Appendix A. Ecological Effects Characterization

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

With respect to the open literature, studies may be classified as either qualitative, quantitative, or invalid. The degree to which open literature data are quantitatively or qualitatively characterized is dependent on whether the information is relevant to the assessment endpoints (i.e., maintenance of the survival, reproduction, and growth of the assessed listed species) identified in the problem formulation. Open literature studies classified as qualitative are not appropriate for quantitative use but are of good quality, address issues of concern to the risk assessement, and, when appropriate, are discussed qualitatively in the risk characterization discussion. Those open literature studies that are classified as quantitative are appropriate for quantitative use in the risk assessment including calculation of RQs. It should be noted that this appendix includes all relevant data taken from the 2003 IRED atrazine effects appendix. In addition, ECOTOX information was obtained on May 31, 2007. The May 2007 ECOTOX search included all open literature data for atrazine (i.e., pre- and post-IRED). Data that pass the ECOTOX screen described in Section 4.1 of the assessement are evaluated along with the registrant-submitted data, and may be incorporated qualitatively or quantitatively into this endangered species assessment. In general, effects data in the open literature that are more conservative than the registrant-submitted data are considered for quantitative use.

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

A.1 Toxicity to Birds / Reptiles

Given limited 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. No studies in birds that were more sensitive than currently available data were located in the most recent ECOTOX search (October, 2008).

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 these studies 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 sensitive 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. ($p \leq 0.05$) 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
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 consumptio	425471-01 Pedersen & DuCharme 1992	Acceptable

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 # 42547102). 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.

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 00024723).

A.1.4 Birds/Reptiles: Open Literature

A.1.4a Birds: New Open Literature Data

Three 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). However, Wilhelms (2006b) did not observe any effects on body weight, food intake, mortality, circulating corticosterone levels, or weights of liver, ovaries, or oviducts at dietary concentrations up to 1000 ppm in female Japanese quail.

These data study suggest that atrazine was associated with evidence of toxicity at dietary concentrations of 1000 ppm in Japanese quail. However, these open literature studies produced less sensitive LOAECs than the submitted data summarized in Table A-5 and were, therefore, not used to derive risk quotients.

Table A-6. Avian Reproduction/Growth Effects Tests from Open Literature (2007 Review)

Study type/ Test material	Test Organism (Common and Scientific Name) and Age and/or Size	Test Design	Endpoint Concentration	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: 42547101 produced a more sensitive LOAEC
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., 2006a (82035)	Qual: 42547101 produced a more sensitive LOAEC.

Table A-6. Avian Reproduction/Growth Effects Tests from Open Literature (2007 Review)					
Study type/ Test material	**Test Organism (Common and Scientific Name) and Age and/or Size**	**Test Design**	**Endpoint Concentration**	**Citation (EcoRef. #)**	**Rationale for Use in Risk Assessment[(1)]**
Reproduction toxicity study in birds	Japanese quail	Birds were exposed to atrazine in the diet at concentrations that ranged from 0.001 ppm to 1000 ppm.. No effects on body weight, food intake, mortality, circulating corticosterone levels, or weights of liver, ovaries, or oviducts.	NOAEC: 1000 ppm	Wilhelms et al., 2006b	QUAL. No effects were observed in the study.

(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: Open Literature Data (2007 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 (2007 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: 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 treatment-related effects occurred in the study (study did not provide a sensitive endpoint for use in risk assessment). Relevance of exposure pathway (direct application to eges) to the current assessment is questionable.

[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.

No relevant studies in reptiles were located in the October, 2008 Ecotox search.

A. 1.5. Toxicity to Mammals

Acute Exposure (Mortality) Studies

Atrazine is slightly toxic to mammals on an acute oral basis. LD50s are also available for two degradates (DEA and DIA). These degradates are also slightly toxic to rats on an acute oral basis; however, LD50s for both degradates were lower than the LD50 for atrazine. Acute oral toxicity data are summarized in the following table.

Table A-8. Summary of Acute Toxicity of Atrazine and its Degradates of Concern

Chemical	Acute Mammal LD50 (mg/kg-bw)
Atrazine	1900 (MRID 00024709)
HA	Not available
DEA	1240 (MRID 43013201)
DIA	1100 (MRID 43013202)
DACT	Not available

Reproduction Toxicity

In a 2-generation reproduction study (MRID 40431303) technical grade atrazine was administered to Charles River (CRCD, VAF/PLUS) rats 30/sex/dose in the diet at concentrations of 0, 10, 50, and 500 ppm. Parental body weights, body weight gain, and food consumption were statistically significantly reduced at the 500 ppm dose in both sexes and both generations throughout the study. Compared to controls, body weights for F_0 high dose males and females at 70 days into the study were decreased by 12% and 15%, respectively while F_1 body weight for the same time period was decreased by 15% and 13% for males and females, respectively. The only other parental effect which may have been treatment related was a slight, but statistically significant, increase in relative testes weight which occurred in both generations of the high dose. There did not appear to be any reproductive effects from compound exposure. Measured reproductive parameters from both generations did not appear to be altered in a dose-related manner.

The LOAEL was 500 ppm (39 mg/kg/day in males, 43 mg/kg/day in females) based on decreased body weights, body weight gains, and food consumption. The NOAEL was 50 ppm (3.8 mg/kg/day in males, 3.7 mg/kg/day in females).

Reproduction studies are not available on the degradates of concern. However, the degradates have been tested in prenatal developmental studies in rodents. These studies are summarized below. Results for atrazine from the same guideline study are also presented for comparison. The data suggest that the degradates are approximately as toxic as atrazine in these studies. All NOAELs of the degradates are within a factor of 5 of the atrazine NOAEL, although the NOAEL

for DEA and DIA are 2-fold lower, and the NOAEL for DACT is approximately 4-fold lower than the NOAEL for atrazine in the prenatal developmental study.

Table A-.9. Comparison of Toxicity Reference Values for Atrazine and Degradates of Concern in Guideline Prenatal Developmental Toxicity Studies

Chemical	Study type/Data Source	Study Summary
Atrazine	Prenatal developmental in rodents / 40566302	Maternal NOAEL = 10 mg/kg/day. Maternal LOAEL = 70 mg/kg/day, based on reduced body weight gain Developmental NOAEL = 10 mg/kg/day Developmental LOAEL = 70 mg/kg/day based on delayed or no ossification at several sites
	MRID 41065201	Maternal NOAEL = 25 mg/kg/day. Maternal LOAEL = 100 mg/kg/day based on reduced body weight gain and food consumption. Developmental NOAEL = 25 mg/kg/day. Developmental LOAEL = 100 mg/kg/day based on increased incidence of delayed ossification of skull bones.
HA	Prenatal developmental in rodents / MRID 41065202	Maternal NOAEL = 25 mg/kg/day Maternal LOAEL = 125 mg/kg/day based on decreased food consumption during the dosing period and enlarged and mottled kidneys. Developmental NOAEL = 25 mg/kg/day. Developmental LOAEL = 125 mg/kg/day based on increased incidence of partially ossified interparietal and hyoid bones and decreased fetal body weight.
DIA	Prenatal developmental in rodents / MRID 43013209	Maternal NOAEL = 5 mg/kg/day Maternal LOAEL = 25 mg/kg/day based decreased food consumption, body weight/weight gain and food consumption Developmental NOAEL = 25 mg/kg/day Developmental LOAEL = 100 mg/kg/day based on increased fetal and litter incidences of fused sternebrae 1 and 2 and increased fetal incidence of poor ossification of the proximal phalanx of posterior digit 5 (a skeletal variation)
DEA	Prenatal developmental in rodents / MRID 43013208	Maternal NOAEL= 5 mg/kg/day Matenal LOAEL= 25 mg/kg/day based on decreased body weight gain and food consumption Developmental NOAEL= 5 mg/kg/day Developmental LOAEL 25 mg/kg/day based on . increased fetal and litter incidences of fused sternebrae 1 and 2
DACT	Prenatal developmental toxicity in rodents /	Maternal NOAEL = 25 mg/kg/day Maternal LOAEL = 75 mg/kg/day, based on decreased body weight

	MRID 41392402	gain during dosing. Developmental NOAEL is 2.5 mg/kg/day. Developmental LOAEL = 25 mg/kg/day, based on increases in incidences of incompletely ossified parietals, interparietals and unossified hyoids.

Metabolism in Mammals

Metabolism data are summarized in Table A-10. These data suggest that that atrazine is not likely to bioaccumulate and that it is excreted relatively rapidly. However, small mammals may retain atrazine for a sufficient duration such that secondary exposure may occur.

Table A-10. Summary of Available Metabolism Data for Atrazine in Rodents

Guideline No./ Study Type	MRID No. (year) /Doses	Results
870.7485 Metabolism and pharmacokinetics	40431304 (1987) 0, 1, and 100 mg/kg for a single dose given through oral gavage. 1.0 mg/kg/day for 15 days by oral gavage.	*Distribution, accumulation* Distribution was dose-dependent and independent of sex. Distribution appeared to follow first-order kinetics and the half-life in the tissues was 31.3 hours. *Excretion* Approximately 95% of the atrazine excreted within 7 days of dosing. Urinary route accouted for about 75% of the excretion feces accounted for 20%. Route of excretion did not seem to vary among sexes or with dose.
870.7485 Metabolism and pharmacokinetics	MRID 40431305 (1987) The animals were dosed daily for 10 days through a stomach tube with dose levels of 0, 1, 3, 7, 10, 50 or 100 mg/kg/day.	*Distribution, accumulation* Distribution was highest in the red blood cell, followed by the liver, ovary and kidney. When the dose increased the amount distributed in the tissues increased. The distribution appeared to follow first-order kinetics and the tissue half-life was 38.6 hours. This indicates that atrazine, with possible exception of the red blood cell, does not bioaccumulate.
870.7485 Metabolism and pharmacokinetics	MRID 40431306 (1987) Rats were given test 100 mg/kg article was given through the stomach tube in a single oral dose. Other rats were given 16.18 and 19.64 mg/kg and urine was collected over a 24 hr period. The urine was analyzed for metabolites.	*Excretion* In the rats given 100 mg/kg greater than 100% of the administered radioactivity was recovered within 3 days of dosing. Urine was found to contain 47.3% of the radioactivity and the feces 49.3%. The tissues contained 5.75% and 1.4% was found in the blood. *Metabolism* Metabolites indicate that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alky substituents of atrazine appears to be of minor metabolic importance.
870.7485 Metabolism and	MRID 42165503 (1993) Fecal and urinary samples	*Metabolic profile*

Guideline No./ Study Type	MRID No. (year) /Doses	Results
pharmacokinetics	from rats exposed in a seprate metabolism study (MRID 40431304) were obtained and analyzed to determine metabolism profiles.	No sex differences in metabolic profiles were evident. The major fecal metabolite was DACT which accounted for 40% of the total fecal radioactivity.
870.7485 Metabolism and pharmacokinetics	MRID 44713802 (1993) single oral dose of 1 or 100 mg/kg through oral gavage	*Distribution, accumulation* Time to maximum blood concentration (t_{cmax}) was 2 hours and 24 hours for the low and high dose groups, respectively. With exception of red blood cells, whole blood, and skeletal muscle, tissue burden for any specific tissue or organ represented less than 1% of the administered dose by 14 days post dosing *Excretion* Urinary excretion was 64.72% of the total administered low dose over a 48-hour period and 66.16% of the total administered high dose over a 168-hour period. Within 48 hours urinary excretion was 100% and 94% complete for the low-dose and high-dose group, respectively. Fecal elimination accounted for 10.80% and 19.69% of the total dose for the low and high dose groups, respectively.

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-11.

Table A-11. 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)

Table A-11. 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
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-12 presents fish and amphibian toxicity data for formulated products.

Table A-12. 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 for hydroxyatrazine (HA). Acute studies in rainbow trout have also been submitted for DACT and DIA degradates. Table A-13 presents freshwater fish toxicity data for HA, DIA, and DACT.

Table A-13. Freshwater Fish Acute Toxicity

Surrogate Species/ Flow-through or Static	% ai formul.	96-hour LC_{50} (ppb) (measured/nominal)	Toxicity Category	MRID No. Author/Year	Study Classification
		HA			
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
		DIA			
Rainbow trout (*Oncorhynchus mykiss*); 1.5 grams Static test; 14 °C 165 mg/L hardness	Not reported	17,000 (measured dissolved)	Slightly toxic	47046103 Vial, 1991a	Supplemental
		DACT			
Rainbow trout (*Oncorhynchus mykiss*); 1.5 g Static test; 14 °C 164 mg/L hardness	Not reported	>100,000 (measured dissolved)	Practically non-toxic	47046104 Vial, 1991b	Supplemental

* 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-14 presents the chronic toxicity data for freshwater fish.

Table A-14. 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, 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-15. 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-15. 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	00024377 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.	00024377 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)	42547103 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-16. 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-18. 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-16). 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. Atrazine treatments did not affect hatchability of embryos or 96-h posthatch mortality of leopard frog larvae. In addition, atrazine had no effect on swimming speed 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%). The corresponding NOAEL for deformaties was 2.59 mg/L. Similar incidences of deformities were observed for all species tested. It should be noted, however, that atrazine was detected at low levels (0.01 to 0.06 ug/L) in a number of the water samples where the egg masses were originally collected; therefore, "control" embryos may have been exposed to low levels of atrazine prior to the experiment

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 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. Reported malformations in bullfrogs and American toads were less than 10% at atrazine concentrations of 6,300 and 24,800 ug/L Although an LC_{50} of 410 ug/L was reported in bullfrogs, 92% survival was observed at 410 ug/L in the study. Therefore, there is considerable uncertainty in the LC_{50} reported by Birge et al. (1983) of 0.41 mg/L (410 ug/L). Bullfrog survival was reported as 54% at an atrazine concentration of 14,800 ug/L. There is also uncertainty associated with the American toad LC_{50} of >4800 ug/L as survival was reported as $\geq$90% at atrazine exposure concentrations of 24,800 ug/L and below. Reporting defeciences included the following: raw data was not provided, no data on mortality or frequency of malformations were provided for the control groups, and water quality data on the test solutions was not provided. 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 of the higher treatment groups. Significant differences in survivorship within the 2.84 ug/L 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-16. Amphibian Mortality/Survivorship Toxicity Tests from Open Literature (2007 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, the, and study did not provide a more sensitive endpoint than the freshwater fish data. In addition, atrazine was detected at low levels (0.01 to 0.06 ug/L) in a number of water samples where the embryos were collected; therefore, control animals may have been exposed to atrazine.
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 $\leq$ 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

Table A-16. Amphibian Mortality/Survivorship Toxicity Tests from Open Literature (2007 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, 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 early life stage LC_{50}: 410 ug/L American toad LC_{50}: >4800 ug/L	Birge et al., 1983. (19124)	QUAL: No water quality data provided., no data on mortality or frequency of malformations was provided for the control groups. 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.

The embryo and larval stages of several amphibian species were exposed to atrazine (Birge et al.1980), the results of which are different between species. LC50 values (mortality + teratogenic effects) for continuous exposure of embryos (eggs exposed within several hours of fertilization) and larvae through 4 days post-hatch were 410 ug/L for the bullfrog (*Rana catesbeiana*), 7,680 ug/L for the leopard frog (*Rana pipiens*), 17,960 ug/L for the pickerel frog (*Rana palustris*), and >48,000 ug/L for the American toad (*Bufo americanus*). In most of these species, concentrations of atrazine in excess of 5,000 ug/L were required to cause an incidence of teratic larvae in excess of 7 percent.

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, although 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 ($\geq$ 1,500 µg/L), liver (almost normal at 1,500 µg/L and vacuolization of hepatocytes at $\geq$ 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 (2007 review) Open Literature Data)

Four 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-17. 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 full salinity

seatwater 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 the 5 ug/L atrazine exposure level and higher after 24 hours exposure in uncontaminated seawater; 15% of fish exposed to atrazine at 1 ug/L died (all controls survived). In a separate experiment, however, transfer to seawater did not produce mortality after atrazine exposure at 6.5 ug/L and lower in freshwater. However, it is uncertain if the effects observed in this study are applicable to environmental conditions inhabited by the assessed species. For example, salmon were exposed to atrazine in freshwater then to full salinity sea water. It is uncertain if more gradual changes in salinity or if exposure to less than full salinity seawater after freshwater exposures would also produce similar effects. Also, a non-recommended solvent (industrial methylated solvents) was used. Taken together, these data provide evidence that atrazine exposure may affect gill physiology; however, toxicity values from this study are not used to derive risk quotients 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.

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 (0.5 and 1.0 ug/L) significantly reduced the olfactory response to the female priming pheromone, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). After parr were exposed to atrazine, the levels of plasma testosterone and 17,20β-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. Although this study produced a NOAEC that is lower than the fish full life-cycle test of 65 ppb, this study was not considered appropriate for RQ calculation for the following reasons:

(1) A negative control was not used; therefore, potential solvent effects cannot be evaluated;

(2) The study did not determine whether the decreased response of olfactory epithelium to specific chemical stimuli would likely impair similar responses in intact fish.

(3) A quantitative relationship between the magnitude of reduced olfactory response of males to the female priming hormone observed in the laboratory and reduction in salmon reproduction (i.e., the ability of male salmon to detect, respond to, and mate with ovulating females) in the wild is not established.

Birge et al. (1983; Ecotox Reference # 19124) suggested that atrazine exposure to eggs for approximately 10 to 49 days (depending on the species) fish may induce abnormalities. Specific types of abnormalities associated with atrazine exposure were not reported 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 estated at 870 ug/L and 220 ug/L, respectively. Developmental abnormalities were generally observed at atrazine levels that also induced mortality. These data were not used to derive risk quotients because the endpoint from this study was less sensitive than the most sensitive life-cycle study NOAEC of 65 ppb. In addition, no data on mortality or frequency of malformations were provided for the control groups, water quality data on the test solutions were not provided, and it is uncertain if a solvent was used. A solvent was presumably used given that concentrations tested exceeded atrazine's solubility limit. However, use of a solvent control was not indicated.

Tierney et al. (2007) studied the behavioral and neurophysiological responses of juvenile rainbow trout to an amino acid odorant (L-histidine at 10^{-7} M) and how those responses were altered by 30 minute exposure to atrazine at 1, 10, and 100 ug/L and a solvent control (no negative control was tested). L-histidine was chosen because it has been shown to elicit an avoidance response in salmonids; however, control fish exposed to L-histidine at 10^{-7} M showed a slight preference (1.2 response ratio). Although the study authors conclude that L-histidine preference behavior was altered by atrazine at exposures $\geq$ 1 ug/L, no significant decreases in preference behavior were observed at 1 ug/L. Furthermore, no dose response relationship was observed in the behavioral response following pesticide exposure. At 1 and 100 ug/L, non-significant decreases in L-histidine preference were observed; however a statistically significant avoidance of L-histidine was observed at 10 ug/L, but not 100 ug/L. Hyperactivity (measured as the number of times fish crossed the centerline of the tank) was observed in trout exposed to 1 and 10 ug/L atrazine. In the study measuring neurophysiological responses following atrazine exposure, electro-olfactogram (EOG) response was significantly reduced (EOG measures changes in nasal epithelial voltage due to response of olfactory sensory neurons). Although this study produced a more sensitive effects endpoint for freshwater fish, the data were not used quantitatively in the risk assessment because of the following reasons: 1) A negative control was not used; therefore, potential solvent effects cannot be evaluated; 2) The study did not determine whether the decreased response of olfactory epithelium to specific chemical stimuli would likely impair similar responses in intact fish; and 3) A quantitative relationship between the magnitude of reduced olfactory response to an amino acid odorant in the laboratory and reduction in trout imprinting and homing, alarm response, and reproduction (i.e., the ability of trout to detect, respond to, and mate with ovulating females) in the wild is not established.

Table A-17. Freshwater Fish Sublethal Effects Tests from Open Literature (2007 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]**
Gill physiology and survival after transfer to full salinity seawater.	Salmon smolts	Fish were exposed to atrazine for 7 days at atrazine concentrations of 1 – 23 ug/L under flow through conditions. Endpoints evaluated included gill physiology and survival after transfer to full salinity sea water. **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 full salinity seawater resulted in mortality; 43% mortality was observed at 5 ug/L and higher after 24 hours.	Waring and Moore 2004 (72625)	Qual: Relevance of environmental conditions used in this study to the assessed species is questionable because the assessed fish species do not enter full salinity seawater; an unacceptable solvent was used (industrial methylated spirits)
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) was observed at 0.5 ug/L.	Moore, A., and N. Lower, 2001 (67727)	Qual: A solvent control, but no negative control, was used; therefore, potential solvent effects cannot be evaluated; Study conducted on olfactory epithelium; therefore it is unclear whether response to chemical stimuli would impair similar responses in intact fish. Relationship between the magnitude of effects on the endpoints evaluated and reproduction or survival has not been established.
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 control responses were reported; limited water quality parameters were provided; use of a solvent is uncertain; Reported toxicity value is less sensitive than the available life-cycle NOAEC of 65 ppb. LC_{50}s were based on combination of abnormalities and mortality.

Table A-17. Freshwater Fish Sublethal Effects Tests from Open Literature (2007 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]**
Olfactory-mediated behavioral and neurophysiological response in FW fish 30 min exposure Atrazine (97.4% ai)	Juvenile rainbow trout (mass = 32.7 +1.2 g, length = 14.7 $\pm$ 0.18 cm) Source: Sun Valley Trout Farm (Mission BC)	Fish statically exposed to 1, 10, and 100 ug/L atrazine (6 per treatment level) and acetone control. Preference/avoidance to 10^{-7} L-histidine was measured. Electro-olfactograms (EOGs) of olfactory rosettes were measured (EOGs measure electrical responses of olfactory neurons) N = 6 fish/treatment level and solvent control Filtered, dechlorinated municipal water used. pH = 6.8 Hardness = 6.12 CaCO3 Oxygen = > 90% sat. Light/Dark = 12:12 h Food: salmon pellets ad libitum Temp: 12°C	Exposure to 10 ppb atrazine resulted in L-histidine avoidance Hyperactivity observed at atrazine concentrations of 1 and 10 ppb. L-histidine evoked EOGs were significantly reduced at 10 ppb	Tierney et al., 2007 (89625)	Qual: A solvent control, but no negative control, was used; therefore, potential solvent effects cannot be evaluated. Study conducted on olfactory epithelium; therefore it is unclear whether response would impair similar responses in intact fish. Relationship between the magnitude of effects on the endpoints evaluated and reproduction, survival, or growth has not been established.

