Anabaena variabilis		9,200	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		4,700	50 % red. photosynthesis		unavailable)
Fresh. Blue-Green - Cyanophyceae	> 95	> 10,000	50% red. cell count		Supplemental
Anabaena cylindrica		> 10,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		9,300	50% red. photosynthesis		unavailable)

Table A-47. Degradate Diamino-Atrazine 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	> 95	7,000	50% red. cell count		Supplemental
Anabaena inaequalis		>10,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days[1]; nominal)		>100,000	50% red. photosynthesis		unavailable)
Freshwater Green - Chlorophyceae	> 95	4,600	50% red. cell count		Supplemental
Scenedesmus quadricauda		10,000	50% red. Growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		>100,000	50% red. photosynthesis		unavailable)
Freshwater Green - Chlorophyceae	> 95	>10,000	50% red. cell count		Supplemental
Chlorella pyrenoidosa		>10,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days[1]; nominal)		>100,000	50% red. photosynthesis		unavailable)

Table A-47. Degradate Diamino-Atrazine 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 variabilis* (12-14 days; nominal)	> 95	>10,000 >10,000 100,000	50% red. cell count 50% red. growth rate 50 % red. photosynthesis	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	Stratton 1984	Supplemental (NOAEC and raw data unavailable)

Table A-48. Degradate Hydroxyatrazine 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	>10,000 >10,000 >100,000	50% red. cell count 50% red. growth rate 50% red. photosynthesis	Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Freshwater Green - Chlorophyceae *Scenedesmus quadricauda* (12-14 days; nominal)	> 95	>10,000 >10,000 >100,000	50% red. cell count 50% red. Growth rate 50% red. photosynthesis	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	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	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	Stratton 1984	Supplemental (NOAEC and raw data unavailable)

The Tier II results for atrazine degradates indicate that deethylatrazine is more toxic than the other four degradates, and the most sensitive algae of the five species is generally the blue-green alga *Anabaena inaequalis* with EC_{50} values ranging from 100 to > 100,000 ppb. Atrazine is more toxic to these algal species than any degradate. The order of descending toxicity for these algal species are atrazine > deethylatrazine > deisopropylatrazine > diamino-atrazine > hydroxy-atrazine.

A.5 Effects of Environmental Factors and Life-Stage on Aquatic Atrazine Toxicity

A.5.1 Interaction Effects on Atrazine Toxicity to Plants

Some intra-laboratory studies suggest that atrazine toxicity is affected by environmental parameters, such as temperature, light intensity and salinity levels. Mayer *et al*. (1998) concluded that a temperature difference of 1 ^{o}C will cause a difference in algal growth rate in the range of 7 to 9 percent at the typical rate increase for 10 ^{o}C temperature increase (Q_{10}) of 2 to 2.3.

In general, the toxicity of pesticides increase with increasing temperature. Mayasich, Karlander and Terlizzi, Jr. (1986) tested two algal species in 27 combinations of temperature (15, 20 and 25 ^{o}C), light intensity (0.208, 0.780 and 1.352 mW/cm^2) and atrazine concentrations of 0, 50 and 100 µg/L) for 7-day periods. Toxic effects of atrazine on *Nannochloris oculata* growth rates were significantly ($p \leq 0.01$) dependent on both temperature and light intensity as determined by the 3-way interactions. Atrazine toxicity increased to *N. oculata* with both increasing temperature and increasing light intensity, except at 15 ^{o}C and 1.352 mW/cm^2 where growth was intermediate. Previous results yielded a similar anomaly and suggest that 15 ^{o}C is near the lower limit for growth of this algal species. With *Phaeodactylum tricornutum*, growth rates were significant ($p \leq 0.01$) for light intensity and atrazine concentrations, and also significant ($p \leq 0.05$) for temperature, but only light intensity was significantly ($p \leq 0.01$) related to an increase in atrazine toxicity. Atrazine toxicity was highest at the lowest light intensity. "The response of *P. tricornutum* to atrazine at light intensities of 0.780 and 1.352 mW/cm^2 may be a reflection of primary effects only, while at 0.208 mW/cm^2, light intensity includes secondary effects" (Mayasich *et al*., 1986). With respect to the insignificant effect of temperature on growth, Ukeles (1961) and Fawley (1984) found that the growth of *P. tricornutum* was unchanged by temperatures in the range of 14 to 25 ^{o}C.

