MEETING OF THE FIFRA SCIENTIFIC ADVISORY PANEL

ON THE

PROBLEM FORMULATION FOR THE ENVIRONMENTAL FATE AND ECOLOGICAL RISK ASSESSMENT FOR ATRAZINE

JUNE 12-15, 2012

Preamble

This document is a preliminary problem formulation for atrazine to fulfill Registration Review requirements under FIFRA. In a problem formulation, available information, including stressor sources and characteristics, exposure, ecological effects on plants and animals (*e.g.*, amphibians, fish, invertebrates, birds, and mammals), and characteristics of the ecosystem(s), is used to identify missing information and assessment endpoints and to develop a preliminary assessment of the problem.

The document consists of two main sections. In the first section is background material on the regulatory history, use and usage, ecotoxicity data, environmental fate data, and environmental monitoring data for atrazine **(Chapter I)**. These data serve as the foundational data for the risk assessment. This section also contains a critical review of published atrazine testing with amphibians (**Chapter II**).

The second section includes the analysis plan (**Chapter III**) for the risk assessment. This section also provides a detailed plan on the methods used to assess risk in light of information on use/usage, ecotoxicity data, and environmental fate data. A key aspect of this section is identifying and addressing uncertainties in the risk assessment. The topics are:

- **Chapter IV** – The methodology for determining the level of concern for atrazine
- **Chapter V** - A strategy for using the PATI-derived CE-LOC for identification of vulnerable watersheds

Charge questions to the FIFRA SAP can be found in the relevant sections of **Chapters I, II, IV, and V.**

Table of Contents

APPENDICES:

- **A.** Supporting Environmental Fate Data
- **B.** Supporting Ecological Toxicity Data
- **C.** Open Literature Review of Amphibian Data
- **D.** Bibliography of Microcosm and Mesocosm Studies and Criteria Used to Screen Studies for Analysis of Atrazine Risks to Aquatic Plant Communities
- **E.** Proposed Methodology for Specifying Atrazine Levels of Concern for Protection of Plant Communities in Freshwater Ecosystems
- **F.** Critique of Syngenta Corporation's Probabilistic Implementation of $CASM_{ATZ}$ for Ecological Risk Assessment of Atrazine
- **G.** ELECTRONIC FILES:
    a. The PATI Model Working Program
    b. Microcosm and Mesocosm and Atrazine Ecological Exposure Monitoring Program Input Files
    c. PATI Output and CE-LOC Calculation
    d. Monitoring Data Processing Macro File
    e. Summary of Monitoring Data, Including Listing of Water Monitoring Sites that Exceed the Aquatic Plant Community Level of Concern
    f. Breakdown of Species Tested in Ecotoxicity Studies
    g. Log Linear 95% C.I. Bias Factor Sensitivity Analysis Using AEEMP Monitoring Data
    h. NAWQA Map Data: 60-day Maximum Average 7 ppb exceeders
    i. NAWQA Map Data: Bias Factor Adjusted 4 ppb exceeders
    j. NAWQA Map Data: Bias Factor Adjusted 7 ppb exceeders
    k. NAWQA Map Data: 60-Day Maximum Average 4 ppb Exceeders
- **H.** Bibliography of ECOTOX Accepted Papers (2003-2011)
- **I.** Bibliography of ECOTOX Rejected Papers (2003-2011)
- **J.** Bibliography of ECOTOX Excluded Papers (2003-2011)
- **K.** Bias Factor Calculations Regression Equations
- **L.** Degradation Product Structures
- **M.** Reanalysis of Toxicity Data for Constructing PATI
- **N.** Evaluation Guidelines for Ecological Toxicity Data in the Open Literature

CHAPTER I. ATRAZINE BACKGROUND, HISTORY, AND ECOTOXICOLOGICAL DATA

1. Purpose

Atrazine is currently registered as an herbicide in the U.S. to control annual broadleaf and grass weeds primarily in corn, sorghum, and sugarcane. In addition to food crops, atrazine is also used on a variety of non-food crops, forests, residential, commercial, and industrial lawn uses, golf course turf, recreational areas, and rights-of-way. It is one of the most widely used herbicides in North America (USEPA, 2003a).

The purpose of this white paper (a problem formulation) is to provide an understanding of what is currently known about the environmental fate and ecological effects of atrazine, in relationship to its registered uses. An understanding of the environmental fate and ecological effects of atrazine will inform the risk assessment, which will be conducted for atrazine under EPA's registration review program, and subsequently published for public comment. The atrazine risk assessment is scheduled for completion in 2017.