A number of studies were also located in the October, 2008 ecotox search that evaluated effects to fish. Most studies located in the open literature search reported endpoints that were not more sensitive than the currently available data. Studies that did report endpoints that were more sensitive than the lowest NOAEC from the submitte data did not evaluate endpoints that are quantitatively associated with assessment endpoints of survival, growth, and reproduction.

Species	Endpoint	Conc.	Units	Media	Duration	Duration Units	Ref #	Reference
Acute studies that did not produce more sensitive LC50								
Bluegill	LC50	approx. 26	mg/L	FW	48	h	80976	Beliles RP;Scott WJ; Atrazine Safety Evaluations on Fish and Wildlife (Bobwhite Quail, Mallard Ducks, Rainbow Trout, Sunfish, and Goldfish). (): 9 p. (Author Communication Used)-. 1965
Brown trout	LC50	27	mg/L	FW	96	h	62367	Grande M;Andersen S;Berge D; Effects of Pesticides on Fish Experimental and Field Studies. Norw J Agric Sci Suppl.13(): 195-209. 1994
Goldfish	LC50	>56 to 60	mg/L	FW	48 - 96	h	80976	Beliles RP;Scott WJ; Atrazine Safety Evaluations on Fish and Wildlife (Bobwhite Quail, Mallard Ducks, Rainbow Trout, Sunfish, and Goldfish). (): 9 p. (Author Communication Used)-. 1965

Species	Endpoint	Conc.	Units	Media	Duration	Duration Units	Ref #	Reference
Rainbow trout,donaldson trout	LC50	4.5 to 10	mg/L	FW	48 - 96	h	80976	Beliles RP;Scott WJ; Atrazine Safety Evaluations on Fish and Wildlife (Bobwhite Quail, Mallard Ducks, Rainbow Trout, Sunfish, and Goldfish). (): 9 p. (Author Communication Used)-. 1965
Sheepshead minnow	LC50	>22	mg/L	SW	24 - 72	h	71608	Machado MW; Atrazine Technical - Acute Toxicity to Sheepshead Minnow (Cyprinodon variegatus) Under Flow-Through Conditions. Final SLI Rep No 94-7-5384, Springborn Lab Inc , Wareham, MA(): 4-36. 1994
Sheepshead minnow	LOEC	4.6	mg/L	SW	48	h	71608	Machado MW; Atrazine Technical - Acute Toxicity to Sheepshead Minnow (Cyprinodon variegatus) Under Flow-Through Conditions. Final SLI Rep No 94-7-5384, Springborn Lab Inc , Wareham, MA(): 4-36. 1994
Zebra danio	LC50	1255	uM	FW	96	hpf	93401	Ton C;Lin Y;Willett C; Zebrafish as a Model for Developmental Neurotoxicity Testing. Birth Defects Res Part A76(7): 553-567. 2006
Zebra danio	EC50	440	uM	FW	96	hpf	93401	Ton C;Lin Y;Willett C; Zebrafish as a Model for Developmental Neurotoxicity Testing. Birth Defects Res Part A76(7): 553-567. 2006
Zebra danio	LOEC	5 to 40	mg/L	SW	12 to 48	h	62460	Wiegand C;Krause E;Steinberg C;Pflugmacher S; Toxicokinetics of Atrazine in Embryos of the Zebrafish (Danio rerio). Ecotoxicol Environ Saf 49(3): 199-205. 2001
Blennie	LC50	>0.34	mg/L	FW	10	d	4442	Davies PE;Cook LSJ;Goenarso D; Sublethal Responses to Pesticides of Several Species of Australian Freshwater Fish and Crustaceans and Rainbow Trout. Environ Toxicol Chem 13(8): 1341-1354 (OECDG Data File). 1994
Common jollytail	LC50	>0.34	mg/L	FW	10	d	4442	Davies PE;Cook LSJ;Goenarso D; Sublethal Responses to Pesticides of Several Species of Australian Freshwater Fish and Crustaceans and Rainbow Trout. Environ Toxicol Chem 13(8): 1341-1354 (OECDG Data File). 1994
Rainbow trout,donaldson trout	LC50	>0.34	mg/L	FW	10	d	4442	Davies PE;Cook LSJ;Goenarso D; Sublethal Responses to Pesticides of Several Species of Australian Freshwater Fish and Crustaceans and Rainbow Trout. Environ Toxicol Chem 13(8): 1341-1354 (OECDG Data File). 1994

Species	Endpoint	Conc.	Units	Media	Duration	Duration Units	Ref #	Reference
Sheepshead minnow	NOEC	3.2	mg/L	SW	48	h	71608	Machado MW; Atrazine Technical - Acute Toxicity to Sheepshead Minnow (Cyprinodon variegatus) Under Flow-Through Conditions. Final SLI Rep No 94-7-5384, Springborn Lab Inc , Wareham, MA(): 4-36. 1994
Sheepshead minnow	NOEC	3.2	mg/L	SW	48	h	71608	Machado MW; Atrazine Technical - Acute Toxicity to Sheepshead Minnow (Cyprinodon variegatus) Under Flow-Through Conditions. Final SLI Rep No 94-7-5384, Springborn Lab Inc , Wareham, MA(): 4-36. 1994
Fathead minnow	NOEC	4.1	mg/L	FW	96	h	81782	Jop KM; (Atrazine Technical) - Acute Toxicity to Fathead Minnows (Pimephales promelas) Under Static Conditions. SLI Rep No 91-1-3630, Springborn Lab Inc , Environ Sci Div , Wareham, MA(): 46 p.-. 1991
Guppy	NR-LETH	106.1	mg/L	FW	72	h	61069	Bogacka T;Trzcinska B;Groenwald M; Toxicity and Biodegradation of Atrazine and Symazine in Water Medium (Toksycznosc i Biodegradacja Atrazyny i Symazyny w Srodowisku Wodnym). Bromatol Chem Toksykol 23(1/2): 26-34 (POL) (ENG ABS). 1990
Zebra danio	NOEC	5 to 30	mg/L	SW	12 to 48	h	62460	Wiegand C;Krause E;Steinberg C;Pflugmacher S; Toxicokinetics of Atrazine in Embryos of the Zebrafish (Danio rerio). Ecotoxicol Environ Saf 49(3): 199-205. 2001
Studies that did not produce sensitive chronic endpoint								
Goldfish	LOEC	0.859	mg/L	FW	7 to 21	d	73297	Spano L;Tyler CR;Van Aerle R;Devos P;Mandiki SNM;Silvestre F;Thome JP;Kestemont P; Effects of Atrazine on Sex Steroid Dynamics, Plasma Vitellogenin Concentration and Gonad Development in Adult Goldfish (Carassius auratus). Aquat Toxicol 66(4): 369-379. 2004
Channel catfish	NR-LETH	46.7	mg/L	FW	8.0 to 8.5	d	563	Birge WJ;Black JA;Bruser DM; Toxicity of Organic Chemicals to Embryo-Larval Stages of Fish. EPA-560/11-79-007, U S EPA, Washington, D C (): 60 p. (OECDG Data File) (NTIS PB80-101637)-. 1979
Channel catfish	NR-LETH	46.7	mg/L	FW	4.5	d	563	Birge WJ;Black JA;Bruser DM; Toxicity of Organic Chemicals to Embryo-Larval Stages of Fish. EPA-560/11-79-007, U S EPA, Washington, D C (): 60 p. (OECDG Data File) (NTIS PB80-101637)-. 1979

Species	Endpoint	Conc.	Units	Media	Duration	Duration Units	Ref #	Reference
Rainbow trout,donaldson trout	NR-LETH	50	mg/L	FW	23	d	563	Birge WJ;Black JA;Bruser DM; Toxicity of Organic Chemicals to Embryo-Larval Stages of Fish. EPA-560/11-79-007, U S EPA, Washington, D C (): 60 p. (OECDG Data File) (NTIS PB80-101637)-. 1979
Common jollytail	NOEC	>0.34	mg/L	FW	10	d	4442	Davies PE;Cook LSJ;Goenarso D; Sublethal Responses to Pesticides of Several Species of Australian Freshwater Fish and Crustaceans and Rainbow Trout. Environ Toxicol Chem 13(8): 1341-1354 (OECDG Data File). 1994
Zebra danio	NOAEL	7.5	uM	FW	120	hpf	95954	Muncke J;Junghans M;Eggen RIL; Testing Estrogenicity of Known and Novel (Xeno-)Estrogens in the MolDarT Using Developing Zebrafish (Danio rerio). Environ Toxicol 22(2): 185-193. 2007
Zebra danio	NOAEL	500	uM	FW	48	hpf	93401	Ton C;Lin Y;Willett C; Zebrafish as a Model for Developmental Neurotoxicity Testing. Birth Defects Res Part A76(7): 553-567. 2006
Zebra danio	NOAEL/LOAEL	200/500	uM	FW	96	hpf	93401	Ton C;Lin Y;Willett C; Zebrafish as a Model for Developmental Neurotoxicity Testing. Birth Defects Res Part A76(7): 553-567. 2006
Studies that did not produce acute or chronic endpoint quantitatively related to assessment endpoints								
Atlantic croaker	NOAEL/LOAEL	1 to 10	uM	FW	12	h	103377	Thomas P;Sweatman J; Interference by Atrazine and Bisphenol-A with Progestin Binding to the Ovarian Progestin Membrane Receptor and Induction of Oocyte Maturation in Atlantic Croaker. Mar Environ Res 66(1): 1-2. 2008
Atlantic salmon	NOAEL	0.0005/0.005	mg/L	FW	81	d	103267	Moore A;Lower N;Mayer I;Greenwood L; The Impact of a Pesticide on Migratory Activity and Olfactory Function in Atlantic Salmon (Salmo salar L.) Smolts. Aquaculture273(2/3): 350-359. 2007
Atlantic salmon	NOAEL	0.000495	mg/L	FW	5	d	103267	Moore A;Lower N;Mayer I;Greenwood L; The Impact of a Pesticide on Migratory Activity and Olfactory Function in Atlantic Salmon (Salmo salar L.) Smolts. Aquaculture273(2/3): 350-359. 2007
Atlantic salmon	NOAEL	0.00495	mg/L	FW	81	d	103267	Moore A;Lower N;Mayer I;Greenwood L; The Impact of a Pesticide on Migratory Activity and Olfactory Function in Atlantic Salmon (Salmo salar L.) Smolts. Aquaculture273(2/3): 350-359. 2007

Species	Endpoint	Conc.	Units	Media	Duration	Duration Units	Ref #	Reference
Atlantic salmon	NOAEL/LOAEL	0.0085 to 0.084 / 0.084	mg/L	FW	10 to 21	d	93473	Nieves-Puigdoller K;Bjornsson BT;McCormick SD; Effects of Hexazinone and Atrazine on the Physiology and Endocrinology of Smolt Development in Atlantic Salmon. Aquat Toxicol 84(): 27-37. 2007
Blennie	NOEC/LOEC	0.003 to 0.01	mg/L	FW	10	d	4442	Davies PE;Cook LSJ;Goenarso D; Sublethal Responses to Pesticides of Several Species of Australian Freshwater Fish and Crustaceans and Rainbow Trout. Environ Toxicol Chem 13(8): 1341-1354 (OECDG Data File). 1994
Coho salmon, silver salmon	NOAEL	0.00034	mg/L	FW	30	d	2873	Walsh AH; The Pathology of Pesticide Poisoning in Fish. Ph D Thesis, Univ of Wisconsin, Madison,WI(): 205 p.; Weed Abstr.24(9): 241 / Toxicol.Appl.Pharmacol.25(3): 485 (ABS)-. 1975
Goldfish	NOEC	0.1 to 0.9	mg/L	FW	7 to 21	d	73297	Spano L;Tyler CR;Van Aerle R;Devos P;Mandiki SNM;Silvestre F;Thome JP;Kestemont P; Effects of Atrazine on Sex Steroid Dynamics, Plasma Vitellogenin Concentration and Gonad Development in Adult Goldfish (Carassius auratus). Aquat Toxicol 66(4): 369-379. 2004
Mummichog	NOAEL/LOAEL	0.05/0.5	mg/L	FW	96	h	103349	Fortin MG;Couillard CM;Pellerin J;Lebeuf M; Effects of Salinity on Sublethal Toxicity of Atrazine to Mummichog (Fundulus heteroclitus) Larvae. Mar Environ Res 65(2): 158-170. 2008
Nile tilapia	LOAEL	0.006100625	mg/L	FW	72	h	103350	De campos Ventura B;de Transceschi de Angelis D;Marin-Morales MA; Mutagenic and Genotoxic Effects of the Atrazine Herbicide in Oreochromis niloticus (Perciformes, Cichlidae) Detected by the Micronuclei Test and the Comet Assay. Pestic Biochem Physiol 90(1): 42-51. 2008
Rainbow trout,donaldson trout	LOEC	0.34	mg/L	FW	10	d	4442	Davies PE;Cook LSJ;Goenarso D; Sublethal Responses to Pesticides of Several Species of Australian Freshwater Fish and Crustaceans and Rainbow Trout. Environ Toxicol Chem 13(8): 1341-1354 (OECDG Data File). 1994

Species	Endpoint	Conc.	Units	Media	Duration	Duration Units	Ref #	Reference
Rainbow trout,donaldson trout	NOEC	0.05 to >0.34	mg/L	FW	10	d	4442	Davies PE;Cook LSJ;Goenarso D; Sublethal Responses to Pesticides of Several Species of Australian Freshwater Fish and Crustaceans and Rainbow Trout. Environ Toxicol Chem 13(8): 1341-1354 (OECDG Data File). 1994
Red drum	NOAEL/LOAEL	0.06 to 0.08 / 0.03333 TO 0.03743	mg/L	SW	8	d	103059	McCarthy ID;Fuiman LA; Growth and Protein Metabolism in Red Drum (Sciaenops ocellatus) Larvae Exposed to Environmental Levels of Atrazine and Malathion. Aquat Toxicol 88(): 220-229. 2008
Red drum	NOAEL	0.05871 TO 0.08051	mg/L	SW	8	d	103059	McCarthy ID;Fuiman LA; Growth and Protein Metabolism in Red Drum (Sciaenops ocellatus) Larvae Exposed to Environmental Levels of Atrazine and Malathion. Aquat Toxicol 88(): 220-229. 2008
Spotted seatrout	LOAEL	0.1	uM	FW	12	h	103377	Thomas P;Sweatman J; Interference by Atrazine and Bisphenol-A with Progestin Binding to the Ovarian Progestin Membrane Receptor and Induction of Oocyte Maturation in Atlantic Croaker. Mar Environ Res 66(1): 1-2. 2008
Channel catfish	LC01	0.029 to 0.0772	mg/L	FW	27	d	563	Birge WJ;Black JA;Bruser DM; Toxicity of Organic Chemicals to Embryo-Larval Stages of Fish. EPA-560/11-79-007, U S EPA, Washington, D C (): 60 p. (OECDG Data File) (NTIS PB80-101637)-. 1979
Acute study that used uncertain test substance								
Harlequinfish, red rasbora	LC50*	0.5	mg/L	FW	48	h	542	Alabaster JS; Survival of Fish in 164 Herbicides, Insecticides, Fungicides, Wetting Agents and Miscellaneous Substances. Int Pest Control11(2): 29-35 (Author Communication Used). 1969
Harlequinfish, red rasbora	LC50*	0.55	mg/L	FW	24	h	542	Alabaster JS; Survival of Fish in 164 Herbicides, Insecticides, Fungicides, Wetting Agents and Miscellaneous Substances. Int Pest Control11(2): 29-35 (Author Communication Used). 1969

A.2.4c Sublethal Effects: Amphibians (Summary of the 2003 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 and 2008 open literature searches that were not reviewed as part the white paper discussed above.

Frogs (Anurans)

A total of nine studies on potential sublethal effects of atrazine to frogs were reviewed as part of the open literature. Five of the nine studies were classified as acceptable to use in qualitative sense and the other four were classified as unacceptable. Two of the five qualitative studies are microcosm/mesocosm tests (one of which includes data for both frogs and salamanders), and three are chronic lab studies. A review of the qualitative studies is provided below and summarized in Table A-18. 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 limitations in the study design and/or they provide less sensitive endpoints than studies which are used for quantitative derivation of risk quotients. In summary, the majority of 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. However, one chronic lab study by Hayes et al. (2006) indicates that leopard frog size at metamorphosis (i.e., body weight and snout-vent length) is significantly lower than the ethanol control at an atrazine exposure concentration of 0.1 ppb, which is three orders of magnitude lower than the lowest effect level observed in the other open literature studies. It should be noted, however, that there are a number of uncertainties associated with the Hayes et al. (2006) study design, which confound the ability to interpret the study results. These uncertainities are discussed in further detail below. 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 the 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 between 20 and 200 ppb. Uncertainties associated with the study design included the following: no data on water quality were provided and one of the negative control microcosms was removed from the analysis due to an unexplained zooplankton bloom which resulted in the removal of phytoplankton. In addition pre-metamorphosis weight and length were not determined; therefore it was not possible to establish similar size and weight disparity of larvae.

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. Limitations associated with this study include the following: only one concentration of atrazine was tested; the percentage difference in effects of the atrazine treated group relative to the control group was not presented; and tap water was used in all control and treatment test solutions; however, the chlorine content and other water quality parameters of the tap water were not specified.

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 male frogs exposed to 250 ppb atrazine may affect reproductive functions in the wild is unclear; therefore, it is not possible to quantitatively link this sublethal effect to the identified assessment endpoint of fecundity (or survival and growth). 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-18) 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.

Hayes et al. (2006) assessed the effect of 9 individual pesticides, including atrazine at 0.1 ppb (and metolachlor, alchlor, nicosulfuron, cyfluthrin, cyhalothrin, tebupirimphos, methalaxyl, and propiconizole also at 0.1 pbb), and three different mixtures containing atrazine, to mortality, growth and development, gonadal development, thymus histology, and disease rates (i.e., immune function) in larval leopard frogs (*R. pipiens*). The three mixtures included atrazine and S-metalachlor at 0.1 and 10 ppb, Bicep II Magnum (reported as 33.3% atrazine, 0.7% atrazine-related products, 26.1% TGAI of S-metolachlor, and 40.2% inert ingredients), and a mixture of the 9-pesticides cited above at individual concentrations of 0.1 pbb. Ethanol was used as a solvent for all pesticide treatments and was included in the [solvent] control, although no negative control was tested. Each treatment, which included 30 larve per test container, was replicated 3 times. Test containers were reported as "plastic mouse boxes" and size of the containers was not specified. The exposure period lasted throughout the larval period from 2 days post-hatch until complete tail resorption (TR; Gosner stage 46). Nominal treatment concentrations were confirmed via lab analysis. Histological analysis of the gonads and the thymus (to measure immunocompentence) was also completed. Thymus histology was completed after the study authors noted that animals exposed to the 9-compound pesticide mixture experienced increased incidence of bacterial infection with *Chryseobacterium (Flavobacterium) menigosepticum*. Effects of single pesticides (20 animals each), the 0.1 ppb Bicep mixutre, and the 9-compound mixture to the thymus were examined. No single pesticide affected mortality or time to metamorphosis; however, animals exposed to the 9-compound pesticide mixture at 0.1 ppb had significantly longer larval periods. Size at metamorphosis (SVL and BW) was significantly less in the 0.1 ppb atrazine treatment, as compared to the ethanol control; however, no negative control was tested. The lack of testing with a negative control confounds the ability to discriminate between potential treatment-related and solvent impacts and adds a high degree of uncertaintiy to the results of the study. All mixtures resulted in reduced growth (SVL and BW), as compared to the solvent control, with the atrazine and S-metolachlor mixture having the greatest negative effect. With respect to gonadal development, the gonads and gametes were underdeveloped in both the control and treatment groups; therefore, it was not possible for the study authors to assess the affects of atrazine or mixtures on sex differentiation. It should be noted that the study authors were unable to replicate the results of previous experimental findings of ovarian tissues in testes, instead attributing the disparate findings [on atrazine gonadal development effects] to population variability. Exposure to the Bicep mixture (atrazine and S-metolachlor) and the 9-compound mixture resulted in damage to the thymus as measured by thymic plaques; however, the ecological relevance of thymic plaques is not discussed. Given the increased incidence of disease and evidence of histological effects on the thymus in animals exposed to the mixtures, the study authors suggest that exposure to pesticide mixtures renders amphibians more susceptible to disease as a result of immunosuppression. However, there are a number of uncertainties associated with this study. In addition to the lack of negative control testing and the inability of the study authors to replicate the results of previous experiments showing impacts to gonadal development, the following additional limitations were observed in the study design and reporting of data: no raw data or water quality

data were provided; the use of plastic test containers that may leach varying amounts of plasticizers, feeding rates, and the quality of food were not described; and only one exposure concentration was tested for the individual pesticides. In addition, the study author's use of "open" literature to support the contention that atrazine affects time to metamorphosis and weight at metamorphosis is misleading. The work by Carr et al. (2003), supposedly substantiating the effects, was previously demonstrated to be a result of inadequate husbandry. In the Carr et al. (2003) study, the animals were starving and were exposed to poor environmental conditions; thus, the larvae's physical resources were likely focused on survival, rather than growth and development.

As part of the same study, Hayes et al. (2006) also examined the effects of the 9-compound mixture on plasma corticosterone levels (stress hormone) in adult male African clawed frogs (*X. laevis*). African clawed frogs were used as a surrogate because metamorphic leopard frogs are too small to obtain repeated blood samples and because *X. laevis* are available year-round. Five males were treated with the 9-compound pesticide mixture (including atrazine at 0.1 ppb) and five males were exposed to ethanol only (no negative control was tested). During the 27 day exposure period, no aeration was provided, the animals were fed Purina trout chow daily, and solutions were changed and treatments renewed every three days. Blood was collected by cardiac puncture. The study authors report a clear effect on corticosterone levels in male African clawed frogs with corticosterone levels increasing 4-fold in pesticide-exposed males. However, there are several flaws in the study design that add a high degree of uncertainty to the results. First, water quality parameters, including ammonia (which could be a major source of stress) were not measured as part of this study. Secondly, only one single treatment concentration was tested; therefore, it is unclear if there is a dose response. Thirdly, the study author's fail to mention whether the animals were housed in one or separate tanks. If the animals were housed in one tank, the treatment unit would be the tank Most importantly, the collection of repeated blood samples via cardiac puncture is likely to cause severe trauma to the animals; therefore, the study conditions are conducive to elevating the very endpoint the researchers are attempting to measure (i.e., elevation of blood corticosterone). In summary, many of the confounding effects identified in previous studies by the FIFRA SAP limit the utility of this study.