Mayasich *et al*. (1987) repeated the above algal study with lower atrazine concentrations (0, 15, 30 and 50 µg/L and fewer temperatures (15 and 25 ^{o}C) and light intensities (0.208 and 1.352 mW/cm^2) in unialgal and bialgal assemblages. Generally *Phaeodactylum tricornutum*'s presence significantly ($p \leq 0.01$) depressed the growth of *Nannochloris oculata*, but it did not alter the magnitude of the responses to temperature, light intensity or atrazine concentrations. In contrast, the presence of *N. oculata* generally resulted in significant ($p \leq 0.01$) enhancement of *P. tricornutum* growth. The bialgal assemblage produced magnitudes of interactions between temperature and light intensity, and temperature and atrazine were both significantly ($p \leq 0.01$) greater for *N. oculata*. *P. tricornutum* dominated the assemblage over all concentrations of atrazine under simultaneously low levels of temperature (15 ^{o}C) and light intensity (0.208 mW/cm^2). At simultaneous high levels of temperature and light intensity and the absence of atrazine, *P. tricornutum* and *N. oculata* tended to be co-dominant. At increased atrazine concentrations, *P. tricornutum* became the dominant of the two algal species. The authors concluded that the enhanced sensitivity of *N. oculata* to atrazine relative to that exhibited by *P. tricornutum* posed a threat to the diversity and structure of natural phytoplankton populations. Thus, a nutritious algal species for larval oysters (Dupry, 1973) is replaced by what is considered to be a poor food source for larval bivalves (Walne, 1970).

Mayer *et al*. (1998) tested the effect of four main environmental factors on the toxicity of atrazine to the green alga *Selenastrum capricornutum* in 3 day tests. The four factors tested were light intensity (44 and 198 µE/m^2), temperature (16 and 26 ^{o}C), nitrogen source (NH_4+ and NO_3-) and pH (7.6 and 8.6). Temperature influenced growth indirectly by interacting with light intensity. Algal growth measured as the atrazine EC_{50} values was marginally reduced under low light intensity at high and low temperatures (158 and 159 µg/L, respectively versus the atrazine control, 164 µg/L). High light intensity at the low temperature reduced the toxicity of atrazine to the alga by about two fold (LC_{50} 300 µg/L) while high light intensity and high temperature reduced the

toxicity of the atrazine by about 118 fold (LC_{50} 191 µg/L). Nitrogen source and pH had no significant effect on atrazine toxicity affecting algal growth rates.

The above studies indicate that the toxicity of atrazine to plants can be affected by environmental parameters, but differences in effects are dependant on the algal species. Hence, increases in temperature may increase, decrease or have no effect on atrazine toxicity to algal growth. Light intensity generally has a stronger effect on atrazine toxicity to algal growth and may, short of the point of photo-inhibition, increase the toxicity of atrazine. Nitrogen source and pH do not have any effect on the toxicity of atrazine to algae.

A.5.2 Interaction Effects on Atrazine Toxicity to Aquatic Animals

A number of intra-laboratory studies suggest that atrazine toxicity to aquatic animals is affected by environmental parameters, such as water hardness, salinity and differences in the life-stages of organisms.

High levels of water hardness usually reduce the toxicity of pesticides. Intra-laboratory studies on two fish species provide comparative LC_{50} values for two levels of water hardness (Birge, Black and Bruser, 1979). Embryo-larval rainbow trout were exposed to atrazine for 27 days at water hardness levels of 50 and 200 mg/L and produced LC_{50} values of 0.66 and 0.81 mg/L, respectively. Channel catfish were tested at the same water hardness levels for 8 days and yielded LC50 values of 0.22 and 0.23 mg/L. With rainbow trout embryo-larvae, the soft water increased toxicity by about 19 percent, while the LC_{50} values for embryo-larval catfish were the same. It is uncertain if the shorter exposure period, yolk sac, or differences in species sensitivity, account for the difference in water hardness effects between embryo-larvae of channel catfish and rainbow trout.

Salinity effects at 5, 15 and 25 g/L on the toxicity of atrazine are opposite for the estuarine fish larvae, sheepshead minnow and the copepod nauplii, *Eurytemora affinis* (Ziegenfuss, Anderson, Spittler and Leichtweis, 1994). The 96-hour LC_{50} values (16.2, 2.3 and 2.0 mg/L) for sheepshead minnow consistently increased with increasing salinity. In the case of the copepod nauplii, the 96-hour LC_{50} values (i.e., 0.5, 2.6 and 13.3 mg/L) consistently decreased with increasing salinity. The consistency of the two data sets suggest that salinity effects the toxicity of atrazine. Statistical tests for both species indicate significant differences between the LC_{50} valves at 5 and 25 g/L, but not at 15 g/L. The authors concluded that the two species may be more physiologically effective in metabolizing and mitigating toxic effects of atrazine at various salinities. The increase in LC_{50} values for rainbow trout and sheepshead minnow are consistent for increasing water hardness and increasing salinity.

For many pesticides, the earlier life-stages are normally more sensitive than later life-stages. Contrary to most pesticides, the aquatic toxicity data for toad and frog tadpoles suggest that the late stages are more sensitive to atrazine than early tadpole stages (Howe *et al*., 1998). The late stage of the American toad tadpole is about 2.5 times more sensitive to atrazine than the early

stage (10.7 versus 26.5 mg/L). For the northern leopard frog tadpoles, the later stage is about 3.3 times more toxic than the early tadpole stage (14.5 versus 47.6 mg/L).

The above studies suggest that decreases in water hardness and salinity can increase the toxicity of atrazine to fish, but increasing salinity may mitigate atrazine toxicity to copepods. Life stages show differences in sensitivity to atrazine. The later stages in frog and toad tadpole development show an increased sensitivity to atrazine over early tadpole stages.

A.6 References

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