This white paper will describe EPA's plan for analyzing data relevant to atrazine and its degradates and for conducting the environmental fate and ecological risk assessment for atrazine's registered uses. This document also contains charge questions to the Scientific Advisory Panel (SAP), and supporting reference materials.

2. Regulatory Actions and History

2.1. Nature of Regulatory Action

Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), all pesticides intended for use in the United States must be registered (licensed) by the Environmental Protection Agency (EPA) before they can be sold or distributed in commerce. EPA will register a pesticide if scientific data provided by the registrant show that, when used according to label directions, it will not cause unreasonable adverse effects on human heath or the environment. In 1996, when FIFRA was amended by the Food Quality Protection Act, EPA was mandated to implement a program for the periodic review of pesticides (registration review) (http://www.epa.gov/oppsrrd1/registration_review/). The registration review program is intended to ensure that, as the ability to assess risk evolves and as policies and practices change, all registered pesticides continue to meet contemporary standards of health, safety, and product labeling and that their risks are adequately mitigated.

As part of the implementation of the registration review program pursuant to Section 3(g) of the FIFRA, the EPA is intitiating its evaluation of atrazine to determine whether it continues to

meet the statutory standard for no unreasonable adverse effects to human health and the environment based on current uses.

2.2. Regulatory History, Mitigation of Ecological Risk, and Scientific Advisory Panel Reviews

Atrazine was first registered by the United States Department of Agriculture (USDA) in 1958 as a broad spectrum residual herbicide. Atrazine is used both at plant and post-plant, and is primarily used in corn, sweet corn, sorghum, and sugarcane production. Additional uses include soybeans, wheat (stubble only), oats, macadamia nuts, guava, range grasses, conifer forests, Christmas tree farms, sod farms, ornamental grasses, ornamental plants, ornamental turf, residential lawns, schools, parks, playgrounds, athletic fields, roadsides, rights-of-ways, airfields, vacant lots, roadsides, lumber yards, agricultural buildings, industrial sites and storage sites.

EPA's regulation of atrazine over the past three decades has focused on human and environmental exposures through water, the most significant exposures associated with atrazine's agricultural and residential lawn uses. On November 10, 1983, EPA issued a Registration Standard for atrazine, which noted the EPA's concerns regarding ground and surface water contamination (USEPA, 2003a).

In the early 1990s, the registrant voluntarily instituted several risk reduction measures to address concerns raised about ground and surface water contamination by atrazine. In 1990, the following measures were undertaken to address groundwater exposures:

- Reduction of the application rate for corn and sorghum to 3.0 pounds of active ingredient (lbs a.i.)/acre from 4.0 lbs a.i./acre.
- Reduction of the maximum rate for non-cropland and total vegetation control to 10 lbs a.i./acre from 40 lbs a.i./acre.
- Requirement that post-emergence applications to corn and sorghum be made before the corn and sorghum plants reach 12 inches in height.
- Deletion of rangeland, proso millet, and pineapple uses.
- Prohibition of chemigation (applying atrazine through irrigation systems).
- Institution of a well-head protection plan requiring 50-foot setbacks around all wells for mixing, loading, or applying atrazine-containing products.
- Institution of construction requirements for bulk storage facilities to prevent point source contamination from spills
- Classification of all atrazine-containing products (except for the lawn care, turf, and conifer uses) as Restricted Use Pesticides (RUPs).

In 1992, registrants undertook the following additional measures to address concerns about atrazine exposure in surface water:

- Further reduction of the total seasonal application rates for corn and sorghum to 2.5 lbs a.i./acre per year. This rate includes a 1.5 lbs a.i./acre per year pre-emergence use and a 1.0 lbs a.i./acre per year post-emergence use.

- Deletion of all uses for total vegetation control in non-cropland.
- Expansion of restricted use criteria. Expansion of the setback requirements, included following a 50-foot setback around surface water sources when workers are mixing and loading atrazine-containing products; a 66-foot application (ground and aerial) setback from points of entry where field surface water runoff enters surface water sources; and, a 200-foot application setback around lakes and reservoirs.

In November 1994, EPA initiated a Special Review for the triazine pesticides (atrazine, simazine and cyanazine; USEPA, 1994) that focused on minimizing human exposure to atrazine from ground water and surface water.

Further labeled use restrictions in 1996 reduced environmental exposure from tile-terraced fields containing standpipes, as follows:

- Restrictions against application within 66 feet of standpipes.
- Requirement that applications be incorporated to a depth of 2 to 3 inches.
- Restrictions against application to no-till fields unless practicing high crop residue management.