Table A-18. Frog Toxicity Tests from Open Literature (2007 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]

Table A-18. Frog Toxicity Tests from Open Literature (2007 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 = Between 20 and 200 ppb - Length: 5% reduction ($p < 0.001$) at 200 ppb (LOAEC); NOAEC = Between 20 and 200 ppb - Larval period: no effect; NOAEC = 2000 pbb	Diana et al., 2000 (59818)[2]	QUAL: - pre-metamorphosis weight and length were not determined; therefore similar size and weight disparity cannot be established. - no water quality data provided - one of the negative control microcosms was removed from consideration during the data analysis due to a zooplankton bloom which led to a clearing of phytoplankton - NOAEC value is between 20 and 200 ppb; therefore, it is unclear if this study provides the most sensitive endpoint for chronic effects to freshwater fish and amphibians

Table A-18. Frog Toxicity Tests from Open Literature (2007 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 ºC) 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 and other water quality parameters of the tap water were not specified

Table A-18. Frog Toxicity Tests from Open Literature (2007 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$) at 259 ppb (LOAEC); NOAEC = Between 24.6 and 259 ppb - Testicular aromatase activity, CYP19 gene expression, E2 concentration, and GSI: no effect; NOAEC = 259 ppb	Hecker et al., 2005 (79287)	QUAL: - no raw data provided - mechanism associated with suppression of T is unclear because aromatase activity was not increased - extent to which suppression of T may affect reproductive functions in wild is unclear; therefore it is not possible to quantiatively link this sublethal effect to the assessment endpoint of fecundity - no water quality data provided
Chronic lab study / atrazine (99% pure)	Cricket frogs (*A. crepitans*), wood frogs (*R. sylvatica*), Northern leopard frogs (*R. pipiens*)	Tadpoles were exposed to 30, 300, or 600 ug/L atrazine from the first feeding stage through metamorphosis. Growth rate, days to metamorphosis, metaphorphic success, and juvenile weight and length were evaluated.	A statistically significant ($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 completed in accordance with GLP; however, the study appears to be well reported and well conducted. Not all raw data were included in the report.

Table A-18. Frog Toxicity Tests from Open Literature (2007 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 lab study (2 d post-hatch until complete metamorphosis) / Atrazine (≥ 98% ai); 9-pesticide mixutre including 0.1 ppb atrazine; atrazine and S-metolachlor mixture; and Bicep II Magnum (contains 33.3% atrazine, 26.1% S-metolachlor, and 40.2% inerts)	Leopard frog (*R. pipiens*) larvae and adult male African clawed frogs (*X. laevis*)	**Leopard frog study:** - 4 L aerated Holtfreter's solution (size of test container not specified) - Covered plastic mouse boxes used as test containers - Static renewal (100% test solution changed every 3 days) nominal concentrations of 0.1 ppb atrazine, 0.1 and 10 ppb atrazine/S-met mixture, and 0.1 and 10 ppb atrazine in Bicep mixture - 30 larvae/tank; 3 reps/treatment - Ethanol solvent used in all treatments and in control (no negative control) - Temp = 22-23°C - Photoperiod = 12/12-hr light/dark cycle - Feeding: unspecified amount of Purina rabbit chow -Exposure period: 2-days post hatch to complete TR at met. - Endpoints: larval growth and development (time to foreleg emergence [FLE], time to complete tail resorption [TR], snout-vent length [SVL], and body weight [BW] at metamorphosis), mortality, gonadal development, thymus histology, disease rate (i.e., immune function) **African clawed frog study:** -9-compound pesticide mixture (0.1 ppb atrazine) and ethanol control (no negative control) -5 adult males in treatment group and 5 in ethanol control -Static renewal (100% test solution changed every 3 days); no aeration -Feeding: Purina trout chow daily - Blood collected by cardiac puncture -Exposure period= 27 days - Endpoints: plasma corticosterone levels	**Leopard frog:** **Individual atrazine:** - no effect on mortality, time to met, (NOAEC = 0.1 ppb; LOAEC = >0.1 ppb) - not possible to assess sex differentiation because of delay in gonadal development - BW and SVL decrease rel. to solvent control ($p < 0.05$) (NOAEC = < 0.1 ppb; LOAEC = 0.1 ppb) **Mixtures (9-compound, atrz+S-met, and Bicep)** - no effect on mortality - sig. longer larval periods (days to FLE and TR delayed) - sig. reduced growth (BW and SVL) -animals exposed to 9-cmpd mixture contracted flavobacterial meningitis -animals exposed to atrz and S-met mixture resulted in damage to thymus (i.e., thymic plaques) **African Clawed Frog:** - 9-cmpd mixture resulted in 4-fold increase in plasma corticosterone levels	Hayes et al., 2006 (85815)	QUAL: - No raw data provided; - No negative controls tested - Water quality data not provided - Plastic test containers used in the study with possible confounding effects due to potential confounding effects of plasticizers - Feeding rates and quality of food not described - Only one exposure concentration was tested for the individual pesticides -Study authors unable to replicate results of previous studies showing impacts to gonadal development of amphibians. - In the African clawed frog test, water quality parameters were not measured, it is unclear whether the animals were housed in one or separate tanks, only one treatment level was tested, collection of repeated blood samples on reported increase in plasma corticosterone levels is not addressed.

[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.

In the 2008 Ecotox search, a number of studies were identified that evaluated effects to frogs. Studies previously described by the 2003 and 2007 SAP White Papers are not described in this appendix (DuPreez and Solomon, 2003, Ecotox No. 78091; Heckler et al., 2005, Ecotox No. 79288). Also, papers identified that evaluated amphibian gonadal development in *Xenopus* are not further described in this appendix because this topic was the subject of a recent SAP (October, 2007) (Oka et al., 2008, Ecotox No. 103345). In addition, several studies did not report endpoints that were more sensitive than endpoints already available and were, therefore, not further described in this appendix. These studies include Du Preez et al. (2008, Ecotox No. 103271), Lenkowsi et al. (2008, Ecotox No. 103268), and Storrs and Semlitsch (2008, Ecotox No. 103378).

LaFiandra et al. (2008, Ecotox No. 103336) evaluated effects of an atrazine formulation (Aatrex Nine-O; 88.2% atrazine; 1.8% related compounds) on *Hyla versicolor* (gray treefrogs) with and without the presence of a non-lethal predator (dragonfly larvae). No effects on survival, growth, or development was observed at atrazine concentrations up to 200 ug/L. However, tadpole survival was reduced in the presence of 200 ug/L atrazine and the predator. The author attributed the observed effect to increased stress induced by the predator. This effect level is within the range of LOAEC values observed in chronic fish studies previously described.

In addition, several studies included in the 2008 Ecotox search evaluated the potential for atrazine to affect the immune response in amphibians. Forson and Storfer (2006) reported decreased leukocyte levels at 16 ug/L and 160 ug/L in tiger salamanders. Decreased leukocyte levels were associated with increased infections of Ambystoma tigrinum virus (ATV) in exposed larvae at 16 ug/L but not at 160 ug/L. Brodkin et al. (2007) reported effects on the innate immune response as measured by a decrease in thiglycollate-stimulated recruitment of white blood cells to the peritoneal cavity in *Rana pipiens* at atrazine levels as low as 0.01 ug/L. Last, intensity of infection of *R. clamitans* tadpoles by *E. trivolis* was observed by Koprivnikar et al. (2007) when exposed to a formulated product of atrazine at 30 ug/L (nominal). The effect was observed only when atrazine-exposed tadpoles were exposed to parasites that were not also exposed to atrazine. The effect was not observed when tadpoles were exposed to parasites that were also exposed to atrazine. Therefore, the ecological relevance of thi study is uncertain.

These studies raise concerns regarding potential effects of atrazine on amphibian immune systems. However, the data are not sufficient to make any definitive conclusions, and the effects observed in the available studies are not clearly and quantitatively linked to the assessment endpoints of growth, reproduction, and survival.

Common Name	Duration	Endpoint	Test Level	Units	Ecotox No.	Citation
Study reviewed in the 2007 white paper						
African clawed frog	133 d	LOEC	0.00091	mg/L	78091	Du Preez L;Solomon KR; 2003 Exposure of Xenopus laevis Larvae to Different Concentrations of Atrazine in Semi-natural Microcosms (): 44 p.-
African clawed frog	49 d	NOEC	0.107	mg/L	79288	Hecker M;Kim WJ;Park JW;Murphy MB;Villeneuve D;Coady KK;Jones PD;Solomon KR;Van der Kraak G;Carr JA;Smith EE;Du Preez L;Kendall RJ;Giesy JP; 2005 Plasma Concentrations of Estradiol and Testosterone, Gonadal Aromatase Activity and Ultrastructure of the Testis in Xenopus laevis Exposed to Estradiol or Atrazine Aquat Toxicol 72(4): 383-396
African clawed frog	8 wk	NOAEL / LOAEL	0.000974 / 0.0974	mg/L	103345	Oka T;Tooi O;Mitsui N;Miyahara M;Ohnishi Y;Takase M;Kashiwagi A;Shinkai T;Santo N;Iguchi T; 2008 Effect of Atrazine on Metamorphosis and Sexual Differentiation in Xenopus laevis Aquat Toxicol 87(4): 215-226
Studies that did not produce sensitive endpoint						
African clawed frog	2 yr	NOAEL *(no LOAEL observed)*	0.0248	mg/L	103271	Du Preez LH;Kunene N;Everson GJ;Carr JA;Giesy JP;Gross TS;Hosmer AJ;Kendall RJ;Smith EE;Solomon KR;Van der Kraak GJ; 2008 Reproduction, Larval Growth, and Reproductive Development in African Clawed Frogs (Xenopus laevis) Exposed to Atrazine Chemosphere71(3): 546-552
African clawed frog	24 h	NOAEL / LOAEL	10 / 35	mg/L	103268	Lenkowski JR;Reed JM;Deininger L;McLaughlin KA; 2008 Perturbation of Organogenesis by the Herbicide Atrazine in the Amphibian Xenopus laevis Environ Health Perspect 116(2): 223-230
American toad	7 d	NR-LETH	14.8	mg/L	6187	Birge WJ;Black JA;Kuehne RA; 1980 Effects of Organic Compounds on Amphibian Reproduction Res Rep No 121, Water Resourc Res Inst , University of Kentucky, Lexington, KY(): 39 p. (NTIS PB80-147523)-
Studies that evaluated endpoints that are not quantitatively associated with assessment endpoints of growth, survival, or reproduction						

Leopard frog	8 d	LOAEL	0.021	mg/L	103351	Brodkin MA;Madhoun H;Rameswaran M;Vatnick I; 2007 Atrazine is an Immune Disruptor in Adult Northern Leopard Frogs (Rana pipiens) Environ Toxicol Chem 26(1): 80-84
Wood frog	31 d	NOAEL	0.03	mg/L	103348	Koprivnikar J;Forbes MR;Baker RL; 2007 Contaminant Effects on Host-Parasite Interactions: Atrazine, Frogs, and Trematodes Environ Toxicol Chem 26(10): 2166-2170
American toad	42 to 46 go	NOAEL	0.03 to 0.12	mg/L	103378	Storrs SI;Semlitsch RD; 2008 Variation in Somatic and Ovarian Development: Predicting Susceptibility of Amphibians to Estrogenic Contaminants Gen Comp Endocrinol 156(3): 524-530
Gray tree frog	42 go	NOAEL / LOAEL	0.016 / 0.20	mg/L	103336	LaFiandra EM;Babbitt KJ;Sower SA; 2008 Effects of Atrazine on Anuran Development are Altered by the Presence of a Nonlethal Predator J Toxicol Environ Health Part A71(8): 505-511
Tiger salamander	nr stg	NOAEL / LOAEL	0.0016 / 0.016	mg/L	103072	Forson DD;Storfer A; 2006 Atrazine Increases Ranavirus Susceptibility in the Tiger Salamander, Ambystoma tigrinum Ecol Appl 16(6): 2325-2332
Toxicity to Embryo and Larvae (see text for discussion)						
Bullfrog	8 d	LC01/ LC10	0.0074 / 0.0449	mg/L	6187	Birge WJ;Black JA;Kuehne RA; 1980 Effects of Organic Compounds on Amphibian Reproduction Res Rep No 121, Water Resourc Res Inst , University of Kentucky, Lexington, KY(): 39 p. (NTIS PB80-147523)-
Leopard frog	9 d	LC01 / LC50	0.033 / 0.38	mg/L	6187	Birge WJ;Black JA;Kuehne RA; 1980 Effects of Organic Compounds on Amphibian Reproduction Res Rep No 121, Water Resourc Res Inst , University of Kentucky, Lexington, KY(): 39 p. (NTIS PB80-147523)-

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-18. 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 limitations in the study design and/or they provide less sensitive endpoints than studies which are used for quantitative derivation of risk quotients. 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 – 184 ppb and 40 - 400 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, it is not possible to quantitatively link this behavioral endpoint 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-18, 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. As previously mentioned, limitations associated with this study include the following: only one concentration of atrazine was tested; the percentage difference in effects of the atrazine treated group relative to the control group was not presented; and tap water was used in all control and treatment test solutions; however, the chlorine content and other water quality parameters of the tap water were not specified.

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 cannot be quantitatively linked 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. One of the major limitations of this study is that the solvent control contained both DMSO and acetone, whereas the atrazine treatment groups contained DMSO only.

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. According to the study authors, 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, the study authors suggest that these behaviors may elevate predation risk by increasing conspicuosness and encounters with predators. Larval period was lengthened by food limitation and shortened by 400 ppb atrazine. According to the study authors, 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 smaller size at metamorphosis may result in lower terrestrial survival and lifetime reproduction. This study was classified as qualitative due to the following uncertainties: the ability to discern atrazine treatment-related effects on embryos and hatched larvae is confounded 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; it is unclear how this fungal pathogen may have impacted other reported results from the study; no explanation is provided for the difference in larval survival results for 2002 (no effect) versus 2003 (significant effect for 400 ppb treatment

compared to control); the study authors indicate that metamorphic outliers from the 2003 data set were not included in the analyses, yet no explanation is provided for the outlier data; the exact duration of study is not specified; no negative control was tested; and DMSO is not an acceptable solvent because it accelerates movement of a chemical across cell membranes.

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*). Six-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. The NOAEC value for reduced size at metamorphosis and accelerated metamorphosis is between 18.4 and 184 ppb; therefore it is unclear if this study provides the most sensitive endpoint for chronic effects to amphibians relative to the available data for fish, where the chronic NOAEC value is 65 ppb. As such, this study is evaluated qualitatively relative to the available chronic freshwater fish data for atrazine.

Table A-19. Salamander Toxicity Tests from Open Literature (2007 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)]

Table A-19. Salamander Toxicity Tests from Open Literature (2007 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 cannot be quantitatively linked to the assessment endpoints for this risk assessment

Table A-19. Salamander Toxicity Tests from Open Literature (2007 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 ^{o}C - 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
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 ^{o}C - 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 - No water quality data provided - NOAEC value is between 18.4 and 184 ppb; therefore, it is unclear if this study provides the most sensitive endpoint for chronic effects to freshwater fish and amphibians

(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-20.

Table A-20. 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)
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 1991	Supplemental (raw data are missing)
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 1991	Supplemental (raw data are missing)
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)

Table A-20. Freshwater Invertebrate Acute Toxicity

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)
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 the most sensitive species tested; 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 for hydroxyatrazine (HA). 48-Hour acute studies were also submitted on DIA and DACT. Table A-21 presents freshwater invertebrate toxicity data for the three degradates.

Table A-21. Freshwater Invertebrate Acute Toxicity

Surrogate Species/ Flow-through or Static	% ai formul.	48-hour EC_{50} (ppb) (measured/nominal)	Toxicity Category	MRID No. Author/Year	Study Classification
		HA			
Waterflea (*Daphnia magna*); 1st instar (6-24 h old) Static test	98	>4,100 (measured dissolved)	moderately toxic*	465000-01 Peither, 2005c	Acceptable
		DIA			
Waterflea (*Daphnia magna*); 1st instar (6-24 h old) Static test	Not reported	>100,000 (measured dissolved)	Practically non-toxic	47046101 Vial, 1991c	Supplemental
		DACT			
Waterflea (*Daphnia magna*); 1st instar (6-24 h old) Static test	Not reported	>100,000 (measured dissolved)	Practically non-toxic	47046102 Vial, 1991d	Supplemental

* 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-22.

Table A-22. 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)
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.2.7a Freshwater Invertebrates, Acute Open Literature Data

The result of two acute toxicity tests using juvenile (i.e., glochidial) and mature freshwater mussels suggest that two species of Unionid mussels, *Anodonta imbecillis* and *Utterbackia imbecillis* are less sensitive to atrazine on an acute exposure basis than other freshwater invertebrates commonly used in aquatic toxicity tests (e.g., cladocerans and amphipods) (Johnson et al., 1993; Conners and Black, 2004). The results of the freshwater mussel studies are summarized in Table A-23. Johnson et al. (1993) exposed juvenile mussels (20/concentration) to atrazine under static conditions at nominal concentrations up to 36 mg/L and evaluated survival of exposed individuals for 48 hours. Glochidia (1 to 2 days old and 7 to 10 days old) were exposed in a separate experiment for 24 hours under similar environmental conditions and exposure concentrations and evaluated for survival. The study reported LC50s that were >60 mg/L for all life stages. No acute toxicity was observed at any concentration tested. Using methods similar to the Johnson et al. (1993) study, Conners and Black (2004) report a 24-hr LC_{50} value of 214 for *U. imbecillis* glochidia for a formulated product (Atrazine 4L, 40.8% a.i.).

Table A-23a. 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 mussel *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)	Quan: Although no effects were observed at any test concentration, these data are considered to be directly relevant to risk estimation for freshwater mussels because the study was of good quality and no other studies evaluated potential effects of technical grade atrazine on freshwater mussels.
Acute toxicity study in freshwater mussel / Atrazine 4L 40.8% ai	Freshwater mussel *U.imbecillis* Glochidia	*Utterbackia imbecillis* (100/group) were exposed to atrazine for 24 hours under static conditions and evaluated for survival. LC50 values were estimated	LC_{50} = 241 mg/L for juvenile *U. Imbecillis*	Conners and Black, 2004 (74236)	Qual: Study tested a formulated product at concentrations considerably higher than the solubility limit of atrazine of 33 mg/L. Study also produced a toxicity value that is less sensitive than the Johnson et al. (1993) study.

(1) QUAL = The paper is not appropriate for deriving risk quotients for reasons discussed in the table, but is considered to be of good quality, addresses issues of concern to the risk assessment, and is used in the risk characterization discussion.
(2) QUAN = The paper is appropriate for quantitative use and is deemed appropriate for use in risk calculations.

A.2.7b Freshwater Invertebrates, Chronic Open Literature Data

Chronic studies that evaluated potential effects of atrazine on mollusk species are summarized below.

Table A-23b. Chronic Aquatic Invertebrate Toxicity Tests from Open Literature (2006 Review)

Study type/ Test material	Test Organism	Test Design	Endpoint Concentration in ppb	Citation (EcoRef. #)	Rationale for Use in Risk Assessment[1]
12-week mesocosm study / atrazine, 97.8% pure	*Lymnaea palustris*	Surface of mesocosms (1/treatment level) were treated with technical grade atrazine at target concentrations of 5, 25, and 125 ug/L, and effects were evaluated for up to 12 weeks. Endpoints evaluated included mortality, growth, fecundity, and biochemical parameters (glycogen content, polysaccharide hydrolysis	No effects occurred at any concentration for any of the parameters evaluated..The NOAEC was 125 ug/L (nominal).	Baturo et a. (1995)	QUAL. Replicate mesocosms were not used per concentration; atrazine concentrations were not analytically confirmed; no water/sediment quality data were provided.
40-Day lab study / atrazine 99.2% pure	*Ancylus fluviatilis (river limpet), Glossiphonia complanata (leech), Helobdella stagnalis (leech)*	Test animals were exposed to atrazine at atrazine concentrations of 1, 4, or 16 ppm for up to 27 days and evaluated for food ingestion, growth, and egg production.	Effects observed at all concentrations.	Streit and Peter, 1978	QUAL. NOAEC was not achieved; no information was reported on experimental conditions (e.g., temperature, pH, DO); limited information on study design parameters such as no. of replicates, no. of animals treated per concentration, etc.) was reported.
8-week study	Numerous invertebrates including annelids, arthropods, and mollusks.	Aquaria were treated with atrazine at 9, 130, or 670 ppb (measured) in a flow through saltwater system, and were evaluated for abundance of various taxa.	No effects were observed at any concentration. NOAEC = 670 ppb.	EG&G, 1979	Invalid: Possible control contamination;; unacceptable solvent; a solvent control, but no negative control was used

(3) QUAL = The paper is not appropriate for deriving risk quotients for reasons discussed in the table, but is considered to be of good quality, addresses issues of concern to the risk assessment, and is used in the risk characterization discussion.
(4) QUAN = The paper is appropriate for quantitative use and is deemed appropriate for use in risk calculations.

None of the studies included Table A-23b were considered suitable for RQ calculations for reasons described in the table; however, two of the studies were considered useful as supplemental information to support the risk assessment.

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-24. Summaries of mesocosm and limnocorral studies for freshwater ponds, lakes, reservoirs are included in Table A-25 and natural and artificial stream mesocosm data are summarized in Table A-26. In general, all microcosm/mesocosm data were classified as supplemental in the 2003 IRED because this information is used to provide

context to the effects data seen in individual organism toxicity tests. Data from non-guideline microcosm/mesocosm tests are typically not used quantitatively to derive RQs in the Agency's ecological risk assessments, but rather to provide qualitatative information regarding potential aquatic community-level effects of atrazine.

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-24. 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 40-40% 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 Supplemental
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
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

Table A-24. 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
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
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

Table A-24. 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
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

Table A-24. 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

Table A-25. 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

Table A-25. 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

Table A-25. 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 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

Table A-25. 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 Supplemental

Table A-25. 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

Table A-25. 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

Table A-25. 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
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 $\geq$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 $\geq$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
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 Supplemental
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

Table A-25. 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 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 Supplemental
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
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 Supplemental

Table A-26. 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

Table A-26. 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 5 3.56 10 1.20 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
Artificial stream tests: (14 day; measured) One dose and recirculation; two atrazine levels (40.8% ai): 15.2 ± 1.4 and 155.4 ± 1.4 µg/l atrazine on Day 1; 17.5 ± 1.2 and 135.0 ± 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 Supplemental

Table A-26. 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 Supplemental

Table A-26. 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)
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.

Table A-26. 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 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
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 Supplemental
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 $\geq$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
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

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 endangered mussels. The results of this study, which are summarized as part of Table A-27, 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. However, it is not possible to make a quantitative link between increased searching velocity in snails and the assessment endpoints of survival, fecundity or growth; therefore, data from this study is not used to derive RQs in the risk assessment.

Table A-27. Freshwater Mesocosm Study from Open Literature (2007 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-28.

Table A-28. 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-29 presents estuarine/marine fish toxicity data for hydroxyatrazine.

Table A-29. 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 ($\geq$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-30.

Table A-30. 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 °/₀₀ 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, 49526-06, and 469526-04 Cafarella, 2005a	Acceptable

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)

Alvarez (2005; ECOTOX No. 81672) investigated effects of exposure to atrazine for up to 8 days on growth, swimming behavior, predator response, and respiration rate in red drum (*Sciaenops ocellatus*) larvae. This study reported effects including reduced growth rate at 80 ppb and changes in swimming speed and respiration at 40 ppb. However, a negative control was not used in the evaluation of these endpoints; therefore, potential solvent effects could not be evaluated. Also, mortality in the control and treated groups was high for an exposure study that was approximately 1 week (23% mortality, survival for each test group was not reported). Also, it is uncertain if any relationship between the respiration and behavioral effects observed in this study and reduced survival and reproduction exists. Therefore, these data were not used in derivation of risk quotients.

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-31a and A-31b.

Table A-31a. 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)
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)

Table A-31a. 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 - 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-31b. 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
Mysid Shrimp (*Americamysis bahia*) Static test Salinity +22-25 g/L; T 25-26 ºC	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, 469526-01, and 469526-02 Cafarella, 2005c	Acceptable

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 (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 derive risk quotients. In addition, the relationship between the magnitude of delayed maturity observed in this study and survival and reproductive success is uncertain.

Bejarano and Chandler (2003; ECOTOX No. 73333) reported results from a 2.5 generation reproduction study in copepods. In this study, an increase in reproductive failure occurred at 25 ug/L and higher, and viable offspring production per female was significantly decreased at 2.5 ug/L and higher. These effects only occurred in the F1 generation. No effects on survival, development to reproductive maturinty, time to egg extrusion, or time to egg hatch occurred. A negative control was not used in the evaluation of these endpoints; therefore, potential solvent effects could not be evaluated. The copepod was not considered to be an appropriate surrogate invertebrate species included in this assessment for direct effects or for potential effects to dietary items, and data for more taxonomically appropriate species are available. Therefore, this study was not used to derive risk quotients for this assessment.

Table A-34. Estuarine/Marine Invertebrates Sublethal Effects Toxicity Tests from Open Literature (2007 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)	QUAL. 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.
Reproduction of copepods / 98% pure atrazine	**Study duration:** 41 days **Atz Concs:** 2.5 to 250 ppb **Endpoints:** Reproduction **Solvent:** Acetone (unreported concentration)	Copepod *Amphiascus tenuiremis*	No effects on survival, development to reproductive maturinty, time to egg extrusion, or time to egg hatch. In the F1 generation, % reproductive failure occurred at 25 ug/L and higher, and total viable offspring production per female was significantly decreased at 2.5 ug/L and higher.	Bejarano and Chandler (2003; ECOTOX No. 73333)	QUAL. Negative control was not used; therefore, potential solvent effects could not be evaluated; unacceptable solvent was 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. Similar to the freshwater field studies, the estuarine/marine microcosm and mesocosm field studies were classified as supplemental in the 2003 IRED because this information is used to provide context to the effects data seen in individual organism toxicity tests. Data from non-guideline microcosm/mesocosm tests are typically not used quantitatively to derive RQs in the Agency's ecological risk assessments, but rather to provide qualitatative information regarding potential aquatic community-level effects of atrazine.

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

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

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
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 Supplemental
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 Supplemental

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
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

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
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 Supplemental

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. Data from this study are not used to quantitatively calculate RQs for estuarine/marine macrophytes because a more sensitive endpoint of 4 ppb was reported for wild celery (Cohn, 1985).

Table A-37. Estuarine/Marine Field Study from Open Literature (2007 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 °C - 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

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 - Oat (*Avena sativa*)	97.7	**0.004 / 0.0025**	red. in dry weight	420414-03 Chetram 1989	Acceptable
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]
Spruce	2	0

Table A-39b. Summary of woody plant safety study (Hall, 2006).		
Species	**Application Rate (lbs a.i./Acre)**	**Phytotoxicity (%)**
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)
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)

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
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)	Unk.	NOAEC 2 LOAEC 10	200% incr. dark respiration 33% incr. net photosynthesis	452277-14 Hofmann and Winkler 1990	Supplemental (raw data unavailable)
Pondweed *Potamogeton perfoliatus* (4 weeks; initial conc. nominal, terminal conc. measured)	Unk	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)

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
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 ± 0.316	50% red biomass 50% red frond count	461509-01 Desjardins et al., 2003	Acceptable
Broad Waterweed *Elodea canadensis* (3 weeks; nominal)	Unk.	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)	Unk.	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)
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)
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)

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 Diatom Skeletonema costatum (120 hours; measured)	97.1	53 NOAEC 14 Slope 2.798	50% red. cell growth	430748-01 Hoberg 1993	Acceptable
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)
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)

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 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)
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)
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)

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
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)
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.

In addition, a study suggesting that atrazine toxicity to the vascular plant *Elodea canadensis* is lower when light is attenuated was submitted. In this study, plants were exposed to atrazine at approximately 500 to 2000 ug/L at 3 light intensities (0 lux, 500 lux, and 6000 lux). No effects on survival were observed at any light intensity tested at any atrazine level. Shoot length was affected at all concentrations tested at 500 lux and 6000 lux, although the magnitude of effect was higher at 6000 lux compared with the 500 lux group. Dry weight was only affected in the 6000 lux group (all concentrations tested were affected, 465 ug/L and higher). No other plant species were tested. The relevance of this study to risk assessment is uncertain. Light intensity varies daily and from day to day. It is uncertain if atrazine toxicity may be reduced during short periods of light attenuation. The dark (0 lux) group was kept in the dark 24 hours per day, and the 6000 lux groups were exposed to light at 6000 lux for 16 hours per day for 14 days. Neither of these light schedules are environmentally relevant. Also, no studies in non-vascular plants were submitted that evaluated potential effects of light intensity on atrazine toxicity..

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 *Anabaena inaequalis* (12-14 days[1]; nominal)	> 95	1,000 4,000 2,500	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	1,200 2,000 1,800	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	3,200 7,200 1,800	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	3,500 7,500 700	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	8,500 5.500 4,800	50% red. cell count 50% red. growth rate 50% red. photosynthesis	Stratton 1984	Supplemental (NOAEC and raw data 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 *Anabaena inaequalis* (12-14 days[1]; nominal)	> 95	2,500 7,000 9,000	50% red. cell count 50% red. growth rate 50% red. photosynthesis	Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Freshwater Green - Chlorophyceae *Scenedesmus quadricauda* (12-14 days; nominal)	> 95	6,900 6.500 4,000	50% red. cell count 50% red. Growth rate 50% red. photosynthesis	Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Freshwater Green - Chlorophyceae *Chlorella pyrenoidosa* (12-14 days[1]; nominal)	> 95	> 10,000 > 10,000 3,600	50% red. cell count 50% red. growth rate 50% red. photosynthesis	Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Fresh. Blue-Green - Cyanophyceae *Anabaena variabilis* (12-14 days; nominal)	> 95	5,500 9,200 4,700	50% red. cell count 50% red. growth rate 50 % red. photosynthesis	Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Fresh. Blue-Green - Cyanophyceae *Anabaena cylindrica* (12-14 days; nominal)	> 95	> 10,000 > 10,000 9,300	50% red. cell count 50% red. growth rate 50% red. photosynthesis	Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Freshwater Green Algae (*Scenedesmus subspicatus* (72 hours; nominal)	NR	1,300 (slope = 2.18 ±0.377)	50% red. cell density	470461-05 Vial, 1991e	Supplemental (study duration not sufficient to be classified as Tier II study)
		230	5% red. cell density		
		370	NOAEC for red. cell density		
		1,500 (slope = 2.36±0.382)	50% red. biomass		
		290	5% red. biomass		
		370	NOAEC for red. biomass		

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 inaequalis* (12-14 days[1]; nominal)	> 95	7,000 >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	4,600 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)

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 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 Toxicity to Terrestrial Invertebrates

The available open literature data for the toxicity of atrazine to terrestrial invertebrates is summarized in Table A-49.

Atrazine is practically non-toxic to honey bees (LD50: 97 ug/bee). It also did not cause adverse effects in fruit flies exposed to 15 ug/fly. LC50 values in earthworms ranged from 273 to 926 ppm soil (Mosleh et al., 2003; Haque and Ebing, 1983). Atrazine did not produce statistically significant ($p<0.05$) adverse effects in studies on several beetle species at any level tested, which ranged from application rates of approximately 1 lb a.i./Acre to 8 lbs a.i./Acre (Kegel, 1989; Brust, 1990; Samsoe-Petersen, 1995).

The most sensitive terrestrial invertebrate species tested was the springtail (*Onychiurus apuanicus* and *O. armatus*). Exposure to *O. apuanicus* at 2.5 ppm resulted in 18%

mortality, and exposure to *O. armatus* at 20 ppm resulted in 51% mortality (Mola et al., 1987); lower levels were not tested. These soil concentrations are associated with an application rate of approximately 1 lb a.i./Acre and 7 lbs a.i./Acre, respectively, assuming a soil density of 1.3 grams/cm^3 and a soil depth of 3 cm. Additional details on these studies may be found in Appendix A.

Several field studies reported reduced abundance in terrestrial invertebrates (Fox, 1964; Fretello et al., 1985). However, in these studies, reduced abundance could have been caused either by direct effects or by indirect effects caused by reduced vegetation of the herbicide. Available studies are summarized in Table A-49.

Table A-49. Toxixcity of Atrazine to Terrestrial Invertebrates

Species	Dose	Comment	Citation
Honey bees	LD50: >97 ug/bee	5% mortality occurred at the highest dose tested (97 ug/bee)	MRID 00036935
Ground Beetles (Poecilus) P. versicolor	NOAEC: 8 lbs/Acre	Spiked soil study; no effects occurred at any level tested.	Kegel, 1989 Ecotox No. 64007
P. cupreus	NOAEC: 0.8 lbs/Acre	Spiked soil study; no effects occurred at any level tested.	
P. lepidus	NOAEC: 0.8 lbs/Acre	Spiked soil study; a 25% reduction in survival was observed at the highest level tested that was not significant at the $p<0.05$ level.	
5 species of carabid beetles	NOAEC: 2 lbs/Acre	Beetles were dipped in atrazine solution then placed in treated soil (2.24 kg a.i./Acre); a transient repellency effect occurred for 6 days after treatment.	Brust, 1990 Ecotox No. 70406
Rove beetle	NOAEC: The single level tested was intended to approximate practical application rates.	Exposure occurred via sprayed sand; "no measurable effect" occurred at any concentration tested; dose level was reported to approximate a practical field application rate.	Samsoe-Petersen, 1995 Ecotox No. 63490
Onychiuridae Onychiurus apuanicus	NOAEC: <2.5 ppm soil (<approx. 1 lbs/Acre)a	Exposure occurred by treated soil; 18% mortality occurred at 2.5 ppm compared to 0% in controls.	Mola et al., 1987. Ecotox No. 71417
O. armatus	NOAEC: <20 ppm soil (<approx. 7 lbs/Acre)a	Exposure occurred by treated soil; 51% mortality occurred at 20 ppm compared to 0% in controls.	
Micro arthropods	NOAEC: 0.9 lbs/Acre	Field application of 1 kg/ha; atrazine was not associated with adverse effects.	Cortet et al., 2002 Ecotox No. 75784
Microarthopods	NOAEL = 2 kg/ha (1.05 ppm) LOAEC = 6 kg/ha (3.15 ppm)	Field study testing several species of microarthopods. It could not be determined if reduced abundance was caused by migration (repellency), by toxic effects, or both.	Fratello et. al., 1985 Ecotox No. 59428
Fruit flies Drosphilia	NOAEC: 15 ug/fly	No increased mortality occurred in groups exposed to atrazine alone relative to controls.	Lichtenstein et al., 1973 Ecotox No. 2939
Earthworm Aporrectodea caliginosa	28-day LC50: 381 ppm	Spiked soil study, Slope: 8.37	Mosleh et al., 2003 Ecotox No. 77549
Earthworm Eisenia fetida	14-Day LC50: 273 mg/kg soil (ppm) –	Spiked soil study; endpoints evaluated included mortality and biomass.	Haque and Ebing, 1983

	926 ppm		Ecotox No. 40493
Earthworm Eisenia fetida	4-Day LC50: 2.9 ug/cm2	Filter paper study	Lydy and Linck, 2003 Ecotox No. 71459
Earthworm Wire worm Springtail	LOAEC = 8 lb/acre	Field study examining the impacts of several herbicides on soil invertebrate populations. The endpoint measured was abundance of several species. Study authors suggested that reduced abundance was likely caused by repellency and not direct toxicity.	Fox, 1964 Ecotox No. 36668

An estimation of application rates was made from soil concentration by assuming a soil depth of 3 cm and a soil density of 1.3 g/cm3.

A.6 Effects of Environmental Factors and Life-Stage on Aquatic Atrazine Toxicity

A.6.1 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 ^oC) 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 ^oC), 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.6.2 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.7 Pesticide Toxicity Interactions

A number of authors have reported toxic interactions between atrazine, its dealkylated degradates and other pesticides. Synergism between atrazine and a number of other pesticides has also been reported in aquatic organisms, particularly with organophosphate insecticides, a carbamate insecticide and other herbicides including metolachlor.

A.7.1 Plants

In 1974, Putnam and Penner reported on the effects of interactions of herbicides on higher plants. Atrazine was cited in test combinations with 5 herbicides, 2 insecticides and a fungicide. Synergistic effects (i.e., increased toxicity higher than additivity) was identified in 6 out of the 8 test combinations. Atrazine was synergistic with 4 herbicides (i.e., 2, 4-D (oil), paraquat, EPTC, and alachlor) and 2 insecticides (i.e., diazinon and fensulfothion). Atrazine test combinations with dalapon, a herbicide, and dexon, a fungicide, showed antagonistic interactions.

Torres and O'Flaherty (1976) report additive toxicity of atrazine with simazine at concentrations of 1.0 ug/L and 1 mg/L for *Chlorella vulgaris*, *Stigeoclonium tenue*, *Tribonema* sp., *Vaucheria geminata*, and *Oscillatoria lutea*. Additive toxicity of malathion with atrazine was found in *Chlorella vulgaris*, but could not be assessed with other species, because malathion produced total inhibition of chlorophyll production at 1 ug/L or greater concentrations. At 1 and 1,000 ug/L, pesticides mixtures increased toxicity from 2.4 to 100 percent over the toxic levels of atrazine alone. Mixtures of these pesticides at concentrations of 0.1 and 0.5 ug/L usually enhanced the production of chlorophyll.

Stratton (1984) also tested the most sensitive algal species, *Anabaena inaequalis*, with mixtures of atrazine and its two most toxic degradates, deethylatrazine and deisopropylatrazine. Cell count results indicate that combinations of atrazine/deethylatrazine (1.8) and atrazine/deisopropylatrazine (1.3) are synergistic and deethylatrazine/deisopropyl-atrazine mixtures are additive (1.03). For photosynthesis, results after 3 hour exposures indicate that all mixture combinations for these three chemicals are antagonistic (0.8, 0.86, and 0.89).

Burrell *et al*. (1985) reported 11-day interactions between algal populations and between algal populations and pesticides. Population interactions showed that *Chlorella vulgaris* inhibited population growth of *Ankistrodesmus braunii* by 32 percent. The addition of the bacterium, *Chromobacterium violaceum*, added to the algal mixture further inhibited population growth of *A. braunii* by an additional 17% and bacterial growth was stimulated, but the bacterium had no effect on *Chlorella* populations. The combined effect of the mixtures of atrazine (60 μg/L) and sodium pentachlorophenate (Na-PCP) (0, 300, 800, 1,000 and 1,200 μg/L) and atrazine (40 and 100 μg/L) with Na-PCP (700 and 1,200 μg/L) on *A. braunii* populations were additive over a wide range of concentrations. Similar results of atrazine (10 and 100 μg/L) and Na-PCP (300 and 1,200 μg/L) were obtained with C. *vulgaris.* In mixed algal cultures tested with atrazine (40 and 100 g/L), cell numbers of *A. braunii* were reduced 50 and 80 percent, respectively, which was not significantly different than effects when tested alone. In the same mixed culture test, atrazine inhibited growth of C. *vulgaris* by 79 and 85 percent, respectively, which showed a significant growth inhibition only at the lower test concentration (40 μg/L). The authors concluded that the high atrazine concentration (100 μg/L) did not alter the established population relationship between the two algal species, but at the lower concentration (40 μg/L), *A. braunii* increased the susceptibility of *C. vulgaris* to atrazine.

When mixed cultures of algae were treated with both atrazine (60 μg/L) and Na-PCP (300, 800, 1,000 and 1,200 μg/L), chemical antagonism was observed. The addition of the bacterium, *C. violaceum*, to the microcosm, had no effect on the level of antagonism for *A. braunii*. *C. violaceum* modified the antagonism of atrazine toxicity to *C. vulgaris* by about 40 percent, but the antagonistic effect was not eliminated. The net atrazine toxicity decreased as the Na-PCP concentration increased. The authors found no reason for the modification of atrazine effects by *C. violaceum*.

Carder and Hoagland (1998) reported that pesticide interactions of atrazine (0, 12 and 150 μg/L) and alachlor (0, 5, 90 μg/L) on benthic algal communities in artificial recirculating streams showed significant interaction (i.e., antagonism) only in the first week in the combination of high alachlor and low atrazine test concentrations. The authors concluded that the interaction is most likely anomalous and the lack of significant synergistic effects may be attributed to different modes of action.

A.7.2 Animals

A number of authors have reported synergistic effects of atrazine with terrestrial and aquatic animals with one or more of the following pesticides: (i.e., alachlor, chlorpyrifos, DDT, diazinon, malathion, methyl parathion, parathion, metolachlor, and trichlorfon).

Liang and Lichtenstein (1975) also found atrazine synergism between soil residues of both DDT and parathion using fruit flies, *Drosophila melanogaster* and measured lethal effects versus the age of the pesticide residues in soil. Ten grams of Plainfield sand (1.2 % organic matter) or Plano silt loam (4.7% organic matter) was mixed with parathion (2.3 μg/10 g of soil = 0.23 ppm) or DDT (30 μg/10 g of soil = 3 ppm), then was mixed with 10 g of the same soil type, which contained increasing atrazine levels (40 to 1000 μg/10 g of soil = 4 to 100 ppm) or controls. Fifty fruit flies were placed in 120 ml test jars for 24 hours with the 10-g portions of air-dried soil untreated or treated with atrazine, parathion, DDT or combinations thereof. The resulting 24-hour fruit fly LD_{50} values for constant soil levels of parathion (2.3 ppm) and DDT (3.0 ppm) were as follows: parathion (6.2 ppm atrazine in sand and 92 ppm in loam) and DDT (8.5 ppm atrazine in sand and 68 ppm in loam). Synergistic effects were apparent in all test combinations of soil and pesticides yielding a dose-response effect on fly mortality with increasing atrazine soil concentrations. Fruit fly mortality levels with both parathion and DDT in soils also clearly indicate a strong reduction in toxicity with the silt loam soil with a higher percentage of organic matter (4.7%) compared to sandy soil (1.2%).

Additional loam soil toxicity tests were conducted daily for 4 days, with aged-atrazine soil with an initial 50 ppm aged in the dark at 22EC and both fresh and aging-parathion soil levels (0.35 ppm). In the test with fresh parathion soils and aged-atrazine soils, toxicity to fruit flies decreased linearly from 95% mortality on Day 0 to 43.3% over four days. By the fourth day, atrazine levels had declined to 19 ppm, which was barely enough to synergize parathion in loam soils. In another toxicity test, parathion-treated soils were aged under the same conditions as above and added it daily to the initial 10 g

of atrazine-treated soil (50 ppm). In this test, the toxicity to fruit flies decreased logarithmically from about 68% on Day 0 to 10% mortality on Day 4. The measured concentrations of aging parathion in the silt loam soil decreased at a rate paralleling the logarithmic toxicity curve. The final parathion level on Day 4 was 0.24 ppm.

Liang and Lichtenstein (1975) found atrazine to be synergistic with parathion in 24-hour aquatic tests with third-instar mosquito larvae, *Aeddes aegypti* and also assessed the effects of sand and loam soils on their individual and combined toxicity in 20 ml of pesticide-treated water. Atrazine at 10,000 μg/L showed no toxicity to the mosquito larvae; alone, parathion (15 μg/L) killed 20 ± 7 percent of the larvae; and at these concentrations, the combination of the two pesticides produced significantly ($p = 0.01$) higher mortality (73 ± 18 %). Addition of 5 g of Plainfield sand (1.2% organic matter) with 15 μg/L parathion reduced the toxicity of parathion from 20 ± 7% to 18 ±4%, but when sand was mixed into the water, mortality drop to 5%. Plano silt loam soil (4.7% organic matter) without mixing reduced parathion toxicity from 20 ± 7% to 5 ± 4% and when the loam soil was mixed into the water, no mosquito larvae died. When these two soils were added to the same combination of atrazine and parathion, sand reduced the mortality from 73 ± 14% to 71 ± 14% (unmixed) and to 18 ± 4 % when mixed into the water; loam soil reduced the mortality from 73% to 64 ± 4% (unmixed) and to no mortality with mixing. The combination of atrazine and parathion was significantly ($p = 0.01$) more toxic than the toxicity of parathion or atrazine alone.

The above toxicity test method was repeated using 1 and 5 grams of sand or silt loam to measure the effect of different amounts of soil on toxicity following 24-hour exposures. Atrazine (10 ppm) produced no mortality in 24 hours to mosquito larvae. Parathion (0.015 ppm) produced 24 ± 7% mortality (no soil), 16 ± 7% (1g of sand), 2 ± 2% (5 g of sand), 7 ± 0% (1 g of loam soil) and 0% (5 g of loam soil). The combination of atrazine (10 ppm) and parathion (0.015 ppm) showed synergistic effects on mosquito larvae mortality: 62 ± 8% (no soil), 42 ± 10% (1 g of sand), 2 ± 2% (5 g of sand), 22 ± 4% (1 g of loam soil) and 0% (5 g of loam soil). This test format was repeated using higher pesticide concentrations and again the mortality levels were increased with a mixture of atrazine (20 ppm) and parathion (0.30), but the synergistic increase was much lower than in the previous test. The 24-hour results indicated that atrazine alone was not toxic to mosquito larvae; 0.30 ppm parathion (93 ± 6% mortality with on soil), 62 ± 8% with 5 g of sand and no mortality with silt loam soil. The mixture of 20 ppm atrazine and 0.30 ppm parathion produced 98 ± 4% mortality with no soil, 76 ± 4% dead when shaken with 5 g of sand, and 38 ± 10% lethality when shaken with 5 g of silt loam soil. These studies demonstrate that atrazine is synergistic with parathion and, like single toxicants, organic matter in soils and sediments will modify toxicity of pesticide mixtures, especially if the organic matter is suspended in the water. While this particular study has limited value for risk assessment, because the atrazine levels (10 and 20 ppm) exceed the normal environmental range of atrazine exposures, the study suggests that synergism of atrazine and parathion may occur at lower concentrations, possibly in the range of environment levels of atrazine.

Pape-Lindstrom and Lydy (1997) tested atrazine with 6 pesticides for chemical interactions using 4th instar midges (*Chironomus tentans*). The 96-hour test results for the pesticide mixtures indicated that atrazine was synergistic with the phosphonate insecticide, trichlorfon, (0.26 toxic units) and 3 phosphorothioate insecticides (i.e., malathion (0.36 TU), chlorpyrifos (0.58 TU) and methyl parathion (0.59 TU). The atrazine-mevinophos (a phosphate) mixture was less than additive (1.34 TU), while methoxychlor, a organochlorine insecticide mixture was also less than additive (1.67 TU). The results from these tests are questionable, since DMSO was used as a solvent with atrazine. These tests were repeated by Belden and Lydy (2000) without DMSO and with lower atrazine concentrations (0, 10, 40, 80, and 200 μg/L). Acute 96-hour tests with *Chironomus tentans* were conducted with each pesticide and EC_1, EC_5, EC_{15} and EC_{50} values were determined based on inability of the midge to swim when prodded with forceps. Chemical interactions were tested at each of these EC levels with atrazine levels of 0, 10, 40, 80 and 200 μg/L using 5 replicates of 10 midges each. Atrazine increased the toxicity of chlorpyrifos, diazinon and parathion, but not malathion. The authors concluded that "Interaction terms were not significant for atrazine + methyl parathion and atrazine + diazinon; however, a significant interaction was found for the atrazine + chlorpyrifos test ($p = 0.002$, df - 12, $F = 2.94$)." Synergistic ratios were reported as follows: chlorpyrifos, 1.83 at 40 μg/L and 4.00 at 200 μg/L atrazine; at 200 μg/L diazinon the SR was 2.71 and for methyl parathion, the SR was 1.94. The variety of chemical interactions produced by atrazine mixtures indicates that the effect of atrazine on an organism is dependent on the species, cocontaminant, and the concentration of atrazine. Additional tests with 200 μg/L atrazine and chlorpyrifos showed that atrazine increased the uptake of chlorpyrifos by 42 percent, and that the atrazine induction of cytochrome-P450 increased the formation of the O-analog which increased the toxicity of chlorpyrifos at enviromentally relevant concentrations.

Anderson and Lydy (2002) demonstrated that atrazine concentrations as low as 80 μg/L significantly increased the acute toxicity of diazinon to the amphipod *Hyallela azteca*. Using larvae of the mide *Chironomus tentans*, Belden and Lydy (2000) demonstrated a significant increase in diazinon toxicity when simultaneous exposure to 40 μg/L of atrazine occurred. In another study by Banks et al (2005), the study authors demonstrated that atrazine concentrations as low as 5.0 μg/L in combination with diazinon resulted in significant ($P < 0.05$) increases in the 48-h toxicity of diazinon to *Ceriodaphnia dubia*. Atrazine induces cytochrome P450 and general esterase activities in insects (Kao et al., 1995). It is possible that induction of the P450 system by atrazine may either increase or decrease the toxicity of other chemicals, depending on whether metabolites of the chemical in question are more or less toxic than the parent compound itself. Syngeristic effects of organophosphates and atrazine observed by Pape-Lindstrom and Lydy (1997) for the midge C. tentans suggest that processes involved with oxidation of some organophosphate molecules to more toxic oxon metabolites may be enhanced in the presence of atrazine.

Howe *et al*. 1998 reported synergism between atrazine and alachlor, a herbicide, in tests with young rainbow trout, channel catfish and early and late tadpole stages of the

northern frog and the American toad. The results are presented in the table below. (MRID # 452029-10).

Species (stage)	Time (hour)	Atrazine LC50 (95% CI) mg/L	Alachlor LC50 (95% CI) mg/L	Atrazine-Alachlor LC50[a] (95% CI) mg/L	Additive Index[b] (95% CI)
Rainbow trout	24	31.6 (28.2 - 35.4)	10.6 (9.5 - 11.7)	9.5 (8.3 - 10.9)	-0.20 (-0.53-0.059)
(0.8-1.0-gram juveniles)	96	20.5 (18.3 - 22.9)	9.1 (9.0 - 9.2)	6.5 (5.7 - 7.7)	-0.03 (-0.28-0.15)
Channel catfish	24	51.3 (44.6 - 59.0)	23.8 (22.7 - 25.0)	11.1 (9.6 - 12.4)	0.29 (0.067-0.55)[c]
(0.9-1.1-gram juveniles)	96	23.8 (22.3 - 25.5)	16.7 (15.1 - 18.4)	7.5 (5.3 - 8.4)	0.31 (0.072-0.57)[c]
Northern leopard frog	24	69.7 (63.1 - 77.2)	14.9 (13.3 - 16.6)	12.1 (11.0 - 12.9)	0.015 (-0.17-0.24)
(0.7-0.9-gr early larvae)	96	47.6 (41.4 - 54.8)	11.5 (10.1 - 13.2)	6.5 (5.7 - 7.7)	0.43 (0.054-0.87)[c]
Northern leopard frog	24	45.3 (42.3 - 48.5)	7.3 (6.6 - 8.0)	5.9 (5.5 - 6.4)	0.07 (-0.12-0.25)
(1.4-1.9-gr late larvae)	96	14.5 (11.9 - 17.5)	3.5 (3.1 - 3.8)	2.1 (2.0 - 2.3)	0.34 (0.069-0.56)[c]
American Toad	24	66.4 (58.9 - 74.9)	5.7 (4.7 - 5.8)	4.4 (4.2 - 4.6)	0.19 (-0.057-0.28)
(0.1-0.2-gr early larvae)	96	26.5 (23.0 - 30.5)	3.9 (3.7 - 4.2)	1.8 (1.7 - 1.9)	0.89 (0.68 - 1.2)[c]
American Toad	24	15.8 (13.5 - 18.4)	4.3 (3.8 - 4.8)	2.9 (2.6 - 3.3)	0.17 (0.11 - 0.46)[c]
(0.4-0.5-gr late larvae)	96	10.7 (9.2 - 12.5)	3.3 (2.8 - 3.6)	1.5 (1.4 - 1.6)	0.68 (0.34 - 1.0)[c]

[a] 50:50 mixture of atrazine 4L (40.8% ai.) and alachlor EC (43.0% ai.).
[b] An additive index greater than zero indicates greater than additive toxicity.
[c] Significant chemical synergy interaction between atrazine and alachlor.

Boone and Bridges-Britton (2006) examined the single and interactive effects of atrazine, carbaryl, and ammonium nitrate fertilizer on metamorphosis of tadpoles of the gray treefrog (*Hyla versicolor*). Tadpoles were reared in mesocosms from hatching through metamorphosis and were exposed to the presence or absence of the three contaminants. The results of the study indicated that the presence of multiple, sublethal chemical stressors with different modes of action may not be more determintal than that of one chemical factor alone.

As previously discussed in Section A.2.4d and summarized in Table A-16, Hayes et al. (2006) assessed the effect of three different mixtures containing atrazine, to mortality, growth and development, gonadal development, thymus histology, and disease rates (i.e., immune function) in larval leopard frogs (*R. pipiens*). The three mixtures included atrazine and S-metalachlor at 0.1 and 10 ppb, Bicep II Magnum (reported as 33.3% atrazine, 0.7% atrazine-related products, 26.1% TGAI of S-metolachlor, and 40.2% inert ingredients), and a mixture of the 9-pesticides (atrazine, metolachlor, alchlor, nicosulfuron, cyfluthrin, cyhalothrin, tebupirimphos, methalaxyl, and propiconizole all at 0.1 pbb). The specifics of the study, including uncertainties, which preclude the use of this data quantitatively, are discussed as part of Section A.2.4d. In summary, many of the confounding effects identified in previous studies by the FIFRA SAP limit the utility of this study.

Thymus histology was completed (to measure immunocompetence) after the study authors noted that animals exposed to the 9-compound pesticide mixture experienced increased incidence of bacterial infection with *Chryseobacterium (Flavobacterium) menigosepticum*. Animals exposed to the 9-compound pesticide mixture at 0.1 ppb had significantly longer larval periods. All mixtures resulted in reduced growth (SVL and

BW), as compared to the solvent control, with the atrazine and S-metolachlor mixture having the greatest negative effect. With respect to gonadal development, the gonads and gametes were underdeveloped in both the control and treatment groups; therefore, it was not possible for the study authors to assess the affects of mixtures on sex differentiation. Exposure to the Bicep mixture (atrazine and S-metolachlor) and the 9-compound mixture resulted in damage to the thymus as measured by thymic plaques; however, the ecological relevance of thymic plaques is not discussed. Given the increased incidence of disease and evidence of histological effects on the thymus in animals exposed to the mixtures, the study authors suggest that exposure to pesticide mixtures renders amphibians more susceptible to disease as a result of immunosuppression.

As part of the same study, Hayes et al. (2006) also examined the effects of the 9-compound mixture on plasma corticosterone levels (stress hormone) in adult male African clawed frogs (*X. laevis*). Five males were treated with the 9-compound pesticide mixture (including atrazine at 0.1 ppb) and five males were exposed to ethanol only (no negative control was tested). Following the 27 day exposure period, blood was collected by cardiac puncture. The study authors report a clear effect on corticosterone levels in male African clawed frogs with corticosterone levels increasing 4-fold in pesticide-exposed males. However, there are several flaws in the study design, which are discussed in Section A.2.4d, that add a high degree of uncertainty to the results.

A.8. References

Abou-Waly, Hoda, M. M. Abou-Setta, H. N. Nigg and L. L. Mallory. 1991. Growth response of freshwater algae, *Anabaena flos-aquae* and *Selenastrum capricornutum* to Atrazine and hexazinone herbicides. Bull. Environ. Contam. Toxicol. 46:223-229.

Alazemi, B. M., J. W. Lewis and E. B. Andrews. 1996. Gill damage in the freshwater fish *Gnathonemus petersii* (Family: Mormyridae) exposed to selected pollutants: An ultrastructural study. Environ. Technol. 17:225-238. (MRID # 452029-05).

Allran, J. W. and Karasov, W. H. (2001). Effects of Atrazine on Embryos, Larvae, and Adults of Anuran Amphibians. *Environ.Toxicol.Chem.* 20: 769-775. EcoReference No.: 59251.

Alvarez, M. C. (2005). Significance of Environmentally Realistic Levels of Selected Contaminants to Ecological Performance of Fish Larvae: Effects of Atrazine, Malathion, and Methylmercury. *Ph.D.Thesis, Univ.of Texas, Austin, TX* 141 p. EcoReference No.: 81672.

Anderson, T.D. and M.J. Lydy. 2002. Increased toxicity to invertebrates associated with a mixture of atrazine and organophosphate insecticides. Environ. Toxicol. Chem. 21:1507-1514.

Armstrong, D. E., C. Chester and R. F. Harris 1967. Atrazine hydrolysis in soil. Soil Sci. Soc. Amer. Proc. 31:61-66.

Atkins, E. L., E. A. Greywood and R. L. MacDonald. 1975. Toxicity of pesticides and other agricultural chemicals to honey bees: Laboratory studies. Prepared by Univ. of Calif.,Div. Agric. Ser., Leaflet 2287. 38 p. (MRID No. 000369-35).

Baier, C. H., K. Hurle and J. Kirchhoff. 1985. Datensammlung zur Abschätzung des Gefährdungspotentials von Pflanzenschutzmitteln-Wirkstoffen für Gewässer. Deutscher Verband für Wasserwirtschaft und Kulturbau e. V., Verlag Paul Parey, Hamburg and Berlin, pp. 74-294.

Baird, Donald J., Ian Barber, Amadeu M. V. M. Soares and Peter Calow. 1991. An early life-stage test with *Daphnia magna* Straus: An alternative to the 21-day chronic test? Ecotox. and Environ. Safety 22:1-7. (MRID # 452277-01).

Baker, D. B. 1987. Lake Eire Agro-Ecosystem Program: Sediment, nutrient, and pesticide export studies. US EPA, Great Lakes National Program Office, Chicago, IL.

Baker, D. B., K. A. Kieger, R. P. Richards and J. W. Kramer. 1985. Effects of intensive agricultural land use on regional water quality in northwestern Ohio, p. 201-207. *In*: US EPA. Perspectives on nonpoint source pollution, proceedings of a national conference, Kansas City, MO. EPA-440/5-85-001.

Baker, D. B., K. A. Krieger and J. V. Setzler. 1981. The concentrations and transport of pesticides in northwestern Ohio rivers – 1981. US Army Corps of Engineers, Tech. Rep. Series, No. 19, Buffalo District, Buffalo, NY.

Banks, K.E., P.K. Turner, S.H. Wood, and C. Matthews. 2005. Increased toxicity to Ceriodaphnia dubia in mixtures of atrazine and diazinon at environmentally realistic concentrations. Ecotox. And Env. Safety 60: 28-36.

Baturo, W., L. Lagadic and T. Caquet. 1995. Growth, fecundity and glycogen utilization in *Lymnaea palustris* exposed to atrazine and hexachlorobenzene in freshwater mesocosms. Environ. Toxicol. Chem. 14(3):503-511. (MRID # 450200-13).

Bejarano, A. C. and Chandler, G. T. (2003). Reproductive and Developmental Effects of Atrazine on the Estuarine Meiobenthic Copepod Amphiascus tenuiremis. *Environ.Toxicol.Chem.* 22: 3009-3016. EcoReference No.: 73333.

Belden, J.B. and M.J. Lydy. 2000. Impact of atrazine on organophosphate insecticide toxicity. Environ. Toxicol. Chem. 19(9):2266-2274.

Beliles, R. P. and W. J. Scott, Jr. 1965. Atrazine safety evaluation on fish and wildlife (Bobwhite quail, mallard ducks, rainbow trout, sunfish, goldfish). Prepared by

Woodard Res. Corp.; submitted by Geigy Chemical Co., Ardsley, NY. (MRID No. 000592-14).

Beliles, R. P. and W. J. Scott, Jr. 1965. Atrazine safety evaluation on fish and wildlife (Bobwhite quail, mallard ducks, rainbow trout, sunfish, goldfish): Atrazine: Acute toxicity in goldfish. Prepared by Woodard Res. Corp.; submitted by Ciba-Geigy Corp., Greensboro, NC. (MRID No. 000247-18).

Beliles, R. P. and W. J. Scott, Jr. 1965. Atrazine safety evaluation on fish and wildlife Bobwhite quail, mallard ducks, rainbow trout, sunfish, goldfish): Atrazine: Acute toxicity in rainbow trout. Prepared by Woodard Res. Corp.; submitted by Ciba-Geigy Corp., Greensboro, NC. (MRID No. 000247-16).

Beliles, R. P. and W. J. Scott, Jr. 1965. Atrazine safety evaluation on fish and wildlife (Bobwhite quail, mallard ducks, rainbow trout, sunfish, goldfish): Atrazine: Acute toxicity in sunfish. Prepared by Woodard Res. Corp.; submitted by Ciba-Geigy Corp., Greensboro, NC. (MRID No. 000247-17).

Bentley, R. E. and K. J. Macek. 1973. Acute toxicity of atrazine to mud crab (*Neopanope texana*). Prepared by Bionomics, Inc.; Submitted by Ciba-Geigy Corp., Greensboro, NC. (MRID No. 000247-19).

Benson, B. and G. M. Boush. 1983. Effect of pesticides and PCBs on budding rates of green hydra. Bull. Environ. Contam. Toxicol. 30:344-350. (MRID # 452029-01).

Best, L. B., K. E. Freemark, J. J. Dinsmore and M. Camp. 1995. A review and synthesis of habitat use by breeding birds in Agricultural Landscapes of Iowa. Amer. Midl. Nat. 124:1-29. (MRID # 452051-03).

Bester, K., H. Huhnerfuss, U. Brockmann and H. J. Rick. 1995. Biological effects of triazine herbicide contamination on marine phytoplankton. Arch. Environ. Contam. Toxicol. 29:277-283. (MRID # 450200-21).

Biagianti-Risbourg, S. and J. Bastide. 1995. Hepatic perturbations induced by a herbicide (atrazine) in gray mullet *Liza ramada* (Mugilidae, Teleostei): An ultrastructural study. Aquat. Toxicol. 31:217-229. (MRID # 452049-02).

Birge, W. J., J. A. Black and D. M. Bruser. 1979. Toxicity of organic chemicals to embryo-larval stages of fish. US. EPA, Office of Toxic Substances, EPA-560/11-79-007. 60 p. (MRID # 452029-02).

Birge, W. J., J. A. Black and R. A. Kuehne. 1980. Effects of organic compounds on amphibian reproduction. University of Kentucky, Water Resour. Res. Inst., Res. Rep. 121. 39 p. (USDI, Agreement Numbers: 14-34-0001-7038 (FY 1977), 14-

34-0001-8019 (FY 1978), and 14-34-0001-9091 (FY 1979). (MRID # 452083-02).

Birge, W. J., Black, J. A., Westerman, A. G., and Ramey, B. A. (1983). Fish and Amphibian Embryos - a Model System for Evaluating Teratogenicity. *Fundam.Appl.Toxicol.* 3: 237-242. EcoReference No.: 19124.

Belden, J. B. and M. J. Lydy. 2000. Impact of atrazine on organophosphate insecticide toxicity. Environ. Toxicol. Chem. 19(9):2266-2274. (MRID # 452277-02).

Bond, C. E. 1966. Progress report on aquatic weed research. Projects 773 and 294. Dept. Fish. Wildlife, Oreg. Agr. Exp. Sta., Oreg. State Univ.

Boone, M. D. and James, S. M. 2003. Interactions of an Insecticide, Herbicide, and Natural Stressors in Amphibian Community Mesocosms. *Ecol.Appl.* 13: 829-841. EcoReference No.: 81455

Boone, M.D. and C.M. Bridges-Britton. 2006. Examining Multiple Sublethal Contaminants on the Gray Treefrog (Hyla versicolor): Effects of an Insecticide, Herbicide, and Fertilizer. Env. Tox. And Chem. 25(12):3261-3265. EcoReference No.: 88290.

Braun, F., W. Schűssler and R. Wehrle. 1987. Organische Schadstoffe, Polychlorbiphenyle (PCB) und Pestizide im Kreislauf des Wassers. Bilanzierung und Bewertung. Report of the Bavarian Water Research Institute, Munich, pp. 18-27.

Brockway, D. L., P. D. Smith and F. E. Stancil. 1984. Fate and effects of atrazine in small aquatic microcosms. Bull. Environ. Contam. Toxicol. 32:345-353. (MRID # 450874-07).

Brooke, L. 1991. Results of Freshwater Exposures with the Chemicals Atrazine, Biphenyl, Butachlor, Carbaryl, Carbazole, Dibenzofuran, 3,3'-Dichlorobenzidine, Dichlorvos 1,2-Epoxyethylbenzene (Styrene Oxide), Isophorone, Isopropalin, Oxychlordane, Pentachloroanisole, Propoxur (Baygon), Tetrabromobisphenol A, 1,2,4,5-Tetrachlorobenzene, and 1,2,3-Trichloropropane to Selected Freshwater Organisms. *Ctr.for Lake Superior Environ.Stud., Univ.of Wisconsin-Superior, Superior, WI* 110 p. EcoReference No.: 17138

Buccafusco, R. 1976. Acute toxicity of Atrazina technical to bluegill (*Lepomis macrochirus*). Prepared by EG & G Bionomics, Wareham, MA; submitted by unknown. (MRID No. 001471-25).

Burrell, R. E., W. E. Inniss and C. I. Mayfield. 1985. Detection and analysis of interactions between atrazine and sodium pentachlorophenate with single and

multiple algal-bacterial populations. Arch. Environ. Contam. Toxicol. 14:167-176.

Butler, G. L., T. R. Deason and J. C. O'Kelley. 1975. The effect of atrazine, 2, 4-D, methoxychlor, carbaryl and diazinon on the growth of planktonic algae. Br. Phycol. J. 10:371-376.

Cafarella, M.A. 2005a. Atrazine (G-30027) – Early life-stage toxicity test with sheepshead minnow (*Cyprinodon variegates*). Unpublished study performed by Springborn Smithers Laboratories, Wareham, MA. Laboratory Project No. 1781.6642. Study submitted by Syngenta Crop Protection, Inc., Greensboro, NC. (MRID 466482-03).

Cafarella, M.A. 2005b. Atrazine (G-30027) – Acute Toxicity to Eastern Oysters (*Crassostrea virginica*) Under Flow-Through Conditions. Unpublished study performed by Springborn Smithers Laboratories, Wareham, MA. Laboratory Project No. 1781.6640. Study submitted by Syngenta Crop Protection, Inc., Greensboro, NC. (MRID 466482-01).

Cafarella, M.A. 2005c. Atrazine (G-30027) – Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*). Unpublished study performed by Springborn Smithers Laboratories, Wareham, MA. Laboratory Project No. 1781.6641. Study submitted by Syngenta Crop Protection, Inc., Greensboro, NC. (MRID 466482-02).

Capel, P. D., M. Lin and P. J. Wotzka. 1994. Pesticides in rains in Minnesota 1991-1993: An interim report. Minnesota Dept. Agr., Agronomy Serv. Div., p. 26. (Additional pages on 1994 data).

Carder, J. P. and K. D. Hoagland. 1998. Combined effects of alachlor and atrazine on benthic algal communities in artificial streams. Environ. Toxicol. Chem. 17(7):1415-1420. (MRID # 450200-02).

Carney, E. C. 1983. The effects of atrazine and grass carp on freshwater communities. Thesis Univ. of Kansas, Lawrence, Kansas, USA.

Caux, Pierre-Yves, Lucie Menard, and Robert A. Kent. 1996. Comparative study of the effects of MCPA, butylate, atrazine, and cyanazine on *Selenastrum apricornutum*. Environ. Poll. 92(2):219-225.

Chetram, R. S. 1989. Atrazine: Tier 2 seed emergence nontarget phytotoxicity test. Lab, Study No. LR 89-07C. Prepared by Pan-Agricultural Laboratories, Inc., Madera, CA.; submitted by Ciba-Geigy Corporation, Greensboro, NC. (MRID No. 20414-03).

Chetram, R. S. 1989. Atrazine: Tier 2 seed germination nontarget phytotoxicity test. Lab, Study No. LR 89-07B. Prepared by Pan-Agricultural Laboratories, Inc., Madera, CA.; submitted by Ciba-Geigy Corporation, Greensboro, NC. (MRID No. 12230-01).

Chetram, R. S. 1989. Atrazine: Tier 2 vegetative vigor nontarget phytotoxicity test, Lab, Study No. LR 89-07A. Prepared by Pan-Agricultural Laboratories, Inc., Madera, CA.; submitted by Ciba-Geigy Corporation, Greensboro, NC. (MRID No. 412230-03).

Chetram, R. S. 1989. Atrazine: Tier 2 vegetative vigor nontarget phytotoxicity test, Lab, Study No. LR 89-07A. Prepared by Pan-Agricultural Laboratories, Inc., Madera, CA.; submitted by Ciba-Geigy Corporation, Greensboro, NC. (MRID No. 420414-02).

Coady, K. K., Murphy, M. B., Villeneuve, D. L., Hecker, M., Jones, P. D., Carr, J. A., Solomon, K. R., Smith, E. E., Van der Kraak, G., Kendall, R. J., and Giesy, J. P. (2004). Effects of Atrazine on Metamorphosis, Growth, and Gonadal Development in the Green Frog (Rana clamitans). *J.Toxicol.Environ.Health Part A* 67: 941-957. EcoReference No.: 78295.

Coady, K. K., Murphy, M. B., Villeneuve, D. L., Hecker, M., Jones, P. D., Carr, J. A., Solomon, K. R., Smith, E. E., Van der Kraak, G., Kendall, R. J., and Giesy, J. P. (2005). Effects of Atrazine on Metamorphosis, Growth, Laryngeal and Gonadal Development, Aromatase Activity, and Sex Steroid Concentrations in Xenopus laevis. *Ecotoxicol.Environ.Saf.* 62: 160-173. EcoReference No.: 81457.

Cohn, S. L. Unpublished. An evaluation of the toxicity and sublethal effects of atrazine on the physiology and growth phases of the aquatic macrophyte *Vallisneria americana* L. American Univ., Ph. D Thesis. (1985). (MRID # 450200-01).

Conners, D. E. and Black, M. C. (2004). Evaluation of Lethality and Genotoxicity in the Freshwater Mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) Exposed Singly and in Combination to Chemicals Used in Lawn Care. *Arch.Environ.Contam.Toxicol.* 46: 362-371. EcoReference No.: 74236.

Correll, D. L., J. W. Pierce and T. L. Wu. 1978. Herbicides and submerged plants in Chesapeake Bay, p. 858-877. *In*: Proc. Symp. Tech., Environ. Socioecon. And Regul. Aspects Coastal Zone Manag., ASCE, San Francisco, Calif.

Correll, D. L. and T. L. Wu. 1982b. Atrazine toxicity to submersed vascular plants in simulated estuarine microcosms. Aquatic Botany 14:151-158. (MRID # 450874-08).

Cossarini-Dunier, M. A. Demael, J. L. Riviere and D. Lepot. 1988. Effects of oral doses of the herbicide atrazine on carp (*Cyprinus carpio*). Ambio 17(6):401-405. (MRID # 452029-03).

Crain, D. A., L. J., Guillette, Jr., A. A. Rooney and D. B. Pickford. 1997. Alterations in steroidogenesis in alligators (*Alligator mississippiensis*) exposed to naturally and experimentally to environmental contaminants. Environ. Health Perspectives 105(5):528-533.

Crain, D. A., Spiteri, I. D., and Guillette, L. J. Jr. (1999). The Functional and Structural Observations of the Neonatal Reproductive System of Alligators Exposed In Ovo to Atrazine, 2,4-D, or Estradiol. *Toxicol.Ind.Health* 15: 180-185. EcoReference No.: 70208.

Cunningham, J. J., W. M. Kemp, M. R. Lewis and J. C. Stevenson. 1984. Temporal responses of the macrophyte, *Potamogeton perfoliatus* L., and its associated autotrophic community to atrazine exposure in estuarine microcosms. Estuaries 7(4B):519-530. (MRID # 450874-03).

Dao, T. H., T. L. Lavy and R. C. Sorenson. 1979. Atrazine degradation and residue distribution in soil. Soil Sci. Soc. Amer. J. 43:1129-1134.

Davies, P. E., L. S. J. Cook and J. L. Barton. 1994. Triazine herbicide contamination of Tasmanian streams: Sources, concentrations and effects on biota. Aust. J. Mar. Freshwater Res. 45:209-226. (MRID # 450200-03).

Davies, P. E., L. S. J. Cook and D. Goenarso. 1994. Sublethal responses to pesticides of several species of Australian freshwater fish and crustaceans and rainbow trout. Environ. Toxicol. Chem. 13(8):1341-1354. (MRID # 452029-04).

Davis, D. E. 1980. Effects of herbicides on submerged seed plants. Auburn Univ., Dept. Botany, Plant Pathol. Microbio., USDI, Project A-067-ALA (Oct. 1, 1978 to Sept. 30, 1980. 19 p. (MRID # 452277-04).

Davis, D. E., J. D. Weete, C. G. P. Pillai, F. G. Plumley, J. T. McEnerney, J. W. Everest, B. Truelove and A. M. Diner. 1979. Atrazine fate and effects in a salt marsh. U.S. Environmental Protection Agency EPA-600/3-79-111. 84 p.

Delistraty, D. and C. Hershner. 1984. Effects of the herbicide atrazine on adenine nucleotide levels in *Zostera marina* L. (eelgrass). Aquatic Botany 18:353-369. (MRID # 4522777-05).

DeNoyelles, F., W. D. Kettle and D. E. Sinn. 1982. The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. (MRID # 450200-11).

Desjardins, D., Krueger, H., and Kendall, T. 2003. Atrazine Technical: A 14-Day Static-Renewal Toxicity Test with Duckweed (*Lemna gibba* G3) Including a Recovery Phase. Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Study No. 528A-131A. Study sponsored by Syngenta Crop Protection, Inc., Greensboro, North Carolina. (MRID # 461509-01).

De Solla, S. R., Martin, P. A., Fernie, K. J., Park, B. J., and Mayne, G. (2006). Effects of Environmentally Relevant Concentrations of Atrazine on Gonadal Development of Snapping Turtles (Chelydra serpentina). *Environ.Toxicol.Chem.* 25: 520-526. EcoReference No.: 82032.

Dewey, S. L. 1986. Effects of the herbicide atrazine on aquatic insect community structure and emergence. Ecology 67(1):148-162. (MRID # 452277-06).

Diana, S. G., Resetarits, W. J. Jr., Schaeffer, D. J., Beckmen, K. B., and Beasley, V. R. (2000). Effects of Atrazine on Amphibian Growth and Survival in Artificial Aquatic Communities. *Environ.Toxicol.Chem.* 19: 2961-2967. EcoReference No.: 59818

Dionne, E. 1992. Atrazine technical – Chronic toxicity to the fathead minnow (*Pimephales promelas*) during a full life-cycle exposure. SLI Report No. 92-7-4324. Prepared by Springborn Laboratories, Inc., Wareham, MA; submitted by Ciba-Geigy Corp., Greensboro, NC. (MRID No. 425471-03).

Dodson, S. I., C. M. Merritt, J. Shannahan, and C. M. Shults. 1999. Low exposure concentrations of atrazine increase male production in *Daphnia pulicaria*. Environ. Toxicol. Chem. 18(7):1568-1573.

Douglas, W. S., A. McIntosh and J. C. Clausen. 1993. Toxicity of sediments containing atrazine and carbofuran to larvae of the midge *Chironomus tentans*. Environ. Toxicol. Chem. 12:847-853. (MRID # 452029-05).

Drake, C. H. 1976. Acute toxicity of technical NC 1659 (atrazine) to *Daphnia magna*. Lab. Rep. No. BIOSC/76/E/12. Prepared by Fisons, Ltd., submitted by Fisons Corp., Agricultural Chemicals Div., Bedford, MA. (MRID No. 000272-04).

EG&G. 1979. Effects of atrazine on developing communities of benthic macroinvertebrate estuarine organisms. EG&G Bionomics. Project No. H82-500. Report No. BP-79-11-163R.

Eisler, R. 1989. Atrazine hazards to fish, wildlife, and invertebrates: A synoptic review. USDI, Fish & Wildl. Serv., Biol. Rep. 85(1.18), Contaminant Hazard Rev. Rep. No. 18. 53 p. (MRID # 452029-06).

El-Sheekh, M. M., H. M. Kotkat and O. H. E. Hammouda. 1994. Effect of Atrazine herbicide on growth, photosynthesis, protein synthesis, and fatty acid

composition in the unicellular green alga *Clorella kessleri*. Ecotox. Environ. Safety 29:3490358. (MRID # 452277-07).

Fairchild, J. F., D. S. Ruessler and A. R. Carlson. 1998. Comparative sensitivity of five species of macrophytes and six species of algae to atrazine, metribuzin, alachlor, and metolachlor. Environ. Toxicol. Chem. 17(9):1830-1834. (MRID # 452277-08).

Fairchild, J. F., D. S. Ruessler, P. S. Haverland and A. R. Carlson. 1997. Comparative sensitivity of *Selenastruum capricorntum* and *Lemna minor* to sixteen herbicides. or. Arch. Environ. Contam. Toxicol. 32:353-357. (MRID # 450882-12).

Fawley, M. W. 1984. Effects of light intensity and temperature interactions on growth characteristics of *Phaeodactylum tricornutum* (Bacillariophyceae). J. Phycol. 20:67-72.

Fischer-Scherl, T. A. Veeser, R. W. Hoffmann, C. Kühnhauser, R.-D. Negele and T. Ewringmann. 1991. Morphological effects of acute and chronic atrazine exposure in rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol. 20:454-461. (MRID # 452029-07).

Fleming, W. J., M. S. Ailstock, J. J. Momot and C. M. Norman. 1991. Response of sago pondweed, a submerged aquatic macrophyte, to herbicides in three laboratory culture systems, p. 267-275. *In*: Gorsuch, J. W., W. R. Lower, W. Wang and M. A. Lewis (eds.). Plants for toxicity assessment: Second volume. ASTM STP 1115. (MRID # 450874-09).

Forget-Leray, J., Landriau, I., Minier, C., and Leboulenger, F. (2005). Impact of Endocrine Toxicants on Survival, Development, and Reproduction of the Estuarine Copepod Eurytemora affinis (Poppe). *Ecotoxicol.Environ.Saf.* 60: 288-294. EcoReference No.: 80951

Forney, D. R. and D. E. Davis. 1981. Effects of low concentrations of herbicides on submersed aquatic plants. Weed Sci. 29:677-685. (MRID # 450874-10).

Forson, D. and Storfer, A. (2006). Effects of Atrazine and Iridovirus Infection on Survival and Life-History Traits of the Long-Toed Salamander (Ambystoma macrodactylum). *Environ.Toxicol.Chem.* 25: 168-173. EcoReference No.: 82033

Foy, C. L. and H. Hiranpradit. 1977. Herbicide movement with water and effects of contaminant levels on non-target organisms. Virginia Polytech. Instit. State Univ., VA Water Resources Res. Center, OWRT Proj. A-046-VA. 89 p. (MRID # 000235-43).

Frank, R., H. E. Braun, M. V. Holdrinet, G. J. Sirons and B. D. Ripley. 1982. Agriculture and water quality in the Canadian Great Lakes Basin. V. Pesticide use in 11 agricultural watersheds and presence in stream water 1975-1977. J. Environ. Qual. 11:497-505.

Frank, R. and G. J. Sirons. 1979. Atrazine: Its use in corn production and its loss to stream waters in southern Ontario, 1975-1977. Sci. Total Environ. 12:223-239.

Frank, R., G. J. Sirons, R. L. Thomas and K. McMillan. 1979. Triazine residues in suspended solids (1974-1976) and water (1977) from the mouths of Canadian streams flowing into the Great Lakes. J. Great Lakes Res. 5:131-138.

Frear, D. E. H. and J. E. Boyd. 1967. Use of *Daphnia magna* for the microbioassay of pesticides. I. Development of standardized techniques for rearing *Daphnia* and preparation of dosage-mortality curves for pesticides. J. Econ. Entomol. 60(5):1228-1236. (MRID # 000028-75).

Freemark, K. E. and C. Boutin. 1994. Impacts of agricultural herbicide use on terrestrial wildlife: A review with special reference to Canada. Canadian Wildl. Serv., Tech. Rep. Ser. 196. (MRID # 452049-03).

Gaynor, J. D., D. C. MacTavish and W. I. Findlay. 1995. Organic chemicals in the environment: Atrazine and metolachlor loss in surface and subsurface runoff from three tillage treatments in corn. J. Environ. Qual. 24:246-256.

Giddings, J. M. and L. W. Hall, Jr. 1998. The aquatic ecotoxicology of triazine herbicides. Amer. Chem. Soc. Symp. Ser. 683:347-356.

Gilliom, R. J., J. E. Barbash, D. W. Kolpin and S. J. Larson. 1999. Testing water quality for pesticide pollution. Environ. Sci. Technol. 33:164-169.

Glotfelty, D. E., A. W. Taylor, A. R. Isensee, J. Jersey and S. Glenn. 1984. Atrazine and simazine movement to Wye River estuary. J. Environ. Qual. 13:115-121.

Gluth, G. and W. Hanke. 1985. A comparison of physiological changes in carp, *Cyprinus carpio*, induced by several pollutants at sublethal concentrations: The dependency on exposure time. Ecotoxicol. Environ. Safety 9:179-188. (MRID # 452049-04).

Gonzalez-Murua, C., A. Munoz-Rueda, F. Hernando and M. Sanchez-Diaz. 1985. Effect of atrazine and methabenzthiazuron on oxygen evolution and cell growth of *Chlorella pyrenoidosa*. Weed Research 25:61-66. (MRID # 452277-09).

Görge, G. and R. Nagel. 1990. Toxicity of Lindane, Atrazine, and Deltamethrin to early life stages of zebrafish (*Brachydanio rerio*). Ecotoxicol. Environ. Safety 20:246-255. (MRID # 452029-08).

Grande, M., S. Andersen & D. Berge. 1994. Effects of pesticides on fish: Experimental and field studies. Norwegian J. Agr. Sci., Suppl. 13:195-209. (MRID # 452029-09).

Grenier, G., L. Proteau, J.-P. Marier and G. Beaumont. 1987. Effects of a sublethal concentration of atrazine on the chlorophyll and lipid composition of chlorophyll-protein complexes of *Lemna minor*. Plant Physiol. Biochem. 25(4):409-413. (MRID # 452277-10).

Grobler, E., J. H. J. van Vurin and H. H. du Preez. 1989. Routine oxygen consumption of *Talapia sparrmanii* (Cichlidae) following acute exposure to atrazine. Comp. Biochem. Physiol. 93C(1):37-42. (MRID # 452049-05).

Gross, T. S. 2001. Determination of potential effects of 10 day neonatal exposure of atrazine on histological and hormonal sex determination in incubated American alligator (*Alligator mississippiensis*) eggs. Prepared by University of Florida, Wildlife Reproductive Toxicology Laboratory, Gainesville, FL, NOVA98,02a; submitted by Syngenta Crop Protection, Inc., Greensboro, NC. (MRID No. 455453-02).

Gross, T. S. 2001. Determination of potential effects of 10 day neonatal exposure of atrazine on histological and hormonal sex determination in incubated red-eared slider (Pseudemys elegans) eggs. Prepared by University of Florida, Wildlife Reproductive Toxicology Laboratory, Gainesville, FL, NOVA98,02b; submitted by Syngenta Crop Protection, Inc., Greensboro, NC. (MRID No. 455453-03).

Gruessner, B. 1994. Patterns of herbicide contamination in Vermont streams and the effect of atrazine on communities of stream organisms. M. S. Thesis, Univ. Vermont, Burlington, VT, USA.

Gruessner, B. and M. C. Watzin. 1996. Response of aquatic communities from a Vermont stream to environmentally realistic atrazine exposure in laboratory microcosms. Environ. Toxicol. Chem. 15(4):410-419. (MRID # 450874-11).

Gucciardo, L. S. (1999). The Use of Anuran Larvae to Determine Chronic and Acute Toxicological Effects from Exposure to Atrazine and Metolachlor. *Ph.D.Thesis, Iowa State Univ., Ames, IA* 164 p. EcoReference No.: 78286

Hall, J. K., M. Pawlus and E. R. Higgins. 1972. Losses of atrazine in runoff water and soil sediment. J. Environ. Qual. 1:172-176.

Hall, J. K. 1974. Erosional losses of s-triazine herbicides. J. Environ. Qual. 3:174-180.

Hall, L. W., Jr. and R. D. Anderson. 1991. A review of estuarine aquatic toxicity data for the development of aquatic life criteria for atrazine in Chesapeake Bay. Maryland Dept. of Environ., Baltimore, Maryland. 60 p.

Hall, L. W., Jr., M. C. Ziegenfuss and R. D. Anderson. 1993. An assessment of salinity effects on the toxicity of atrazine to Chesapeake Bay species: Data needs for development of estuarine aquatic life criteria. Final report. Univ. of Maryland, Maryland Agr. Exper. Sta., Wye Res. Educ. Center. 28 p. (MRID # 452277-11).

Hall, L. W., Jr., M. C. Ziegenfuss, and R. D. Anderson. 1994. The influence of salinity on the chronic toxicity of atrazine to an estuarine copepod: Filling a data need for development of an estuarine chronic criterion. Report. Wye Research and Education Center, University of Maryland, Queenstown, MD.

Hall, L. W., Jr., M. C. Ziegenfuss, R. D. Anderson, T. D. Spittler and H. C. Leichtweis. 1994. Influence of salinity on atrazine toxicity to a Chesapeake Bay copepod (*Eurytemora affinis*) and fish (*Cyprinodon variegatus*). Estuaries 17:181-186. (MRID # 452083-03).

Hall, L. W., Jr., M. C. Ziegenfuss, R. D. Anderson and D. P. Tierney. 1995. The influence of salinity on the chronic toxicity of atrazine to an estuarine copepod: Implications for development of an estuarine chronic criterion. Arch. Environ. Contam. Toxicol. 28:344-348.

Hamala, J. A. and H. P. Kollig. 1985. The effects of atrazine on periphyton communities in controlled laboratory ecosystems. Chemosphere 14(9):1391-1408. (MRID # 450874-12).

Hamilton, P. B. 1987. The impact of atrazine on lake periphyton communities, including carbon uptake dynamics using track autoradiography. Environ. Poll. 46:83-103. (MRID # 450200-20).

Hannan, Patrick J. 1995. A novel detection scheme for herbicidal residues. Environ. Toxicol. Chem. 14(5):775-780.

Hartman, W. and D. B. Martin. 1985. Effects of four agricultural pesticides on *Daphnia pulex*, *Lemna minor*, and *Potamogeton pectinatus*. Bull. Environ. Contam. Toxicol. 35:646-651. (MRID # 452277-12).

Hayes, T.B., P. Case, S. Chui, D. Chung, C. Haeffele, K. Haston, M. Lee, V.P. Mai, Y. Marjuoa, J. Parker, and M. Tsui. 2006. Pesticide Mixtures, Endocrine Disruption, and Amphibian Declines: Are We Understanding the Impact? Env. Health Persp. 114(1) p. 40-50.

Heath, R. G., J. W. Spann, E. F. Hill and J. F. Kreitzer. 1972. Comparative dietary toxicities of pesticides to birds. Prepared by U.S. Dept. Interior, Bureau Sport

Fish. Wildlife, Spec. Rep. - Wildlife. No., 152. 57 p.; submitted by Fisons Corp. (MRID No. 000587-46).

Hecker, M., Park, J. W., Murphy, M. B., Jones, P. D., Solomon, K. R., Van der Kraak, G., Carr, J. A., Smith, E. E., Du Preez, L., Kendall, R. J., and Giesy, J. P. (2005). Effects of Atrazine on CYP19 Gene Expression and Aromatase Activity in Testes and on Plasma Sex Steroid Concentrations of Male African Clawed Frogs (Xenopus laevis). *Toxicol.Sci.* 86: 273-280. EcoReference No.: 79287

Herman, D., N. K. Kaushik and K. R. Solomon. 1986. Impact of atrazine on periphyton in freshwater enclosures and some ecological consequences. J. Fish. Aquat. Sci. 43:1917-1925. (MRID # 450200-12).

Hersh, C. M. and W. G. Crumpton. 1987. Determination of growth rate depression of some green algae by atrazine. Bull. Environ. Contam. Toxicol. 39:1041-1048. (MRID # 452277-13).

Hill, E. F., Heath, R. G., Spann, J. W., and Williams, J. D. (1975). Lethal Dietary Toxicities of Environmental Pollutants to Birds. *U.S.Fish and Wildlife Service, Special Scientific Report-Wildlife* 191: 1-61. (MRID # 000229-02). EcoReference No.: 35243

Hoagland, K. L., R. W. Drenner, J. D. Smith and D. R. Cross. 1993. Freshwater community responses to mixtures of agricultural pesticides: Effects of atrazine and Bifenthrin. Environ. Toxicol. Chem. 12:627-637. (MRID # 450200-14).

Hoberg, J. R. 1991. Atrazine technical: Toxicity to the freshwater green alga S*elenastrum capricornutum*. SLI Rep. No. 91-1-3600. Prepared by Springborn Laboratories, Inc., Wareham, MA.; submitted by Ciba-Geigy Corporation, Greensboro, NC. (MRID No. 420607-01).

Hoberg, J. R. 1991. Atrazine technical: Toxicity to the duckweed *Lemna gibba* G3. SLI Rep. No. 91-1-3613. Prepared by Springborn Laboratories, Inc., Wareham, MA.; submitted by Ciba-Geigy Corporation, Greensboro, NC. (MRID No. 420414-04).

Hoberg, J. R. 1993. Atrazine technical: Toxicity to duckweed, (*Lemna gibba*). SLI Rep. No. 93-4-4755. Prepared by Springborn Laboratories, Inc., Wareham, MA.; submitted by Ciba-Geigy Corporation, Greensboro, NC. (MRID No. 430748-04).

Hoberg, J. R. 1993. Atrazine technical: Toxicity to duckweed, (*Lemna gibba*). SLI Rep. No. 93-11-5053. Prepared by Springborn Laboratories, Inc., Wareham, MA.; submitted by Ciba-Geigy Corporation, Greensboro, NC. (MRID No. 430748-03).

Hoberg, J. R. 1993. Atrazine technical: Toxicity to the freshwater green alga, (*Selenastrum capricornutum*). SLI Rep. No. 93-4-4751. Prepared by Springborn

Laboratories, Inc., Wareham, MA.; submitted by Ciba-Geigy Corporation, Greensboro, NC. (MRID No. 430748-02).

Hoberg, J. R. 1993. Atrazine technical: Toxicity to the marine diatom, (*Skeletonema costatum*). SLI Rep. No. 93-4-4753. Prepared by Springborn Laboratories, Inc., Wareham, MA.; submitted by Ciba-Geigy Corporation, Greensboro, NC. (MRID No. 430748-01).

Hofmann, A. and S. Winkler. 1990. Effects of atrazine in environmentally relevant concentrations on submersed macrophytes. Arch. Hydrobiol. 118(1):69-79. (MRID # 452277-14).

Hollister, T. A and G.E. Walsh. 1973. Differential responses of marine phytoplankton to herbicides: Oxygen Evolution, Bull. Environ. Contam. Toxicol. 9(5):291-295. (MRID # 001587-45).

Hopkin, R. and J. M. Kain. 1978. The effects of some pollutants on the survival, growth and respiration of *Laminaria hyperborea*. Estuarine Coast. Mar. Sci. 7:531-553. (MRID # 452277-15).

Hörmann, W. D., J. C. Tournayre and H. Egli. 1979. Triazine herbicides residues in central European streams. Pest. Monit. J. 13:128-131.

Howe, G. E., R. Gillis and R. C. Mowbray. 1998. Effect of chemical synergy and larval stage on the toxicity of atrazine and alachlor to amphibian larvae. Environ. Toxicol. Chem. 17(3):519-525. (MRID # 452029-10).

Huckins, J. N. J. D. Petty and D. C. England. 1986. Distribution and impact of trifluralin, atrazine, and fonofos residues in microcosms simulating a northern prairie wetland. Chemosphere 15(5):563-588. (MRID # 452051-02).

Hughes, J. R. 1986. The toxicity of atrazine, Lot No. FL-850612 to four species of aquatic plants. Lab. Study No. 267-28-1100. Prepared by Malcolm Pirnie, Inc., White Plains, NY.; submitted by Ciba-Geigy Corporation, Greensboro, NC. (MRID No. 410652-03).

Hurlbert, S. H. 1975. Secondary effects of pesticides on aquatic ecosystems. Residue Rev. 57:81-148. (MRID # 452277-16).

Hussein, S. Y., M. A. El-Nasser and S. M. Ahmed. 1996. Comparative studies on the effects of herbicide atrazine on freshwater fish *Oreochromis niloticus* and *Chrysichthyes auratus* at Assiut, Egypt. Bull. Environ. Contam. Toxicol. 57:503-510. (MRID # 452029-11).

Isensee, A. R. 1976. Variability of aquatic model ecosystem-derived data. Internat. J. Environ. Stud. 10:35-41.

Johnson, B. T. 1986. Potential impact of selected agricultural chemical contaminants on a northern prairie wetland: A microcosm evaluation. Environ. Toxicol. Chem. 5:473-485. (MRID # 450874-13).

Johnson I.C., A.E. Keller, and S.G. Zam. 1993. A method for conducting acute toxicity tests with the early life stages of freshwater mussels. Environ Toxicol Risk Assmnt ASTM STP 1179.

Johnson, J. R. and K. T. Bird. 1995. The effects of the herbicide atrazine on *Ruppia maritima* L. growing in autotrophic versus heterotrophic cultures. Botanica Marina 38:307-312. (MRID # 450200-18).

Jones, R. O. 1962. Tolerance of the fry of common warm-water fishes to some chemicals employed in fish culture, 436-445. Proc. 16th Ann. Conf. Southeast. Assoc. Game Fish Comm.

Jones, T. W. and P. S. Estes. 1984. Uptake and phytotoxicity of soil-sorbed atrazine for the submerged aquatic plant, *Potamogeton perfoliatus* L. Arch. Environ. Contam. Toxicol. 13:237-241. (MRID # 450874-04).

Jones, T. W., W. M. Kemp, P. S. Estes and J. C. Stevenson. 1982a. Atrazine uptake, phytotoxicity, release, and short-term recovery for the submersed aquatic plant, *Potamogeton perfoliatus.* Report to U.S. Environmental Protection Agency, Annapolis. NTIS, Springfield, VA.

Jones, T. W., W. M. Kemp, P. S. Estes and J. C. Stevenson. 1986. Atrazine uptake, photosynthetic inhibition, and short-term recovery for the submersed vascular plant, *Potamogeton perfoliatus* L. Arch. Environ. Contam. Toxicol. 15:277-283. (MRID # 452277-18).

Jones, T. W., W. M. Kemp, J. C. Stevenson and J. C. Means. 1982b. Degradation of atrazine in estuarine water/sediment systems and soils. J. Environ. Qual. 11:632-638.

Jones, T. W. and L. Winchell. 1984. Uptake and photosynthetic inhibition by atrazine and its degradation products on four species of submerged vascular plants. J. Environ. Qual. 13(2):243-247. (MRID # 452277-19).

Jooste, A. M., Du Preez, L. H., Carr, J. A., Giesy, J. P., Gross, T. S., Kendall, R. J., Smith, E. E., Van der Kraak, G. L., and Solomon, K. R. (2005). Gonadal Development of Larval Male Xenopus laevis Exposed to Atrazine in Outdoor Microcosms. *Environ.Sci.Technol.* 39: 5255-5261. EcoReference No.: 79286

Jop, K. M. 1991. (Atrazine technical) - Acute toxicity to (*Ceriodaphnia dubia*) under static conditions. SLI Report No. 91-1-3629. Springborn Laboratories, Inc., Wareham, MA. (MRID # 452083-09).

Jop, K. M. 1991. (Atrazine technical) - Acute toxicity to (*Ceriodaphnia dubia*) under static conditions. SLI Report No. 91-2-3665. Springborn Laboratories, Inc., Wareham, MA.

Jop, K. M. 1991. (Atrazine technical) - Acute toxicity to fathead minnow (*Pimephales promelas*) under static conditions. SLI Report No. 91-1-3630. Springborn Laboratories, Inc., Wareham, MA.

Jop, K. M. 1991. (Atrazine technical) - Acute toxicity to fathead minnow (*Pimephales promelas*) under static conditions. SLI Report No. 91-2-3666. Springborn Laboratories, Inc., Wareham, MA.

Jurgensen, T. A. and K. D. Hoagland. 1990. Effects of short-term pulses of atrazine on attached algal communities in a small stream. Arch. Environ. Contam. Toxicol. 19:617-623. (MRID # 450200-04).

Juttner, I., A. Peither, J. P. Lay, A. Kettrup and S. J. Ormerod. 1995. An outdoor mesocosm study to assess ecotoxicological effects of atrazine on a natural plankton community. Arch. Environ. Contam. Toxicol. 29:435-441. (MRID # 450200-22).

Kadoum, A. M. and D. E. Mock. 1978. Herbicide and insecticide residues in tailwater pits: Water and pit bottom soil from irrigated corn and sorghum fields. J. Agric. Food Chem. 26:45-50.

Kaushik, N. K., K. R. Solomon, G. Stephenson and K. Day. 1985. Assessment of sublethal effects of atrazine on zooplankton. Canada Tech. Rep. Fish. Aquat. Sci. 1368:377-379.

Kemp, W. M., W. R. Boynton, J. J. Cunningham, J. C. Stevenson, T. W. Jones and J. C. Means. 1985. Effects of atrazine and linuron on photosynthesis and growth of the macrophytes, *Potamogeton perfoliatus* L. and *Myriophyllum spicatum* L. in an estuarine environment. Mar. Environ. Res. 16:255-280. (MRID # 452277-20).

Kemp, W. M., J. C. Means, T. W. Jones and J. C. Stevenson. 1982. Herbicides in the Chesapeake Bay and their effect on submerged aquatic vegetation, p. 503-567. *In*: Chesapeake Bay Program, Tech. studies, a synthesis. Part IV. US EPA, Washington, DC.

Kettle, W. D., F. DeNoyelles, Jr., B. D. Heacock and A. M. Kadoum. 1987. Diet and reproductive success of bluegill recovered from experimental ponds treated with atrazine. Bull. Environ. Contam. Toxicol. 38:47-52. (MRID # 452029-12).

Klassen, H. E. and A. M. Kadoum. 1979. Distribution and retention of atrazine and carbofuran in farm pond ecosystems. Arch. Environ. Contam. Toxicol. 8:345-353.

Kolpin, D. W. and S. J. Kalkoff. 1993. Atrazine degradation in a small stream in Iowa. Environ. Sci. Technol. 27:134-139.

Korte, F. and H. Greim. 1981. Überprüfung der Durchführbarkeit von Prüfungsvorschriften und der Aussagekraft der Grundprüfung des Ersten Chemikaliengestzes. Bericht der GSF München, Institut für ökologische Chemie und Institut für Biochemie und Toxikologie, Abteilung Toxikologie. An das Umweltbundesamt Berlin, Forschungsbericht Nr. 107 04 006/1.

Kosinski, R. J. 1984. The effect of terrestrial herbicides on the community structure of stream periphyton. Environ. Poll. (Ser. A) 36:165-189. (MRID # 450200-05).

Kosinski, R. J. and M. G. Merkle. 1984. The effect of four terrestrial herbicides on the productivity of artificial stream algal communities. J. Environ. Qual. 13(1):75-82. (MRID # 450200-06).

Krieger, K. A., D. B. Baker and J. W. Kramer. 1988. Effects of herbicides on stream *Aufwuchs* productivity and nutrient uptake. Arch. Environ. Contam. Toxicol. 17:299-306. (MRID # 450200-07).

Lakshminarayana, J. S. S., H. J. O'Neill, S. D. Jonnavithula, D. A. Leger and P. H. Milburn. 1992. Impact of atrazine-bearing agricultural tile drainage discharge on planktonic drift of a natural stream. Environ. Poll. 76:201-210. (MRID # 450200-08).

Lampert, W., W. Fleckner, E. Pott, U. Schober and K-U. Storkel. 1989. Herbicide effects on planktonic systems of different complexity. Hydrobiologia 188/189:415-424. (MRID # 450874-14).

Langan, M. M. and K. D. Hoagland. 1996. Growth responses of *Typhya latifolia* and *Scirpus acutus* to atrazine contamination. Bull. Environ. Contam. Toxicol. 57:307-314. (MRID # 450874-15).

Larsen, D. P., F. DeNoyelles, Jr., F. Stay and T. Shiroyama. 1986. Comparisons of single-species, microcosm and experimental pond responses to atrazine exposure. Environ. Toxicol. Chem. 5:179-190. (MRID # 450200-15).

Larson, D. L., McDonald, S., Fivizzani, A. J., Newton, W. E., and Hamilton, S. J. (1998).

Effects of the Herbicide Atrazine on Ambystoma tigrinum Metamorphosis: Duration, Larval Growth, and Hormonal Response. *Physiol.Zool.* 71: 671-679. EcoReference No.: 60632

Lay, J. P., A Muller, L. Peichl, W. Klein and F. Korte. 1984. Longterm effects of the herbicides atrazine and dichlobenil upon the phytoplankton density and physio-chemical conditions in compartments of a freshwater pond. Chemosphere 13(7):821-832. (MRID # 450200-16).

Liang, T. T. and E. P. Lichtenstein. 1975. Synergism of insecticides by herbicides: Effect of environmental factors. Science

Lorz, H. W., S. W. Glenn, R. H. Williams, C. M. Kunkel, L. A. Norris and B. R. Loper. 1979. Effects of selected herbicides on smolting of coho salmon. US. EPA, Office of Res. Devel., Environ. Res. Lab., Corvallis, OR. EPA-600/3-79-071. 102 p. (MRID # 452051-07).

Lynch, T. R., H. E. Johnson and W. J. Adams. 1985. Impact of atrazine and hexachlorobiphenyl on the structure and function of model stream ecosystems. Environ. Toxicol. Chem. 4:399-413. (MRID # 450200-09).

Lytle, J. S. and T. F. Lytle. 1998. Atrazine effects on estuarine macrophytes *Spartina alterniflora* and *Juncus roemerianus*. Environ. Toxicol. Chem. 17(10):1972-1978. (MRID # 450874-05).

Macek, K. J., K. S. Buxton, S. Sauter, S. Gnilka and J. W. Dean. 1976. Chronic toxicity of atrazine to selected aquatic invertebrates and fishes. US. EPA, Off. Res. Dev., Environ. Res. Lab. Duluth, MN. EPA-600/3-76-047. 49 p. (MRID # 000243-77).

Machado, M. W. 1994. Atrazine technical -- Acute toxicity to sheepshead minnow (*Cyprinodon variegatus*) under flow-through conditions. Prepared by Springborn Laboratories, Inc., Wareham, MA; submitted by Ciba-Geigy Corp., Greensboro, NC. (MRID No. 433449-01).

Machado, M. W. 1994. Atrazine technical -- Acute toxicity to mysid shrimp (*Mysidopsis bahia*) under flow-through conditions. Prepared by Springborn Laboratories, Inc., Wareham, MA; submitted by Ciba-Geigy Corp., Greensboro, NC. (MRID No. 433449-02).

Macinnis-Ng, C. M. O. and Ralph, P. J. (2003). Short-Term Response and Recovery of Zostera capricorni Photosynthesis After Herbicide Exposure. *Aquat.Bot.* 76: 1-15. EcoReference No.: 72996

Madsen, T. J. 2000. Effects of atrazine on the sex ratio of *Daphnia pulicaria*. Prepared by ABC Laboratories, Inc., Columbia, MO, ABC Study No. 45810; submitted by Novartis Crop Protection, Inc., Greensboro, NC. (MRID No. 452995-04).

Marrs, R. H., C. T. Williams, A. J. Frost & R. A. Plant. 1989. Assessment of the effects of herbicide spray drift on a range of plant species of conservation interest. Environ. Poll. 59:71-86. (MRID # 452051-06).

Mayasich, J. M., E. P. Karlander and D. E. Terlizzi, Jr. 1986. Growth responses of *Nannochloris oculata* Droop and *Phaeodactylum tricornutum* Bohlin to the herbicide atrazine as influenced by light intensity and temperature. Aquatic Toxicol. 8:175-184.

Mayasich, J. M., E. P. Karlander and D. E. Terlizzi, Jr. 1987. Growth responses of *Nannochloris oculata* Droop and *Phaeodactylum tricornutum* Bohlin to the herbicide atrazine as influenced by light intensity and temperature in unialgal and bialgal assemblage. Aquatic Toxicol. 8:175-184.

Mayer, F. L., Jr. 1987. Acute toxicity handbook of chemicals to estuarine orgnisms. US. EPA, Off. Res. Dev., Environ. Res. Lab., Gulf Breeze, FL. EPA 600/8-87/017. 274 p. (MRID # 400980-01).

Mayer, F. L., Jr. and M. R. Ellersieck. 1986. Manual of acute toxicity: Interpretation and data base for 410 chemicals and 66 species of freshwater animals. USDI, Fish & Wildl, Serv., Resource publ. 160. 506 p. (MRID # 400980-01).

Mayer, F. L., Jr. 1986. Acute toxicity handbook of chemicals to estuarine organisms. U.S. Environmental Protection Agency, EPA/600/X-86/231. 274 pages. (MRID No. 402284-01).

Mayer, P., J. Frickmann, E. R. Christensen and N. Nyholm. 1998. Influence of growth conditions of the results obtained in algal toxicity tests. Environ. Toxicol. Chem. 17(6):1091-1098. (MRID # 452277-21).

McNamara, P. C. 1991. Atrazine technical - Acute toxicity to the marine copepod (*Acartia tonsa*) under flow-through conditions. SLI Rep. No. 91-2-3662. Springborn Laboratories, Inc. Wareham, MA. (MRID # 4520833-08).

Mitchell, C. A. 1987. Growth of *Halodule wrightii* in culture and the effects of cropping, light, salinity and atrazine. Aquatic Botany 28:25-37. (MRID # 452051-01).

Moody, J. A. and D. A. Goolsby. 1993. Spatial variability of triazine herbicides in the lower Mississippi River. Environ. Sci. Technol. 27:2120-2126.

Moore, A. and Lower, N. (2001). The Impact of Two Pesticides on Olfactory-Mediated Endocrine Function in Mature Male Atlantic Salmon (Salmo salar L.) Parr.

Comp.Biochem.Physiol.B 129: 269-276. EcoReference No.: 67727

Moore, A. and C. P. Waring. 1998. Mechanistic effects of a triazine pesticide on reproductive endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. Pest. Biochem. Physiol. 62:41-50. (MRID # 452049-06).

Moorhead, D. L. and R. J. Kosinski. 1986. Effect of atrazine on the productivity of artifical stream algal communities. Bull. Environ. Contam. Toxicol. 37:330-336. (MRID # 45020010).

Moragua, D. and A. Tauguy. 2000. Genetic indicators of herbicide stress in the Pacific oyster *Crassostrea gigas* under experimental conditions. Environ. Toxicol. Chem. 19(3):706-711. (MRID # 452277-22).

Morrison, Michael L. and E. Charles Meslow. 1984. Response of avian communities to herbicide-induced vegetation changes. J. Wildl. Manage. 48(1):14-22. (MRID # 452051-05).

Muir, D. C. G., J. Y. Yoo and B. E. Baker. 1978. Residues of atrazine and n-deethylated atrazine in water from five agricultural watersheds in Quebec. Arch. Environ. Contam. Toxicol. 7:221-235.

Neckles, H. A. 1992. Indicator development: Seagrass monitoring and research in the Gulf of Mexico. Report of Workshop at Mote Marine Laboratory, Sarasota, FL., January 28-29, 1992. US EPA, Off. Res. Devel., Environ. Res. Lab., Gulf Breeze, FL. EPA/620/R-94/029. 64 p. (MRID # 452277-23).

Nelson, K. J., K. D. Hoagland and B. D. Siegfried. 1999. Chronic effects of atrazine on tolerance of a benthic diatom. Environ. Toxicol. Chem. 18(5):1038-1045. (MRID # 452277-24).

Neskovic, N. K., I. Elezovic, V. Karan, V. Poleksic and M. Budimir. 1993. Acute and subacute toxicity of atrazine to carp (*Cyprinus carpio* L.). Ecotoxicol. Environ. Safety 25:173-182. (MRID # 452029-13).

Neugebaur, K., F.-J. Zieris and W. Huber. 1990. Ecological effects of atrazine on two outdoor artifical freshwater ecosystems. Z. Wasser-Abwasser-Forsch. 23:11-17. (MRID # 450200-17).

O'Kelly, J. C and T. R. Deason. 1976. Degradation of pesticides by algae. US EPA, Athens, GA. EPA-600/3-76-022. 41 pp. (MRID # NA).

Oris, J. T., R. W. Winner and M. V. Moore. 1991. A four-day survival and reproduction toxicity test for *Ceriodphnia dubia*. Environ. Toxicol. Chem. 10:217-224. (MRID # 452083-05).

Pape-Lindstrom, Pamela A. and Michael J. Lydy. 1997. Synergistic toxicity of atrazine and organophosphate insecticides contravenes the response addition mixture model. Environ. Toxicol. Chem. 16(11):2415-2420.

Parrish, R. 1978. Effects of atrazine on two freshwater and five marine algae. Lab. Study No. H82-500. Prepared by E G & G Bionomics, Marine Research Laboratory, Pensacola, FL.; submitted by Ciba-Geigy Corporation, Greensboro, NC. (MRID No. 410652-04).

Pedersen, C. A. and D. R. DuCharme. 1992. Atrazine technical: Toxicity and reproduction study in bobwhite quail. Lab. Proj. ID. No. BLAL No. 102-012-07. Prepared by Bio- Life Associates, Ltd., Neillsville, WI; submitted by Ciba-Geigy Corp., Greensboro, NC. (MRID No. 425471-02).

Pedersen, C. A. and D. R. DuCharme. 1992. Atrazine technical: Toxicity and reproduction study in mallard ducks. Lab. Proj. ID. No. BLAL No. 102-013-08. Prepared by Bio- Life Associates, Ltd., Neillsville, WI; submitted by Ciba-Geigy Corp., Greensboro, NC. (MRID No. 425471-01).

Peither, Armin. 2005a. G 34048 (Hydroxyatrazine): Acute Toxicity to Rainbow Trout (*Oncohrynchus mykiss*) in a 96-Hour Static Test. Unpublished study performed by Environmental Chemistry & Pharmanalytics, RCC Ltd. CH-4452 Itingen, Switzerland. Laboratory Project No. 857052. Study sponsored by Syngenta Crop Protection, Inc., Greensboro, NC. Syngenta Number 2031710. (MRID # 465000-04).

Peither, Armin. 2005b. G 34048 (Hydroxyatrazine): Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) in a 96-Hour Static Test. Unpublished study performed by Environmental Chemistry & Pharmanalytics, RCC Ltd. CH-4452 Itingen, Switzerland. Laboratory Project No. 857054. Study sponsored by Syngenta Crop Protection, Inc., Greensboro, NC. Syngenta Number 2031711. (MRID # 465000-05).

Peither, Armin. 2005c. G 34048 (Hydroxyatrazine): Acute Toxicity to *Daphnia magna* in a 48-Hour Immobilization Test. Unpublished study performed by Environmental Chemistry & Pharmanalytics, RCC Ltd. CH-4452 Itingen, Switzerland. Laboratory Project No. 857056. Study sponsored by Syngenta Crop Protection, Inc., Greensboro, NC. Syngenta Number 2031712. (MRID # 465000-01).

Pereira, W. E., J. L. Domagalski, F. D. Hostettler, L. R. Brown and J. B. Rapp. 1996. Occurrence and accumulation of pesticides and organic contaminants in river sediment, water and clam tissues from the San Joaquin River and tributaries, California. Environ. Toxicol. Chem. 15(2):172-180. (MRID # 452277-25).

Peterson, H. G., C. Boutin, P. A. Martin, K. E. Freemark, N. J. Ruecker and M. J. Moody. 1994. Aquatic phyto-toxicity of 23 pesticides applied at expected environmental concentrations. Aquatic Toxicol. 28:275-292. (MRID # 452277-26).

Petit, F., Le Goff, J. Cravedi, Y. Valotaire and F. Pakdel. 1997. Two complementary bioassays for screening the estrogenic potency of xenobiotics: Recombinant yeast for trout estrogen receptor and trout hepatocyte cultures. J. Molecul. Endocrinol. 19:321-335.

Plumley, F. G. and D. E. Davis. 1980. The effects of a photosynthesis inhibitor atrazine, on salt marsh edaphic algae, in culture, microecosystems, and in the field. Estuaries 3(4):271-277. (MRID # 450874-06).

Plumley, F. G., D. E. Davis, J. T. McEnerney and J. W. Everest. 1980. Effects of a photosynthesis inhibitor, atrazine, on the salt-marsh fiddler crab, *Uca pugnax* (Smith). Estuaries 3(3):217-223. (MRID # 452277-27).

Portmann, J. E. 1972. Results of acute toxicity tests with marine organisms, using a standard method, 212-217. Mar. Poll. Sea Life. (MRID # 452277-28).

Prasad, T. A. V. and D. C. Reddy. 1994. Atrazine toxicity on hydromineral balance of fish, *Tilapia mossambicus*. Ecotoxicol. Environ. Safety 28:313-316. (MRID # 452049-07).

Prasad, T. A. V., T. Srinivas, G. Md. Rafi and C. D. Reddy. 1991. Effect *in vivo* of atrazine on haematology and O_2 consumption in fish, *Talapia mossambica*. Biochem. Internat. 23(1):157-161. (MRID # 452049-08).

Pratt, J. R., N. J. Bowers, B. R. Niederlehner and J. Cairns, Jr. 1988. Effects of atrazine on freshwater microbial communities. Arch. Environ. Contam. Toxicol. 17:449-457. (MRID # 450874-16).

Putam, A. R. and D. Penner. 1974. Pesticide interactions in higher plants. Pesticide Rev. 50:73-110.

Putt, Arthur E. 1991. Atrazine 80% WP: Acute toxicity to daphnids (*Daphnia magna*) underflow-through conditions. Lab. Study No. 91-5-3761. Prepared by Springborn Laboratories, Inc., Wareham, MA.; submitted by Ciba-Geigy Corporation. (MRID # 420414-01).

Putt, Arthur E. 2002. Atrazine Technical SF - Toxicity to Midge (*Chironomus tetans)* Under Flow-Through. Lab. Study No. 1781.6635. Prepared by Springborn Laboratories, Inc., Wareham, MA.; submitted by Syngenta Crop Protection, Inc. (MRID # 459040-01).

Putt, Arthur E. 2003. Atrazine Technical SF - Toxicity to Midge (*Chironomus tetans)* During a 10-Day Sediment Exposure. Lab. Study No. 1781.6636. Prepared by Springborn Laboratories, Inc., Wareham, MA.; submitted by Syngenta Crop Protection. (MRID # 459040-02).

Richard, J. J., G. A. Junk, M. J. Avery, N. L. Nehring, J. S. Fritz and H. J. Svec. 1975. Residues in water. Pest. Monit. J. 9:117-123.

Ritter, W. F., H. P. Johnson, W. G. Lovely and M. Molnau. 1974. Atrazine, propachlor, and diazinon residues on small agricultural watersheds. Environ. Sci. Technol. 8:37-42.

Rohr, J. R., Elskus, A. A., Shepherd, B. S., Crowley, P. H., McCarthy, T. M., Niedzwiecki, J. H., Sager, T., Sih, A., and Palmer, B. D. (2003). Lethal and Sublethal Effects of Atrazine, Carbaryl, Endosulfan, and Octylphenol on the Streamside Salamander (Ambystoma barbouri). *Environ.Toxicol.Chem.* 22: 2385-2392. EcoReference No.: 71723

Rohr, J. R., Elskus, A. A., Shepherd, B. S., Crowley, P. H., McCarthy, T. M., Niedzwiecki, J. H., Sager, T., Sih, A., and Palmer, B. D. (2004). Multiple Stressors and Salamanders: Effects of an Herbicide, Food Limitation, and Hydroperiod. *Ecol.Appl.* 14: 1028-1040. EcoReference No.: 81748

Roses, N., Poquet, M., and Munoz, I. (1999). Behavioural and Histological Effects of Atrazine on Freshwater Molluscs (Physa acuta Drap. and Ancylus fluviatilis Mull. Gastropoda). *J.Appl.Toxicol.* 19: 351-356. EcoReference No.: 60860

Rothstein, E., T. S. Steenhuis, J. H. Peverly and L. D. Geohring. 1996. Atrazine fate on a tile drained field in northern New York: A case study. Agr. Water Manage. 31:195-203.

Roberts, G. C., G. J. Sirons, R. Frank and H. E. Collins. 1979. Triazine residues in a watershed in southwestern Ontario, Canada (1973-1975). J. Great Lakes Res. 5:246-255.

Roberts, S., P. Vassseur and D. Dive. 1990. Combined ffects btween atrazine, copper and pH, on target and non target species. Water res. 24(4):485-491.

Sachsse, K. and L. Ullmann. 1974. Acute oral LD_{50} of technical atrazin (G 30027) in the Japanese quail. Project No. Siss 4407. Prepared by Ciba-Geigy, Ltd., Basle, Switzerland; submitted by Ciba-Geigy Corp., Greensboro, NC. (MRID No. 000247-22).

Sachsse, K. and L. Ullmann. 1975. 8-Day feeding toxicity of technical G30027 (Atrazine) in the Japanese quail. Prepared by Ciba-Geigy, Ltd., Basle,

Switzerland; submitted by Ciba-Geigy Corp., Greensboro, NC. (MRID No. 000247-23).

Saglio, P. and S. Trijasse. 1998. Behavioral responses to atrazine and diuron in goldfish. Arch. Environ. Contam. Toxicol. 35:484-491. (MRID # 452029-14).

Sanderson, J. T., W. Seinen, J. P. Giesy and M. van den Berg. 2000. 2-Chloro-s-triazine herbicides induce aromatase (CYP-19) activity in H295R human adrenocortical carcinoma cells: A novel mechanism for estrogenicity. Toxicol. Sci. 54:121-127.

Sayers, L.E. 2005a. Hydroxyatrazine (G-34084): Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Static Conditions. Unpublished study performed by Springborn Smithers Laboratories, Wareham, MA. Laboratory Study No. 1781.6645. Study submitted by Syngenta Crop Protection, Inc., Greensboro, NC. (MRID # 465000-06).

Sayers, L.E. 2005b. Hydroxyatrazine (G-34084): Hydroxyatrazine (G-34084): Acute Toxicity to Mysids (*Americamysis bahia*) Under Static Conditions. Unpublished study performed by Springborn Smithers Laboratories, Wareham, MA. Laboratory Study No. 1781.6644. Study submitted by Syngenta Crop Protection, Inc., Greensboro, NC. (MRID # 465000-03).

Schober, U. and W. Lampert. 1977. Effects of sublethal concentrations of the herbicide Atrazin^{R} on growth and reproduction of *Daphnia pulex*. Bull. Environ. Contam. & Toxicol. 17(3):269-277. (MRID # 452029-15).

Schulz, A., F. Wengenmayer and H. M. Goodman. 1990. Genetic engineering of herbicide resistance in higher plants. Plant Sci. 9:1-15.

Schwarzschild, A. C., W. G. MacIntyre, K. A. Moore and E. L. Libelo. 1994. *Zostera marina* L. growth response to atrazine in root-rhizome and whole plant exposure exposure experiments. J. Exper. Mar. Biol. Ecol. 183:77-89. (MRID # 452277-29).

Scientific Advisory Panel. 2003. U.S. EPA, Office of Pesticide Programs (OPP), Washington, D.C. Memorandum to James Jones, Director OPP, regarding transmittal of meeting minutes of the FIFRA Scientific Advisory Panel meeting held June 17-20, 2003 on potential developmental effects of atrazine on amphibians. August 4. Available at http://www.epa.gov/scipoly/sap

Shabana, E. F. 1987. Use of batch assays to assess the toxicity of atrazine to some selected cyanobacteria: I. Influence of atrazine on the growth, pigmentation and carbohydrate contents of *Aulosira fertilissima*, *Anabeana oryzae*, *Nostoc muscorum* and *Tolypothrix tenuis*. J. Basic Microbiol. 27(2):113-119.

Solomon, K. R., D. B. Baker, R. P. Richards, K. R. Dixon, S. J. Kline, T. W. La Point, R. J. Kendall, C. P. Weisskopf, J. M. Giddings, J. P. Giesy, L. W. Hall, Jr., and M. Williams. Ecological risk assessment of atrazine in North American surface waters. Environ. Toxicol. Chem. 15(1):31-76. (MRID # 439344-19).

Srinivas, T., T. A. V. Prasad, G. Md. Raffi and D. C. Reddy. 1991. Effect of atrazine on some aspects of lipid metabolism in fresh water fish. Biochem. Internat. 23(3):603-609. (MRID # 452049-09).

Stafford, J.M. 2005a. Desisopropylatrazine (G28279): Acute Oral Toxicity Test (LD50) with Northern Bobwhite Quail (*Colinus virginianus*). Unpublished study performed by Springborn Smithers Laboratories, Snow Camp, NC. Laboratory Project No. 1781.4103. Study submitted by Syngenta Crop Protection, Inc., Greensboro, NC. Syngenta Number T021573-04. (MRID # 465000-07).

Stafford, J.M. 2005b. Hydroxyatrazine (G34048): Acute Oral Toxicity Test (LD50) with Northern Bobwhite Quail (*Colinus virginianus*). Unpublished study performed by Springborn Smithers Laboratories, Snow Camp, NC. Laboratory Project No. 1781.4101. Study submitted by Syngenta Crop Protection, Inc., Greensboro, NC. Syngenta Number T021574-04. (MRID # 465000-08).

Stafford, J.M. 2005c. Desethylatrazine (G30033): Acute Oral Toxicity Test (LD50) with Northern Bobwhite Quail (*Colinus virginianus*). Unpublished study performed by Springborn Smithers Laboratories, Snow Camp, NC. Laboratory Project No. 1781.4102. Study submitted by Syngenta Crop Protection, Inc., Greensboro, NC. Syngenta Number T021575-04. (MRID # 465000-09).

Stallman, Heidi R. and Louis B. Best. 1996. Bird use of an experimental strip intercropping system in northeast Iowa. J. Wildl. Manage. 60(2):354-362. (MRID # 452051-04).

Stay, F. S., A. Katko, C. M. Rohm, M. A. Fix and D. P. Larsen. 1989. The effects of atrazine on microcosms developed from four natural plankton communities. Arch. Environ. Contam. Toxicol. 18:866-875. (MRID # 450874-18).

Stay, F. S., D. P. Larsen, A. Katko and C. M. Rohm. 1985. Effects of atrazine on community level responses in Taub microcosms, p. 75-90. *In:* Boyle, T. P. (ed.). Validation and predictability of laboratory methods for assessing the fate and effects of contaminants in aquatic ecosystem. ASTM STP 865. (MRID # 450874-19).

Steinberg, C. E. W., R. Lorenz and O. H. Spieser. 1995. Effects of atrazine on swimming behavior of zebrafish, *Bachydanio rerio*. Water Research 29(3):981-985. (MRID # 452049-10).

Stevenson, J. C. and N. M. Confer. 1978. Summary of available information on Chesapeake Bay submerged vegetation. U.S. Dept. Interior, Fish Wildl. Ser., Off. Biol. Survey. FWS/OBS-78/66. 335 p.

Stevenson, J. C., T. W. Jones, W. M. Kemp, W. R. Boynton and J. C. Means. 1982. An overview of atrazine dynamics in estuarine ecosystems, p. 79-94. *In*: Proceedings of the workshop on agrochemicals and estuarine productivity, Beaufort, North carolina, September 18-19,1980.

Storrs, S. I. and Kiesecker, J. M. (2004). Survivorship Patterns of Larval Amphibians Exposed to Low Concentrations of Atrazine . *Environ.Health Perspect.* 112: 1054-1057. EcoReference No.: 78290

Stratton, G. W. 1984. Effects of the herbicide atrazine and its degradation products, alone and in combination, on phototrophic microorganisms. Bull. Environ. Contam. Toxicol. 29:35-42. (MRID # 450874-01).

Streit, B., and H. M. Peter. 1978. Long-term effects of atrazine to selected freshwater invertebrates. Arch. Hydrobiol. Suppl. 55:62-77. (MRID # 452029-16).

Sullivan, K. B. and Spence, K. M. (2003). Effects of Sublethal Concentrations of Atrazine and Nitrate on Metamorphosis of the African Clawed Frog. *Environ.Toxicol.Chem.* 22: 627-635. EcoReference No.: 68187

Taylor, E. J., S. J. Maund and D. Pascoe. 1991. Toxicity of four common pollutants to the freshwater macroinvertebrates *Chironomus riparius* Meigen (Insecta: Diptera) and *Gammarus pulex* (L.) (Crustacea: Amphipoda). Arch. Environ. Contam. Toxicol. 21:371-376. (MRID # 452029-17).

Thurman, E. M., D. A. Goolsby, M. T. Meyer, M. S. Mills, M. L. Pomes and D. W. Kolpin. 1992. A reconnaissance study of herbicides and their metabolites in surface water of the midwestern United States using immunoassay and gas chromatography/mass spectroscopy. Environ. Sci. Technol. 26:2440-2447.

Thursby, G. B., D. Champlin and W. Berry. 1990. Acute toxicity of atrazine to copepods. (Memorandum to D. J. Hansen, US. EPA, Narragansett Lab., RI., September 16.). (MRID # 452029-18).

Thursby, G. B. and M. Tagliabue. 1990. Effect of atrazine on sexual reproduction in the kelp, *Laminaria saccharina*. (Memorandum to D. J. Hansen, US. EPA, Narragansett, RI. September 14).

Tierney, K.B., C.R. Singh, P.S. Ross, and C.J. Kennedy. 2007. Relating olfactory neurotoxicity to altered olfactory-mediated behaviors in rainbow trout exposed to three currently-used pesticides. Aquatic Tox. 81:55-64. EcoReference No.: 89625.

Torres, A. M. R. and L. M. O'Flaherty. 1976. Influence of pesticides on *Chlorella*, *Chlorococcum*, *Stigeoclonium* (Chlorophyceae), *Tribonema*, *Vaucheria* (Xanthophyceae) and *Oscillatoria* (Cyanophyceae). Phycologia 15(1):25-36. (MRID # 000235-44).

Tucker, R. K. and D. G. Crabtree. 1970. Handbook of toxicity of pesticides to wildlife. U.S. Dept. Interior, Bureau Sport Fish. Wildlife, Denver Wildlife Res. Center. Submitted by Fison Corp. (MRID No. NA).

Ukeles, R. 196. The effect of temperature on the growth and survival of several marine algal species. Biol. Bull. (Woods Hole, Mass.) 120:255-264.

Union Carbide Corp. 1975. Acute toxicity of SD 12011, Code 4-1-2-1 and Code 4-1-3-1 to fiddler crabs, *Uca pugilator*. Prepared by Union Carbide Corp.; submitted by Shell Chemical Co., Washington, D. C. (MRID No. 000243-95).

Univ. Maryland. 1998. The influence of salinity on the chronic toxicity of atrazine to sago pondweed: Filling a data need for development of an estuarine chronic condition. Univ. Maryland, Agr. Exper. Sta., Wye Res. Educ. Center, Printed by US EPA for Chesapeake Bay Program. EPA 903-R-98-020. (MRID # 450882-31).

U.S. EPA. 2003. White paper on potential developmental effects of atrazine on amphibians. May 29, 2003. Office of Pesticide Programs, Washington D.C. Available at http://www.epa.gov/scipoly/sap.

U.S. EPA. 2004. Interim Guidance of the Evaluation Critieria for Ecological Toxicity Data in the Open Literature: Phases I and II; Procedures for Identifying, Selecting and Acquiring Toxicity Data Published in the Open Literature for Use in Ecological Risk Assessments. Office of Pesticide Programs, Washington D.C. July 16, 2004.

van den Brink, P. J., E. van Donk, R. Gylstra, S. J. H. Crum and T. C. M. Brock. 1995. Effects of chronic low concentrations of the pesticides chlorpyrifos and atrazine in indoor freshwater microcosms. Chemosphere 31(5):3181-3200. (MRID # 450874-17).

Vial, A. 1991a. G28273: Report on The Acute Toxicity Test of G 28273 to Rainbow Trout (*Salmo gairdneri*): Final Report. Unpublished study performed by CIBA-GEIGY Ltd., Product Safety, Ecotoxicology, Basel, Switzerland. Laboratory report number Basel 918139. Study sponsored by CIBA-GEIGY Ltd., Basel, Switzerland and submitted by Syngenta Crop Protection, Inc., Greensboro, NC. Study completed August 6, 1991.

Vial, A. 1991b. G28279: Report on The Acute Toxicity Test of G 28279 to Rainbow Trout (*Salmo gairdneri*): Final Report. Unpublished study performed by CIBA-GEIGY Ltd., Product Safety, Ecotoxicology, Basel, Switzerland. Laboratory report number Basel 918145. Study sponsored by CIBA-GEIGY Ltd., Basel, Switzerland and submitted by Syngenta Crop Protection, Inc., Greensboro, NC. Study completed August 8, 1991.

Vial, A. 1991c. G28273: Report on The Acute Toxicity Test of G 28273 to Daphnia (*Daphnia magna* Straus 1820): Final Report. Unpublished study performed by CIBA-GEIGY Ltd., Product Safety, Ecotoxicology, Basel, Switzerland. Laboratory report number Basel 918141. Study sponsored by CIBA-GEIGY Ltd., Basel, Switzerland and submitted by Syngenta Crop Protection, Inc., Greensboro, NC. Study completed August 7, 1991.

Vial, A. 1991d. G28279: Report on The Acute Toxicity Test of G 28279 to Daphnia (*Daphnia magna* Straus 1820): Final Report. Unpublished study performed by CIBA-GEIGY Ltd., Product Safety, Ecotoxicology, Basel, Switzerland. Laboratory report number Basel 918147. Study sponsored by CIBA-GEIGY Ltd., Basel, Switzerland and submitted by Syngenta Crop Protection, Inc., Greensboro, NC. Study completed August 9, 1991.

Vial, A. 1991e. G28279: Report on the Growth Inhibition Test of G 28279 to Green Algae (*Scenedesmus subspicatus*). Unpublished study performed by CIBA-GEIGY Ltd., Product Safety, Ecotoxicology, Basel, Switzerland. Laboratory report number Basel 918146. Study sponsored by CIBA-GEIGY Ltd., Basel, Switzerland and submitted by Syngenta Crop Protection, Inc., Greensboro, NC. Study completed August 7, 1991.

Waldron, A. C. 1974. Pesticide movement from cropland into Lake Eire. US EPA, Athens, GA, EPA-660/2-74-03.

Walker, C. R. 1964. Simazine and other *s*-triazine compounds as aquatic herbicides in fish habitats. Weeds 12(2):134-139. (MRID # 452029-19).

Walne, P. R. 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea*, *Crassostrea*, *Mercenaria* and *Mytilus*. Fish Invest., London (Series 2) 26:1-62.

Walsh, G. E. 1972. Effects of herbicides on photosynthesis and growth of marine unicellular algae. Hyacinth Control J. 10:45-48. (MRID # 450882-15).

Walsh, G. E. 1983. Cell death and inhibition of population growth of marine unicellular algae by pesticides. Aquatic Toxicol. 3:209-214. (MRID # 452277-31).

Ward, G. S. and L. Ballantine. 1985. Acute and chronic toxicity of atrazine to estuarine fauna. Estuaries 8:22-27. (Estuaries 8(1):22-27. (MRID # 452029-20).

Waring, C. P. and Moore, A. (2004). The Effect of Atrazine on Atlantic Salmon (Salmo salar) Smolts in Fresh Water and After Sea Water Transfer. *Aquat.Toxicol.* 66: 93-104. EcoReference No.: 72625.

Wauchope, R. D. 1978. The pesticide content of surface water draining from agricultural fields – a review. J. Environ. Qual. 7:459-472.

Whale, G. F., D. A. Sheahan and M. F. Kirby. 1994. Assessment of the value of including recovery periods in chronic toxicity test guidelines for rainbow trout (*Onchorhynchus mykiss*), p. 175-187. *In*: R. Muller and R. Lloyd (ed.). Sublethal and chronic effects of pollutants on freshwater fish. Fishing News Books, London, U.K. (MRID # 452083-04).

Wetzel, R. G. 1975. Primary production. p. 230-247. *In*: B. A. Whitton (ed.). River ecology. Univ. of Calif. Press, Berkley.

Wieser, C. M. and T. Gross. 2002. Determination of potential effects of 20 day exposure of atrazine on endocrine function in adult largemouth bass (*Micropterus salmoides*). Prepared by University of Florida, Wildlife Reproductive Toxicology Laboratory, Gainesville, FL, Wildlife No. NOVA98.02e; submitted by Syngenta Crop Protection, Inc., Greensboro, NC. (MRID No. 456223-04).

Wilhelms, K. W., Cutler, S. A., Proudman, J. A., Anderson, L. L., and Scanes, C. G. (2005). Atrazine and the Hypothalamo-Pituitary-Gonadal Axis in Sexually Maturing Precocial Birds: Studies in Male Japanese Quail. *Toxicol.Sci.* 86: 152-160. Ecoreference No. 80632

Wilhelms, K. W., Cutler, S. A., Proudman, J. A., Anderson, L. L., and Scanes, C. G. (2006a). Effects of Atrazine on Sexual Maturation in Female Japanese Quail Induced by Photostimulation or Exogenous Gonadotropin. *Environ.Toxicol.Chem.* 25: 233-240. Ecoreference No. 82035.

Wilhelms, K. W., Cutler, S. A., Proudman, J. A., Carsia, R.V., Anderson, L. L., and Scanes, C. G. (2006b). Lack of effects of atrazine on estrogen-responsive organs and circulating hormone concentrations in sexually immature female Japanese quail (*Coturnix coturnix japonica*). *Chemosphere.* 65: 674-681.

Wu, T. L. 1980. Dissipation of the herbicides atrazine and alachlor in a Maryland corn field. J. Environ. Qual. 9:459-465.

Wu, T. L. 1981. Atrazine residues in estuarine water and the aerial deposition of atrazine into Rhode River, Maryland. Water, Air, Soil Poll. 15:173-184.

Wu, T. L., D. L. Correl and H. E. H. Remenapp. 1983. Herbicide runoff from experimental watersheds. J. Environ. Qual. 12:330-336.