In January 2003, the EPA concluded its ecological risk assessment as part of the Interim Registration Eligibility Decision (IRED) for atrazine (USEPA, 2003a). This assessment was based on laboratory ecotoxicological data as well as microcosm and mesocosm (cosm) field studies found in publicly available literature, a substantial amount of monitoring data for freshwater streams, lakes, reservoirs, and estuarine areas, and incident reports of adverse effects on aquatic and terrestrial organisms associated with the use of atrazine.

Based on this assessment, the January 2003 IRED concluded that atrazine concentrations in watersheds may cause significant changes in aquatic plant community structure and productivity; EPA considered this endpoint to be the most sensitive effect of concern. The IRED also established a framework for developing an aquatic ecosystem level of concern (LOC) to ensure that atrazine concentrations in watersheds will not cause these changes. By focusing on aquatic plant community structural and productivity changes, EPA intended to protect invertebrates, fish, and amphibians from the direct effects of atrazine as well as the effects that atrazine could have on the habitat and food sources of aquatic animals. The January 2003 IRED also established the need for additional data to (1) identify and evaluate water bodies in corn, sorghum, and sugarcane use areas where such changes in aquatic plant community structure and productivity were more likely to occur; and to (2) determine potential amphibian gonadal developmental responses to atrazine.

To mitigate potential ecological risks to aquatic communities identified in the IRED, the EPA required atrazine registrants, in consultation with EPA, to develop a program under which the registrants monitor for atrazine concentrations and mitigate environmental exposures if EPA determines that mitigation is necessary. The program was intended to focus on impacts of

atrazine use at the watershed level in accordance with existing state and federal water quality programs. This monitoring and mitigation program was also agreed upon in a 2003 Memorandum of Agreement (MOA) between EPA and the atrazine registrants (USEPA, 2003b).

On October 31, 2003, EPA issued an addendum that updated the January 31, 2003 IRED (USEPA, 2003c). This addendum described new scientific developments pertaining to monitoring of watersheds and potential effects of atrazine on endocrine-mediated pathways of amphibian gonadal development. As discussed in the October 2003 IRED, the EPA conducted an evaluation of the submitted studies regarding the potential effects of atrazine on amphibian gonadal development and presented its assessment in the form of a white paper for external peer review to a FIFRA Scientific Advisory Panel (SAP) in June 2003. In the white paper dated May 29, 2003, the EPA summarized seventeen studies consisting of both open literature and registrant-submitted laboratory and field studies involving both native and non-native species of frogs (USEPA, 2003d). The EPA concluded that none of the studies fully accounted for environmental and animal husbandry factors capable of influencing endpoints that the studies were attempting to measure. The EPA also concluded that the current lines-of-evidence did not show that atrazine produced consistent effects across a range of exposure concentrations and amphibian species tested.

Based on this assessment, the EPA concluded and the SAP concurred that there was sufficient evidence to formulate a hypothesis that atrazine exposure may impact gonadal development in amphibians, but there were insufficient data to confirm or refute the hypothesis (USEPA, 2003d). Because of the inconsistency and lack of reproducibility across studies and uncertainty in the nature of any dose-response relationship in the currently available data, the EPA determined that the data did not alter the conclusions reached in the January 2003 IRED regarding uncertainties related to atrazine's potential effects on amphibians. The SAP, however, supported EPA in seeking additional data to reduce uncertainties regarding potential effects to amphibians. Subsequent data collection occurred following a multi-tiered process outlined in the EPA's white paper to the SAP (USEPA, 2003d). These data were submitted, and a second SAP was held in 2007 to discuss results of these studies (USEPA, 2007a). These data suggested, and the SAP agreed, that gonadal development of larval *Xenopus laevis* was not affected by atrazine concentrations tested from 0.1 to 100 µg/L. Based on these data, EPA concluded that atrazine, using *Xenopus laevis* as a surrogate, does not consistently affect amphibian gonadal development. EPA acknowledged, however, that there is uncertainty in using *Xenopus laevis* to represent all amphibians.

Since the October 2007 SAP on the potential effects of atrazine on amphibians, several other SAP meetings on atrazine have been held:

- December 4-7, 2007: Interpretation of the Ecological Significance of Atrazine Stream-Water Concentrations Using a Statistically-Designed Monitoring Program.
- May 12-15, 2009: The Ecological Significance of Atrazine Effects on Primary Producers in Surface Water Streams in the Corn and Sorghum Growing Region of the United States.

- November 3, 2009: Presentation of the Approach to Reevaluate Atrazine.
- February 2 - 4, 2010: Incorporation of Epidemiology and Human Incident Data into Human Risk Assessment.
- April 26-29, 2010: Re-Evaluation of Human Health Effects of Atrazine: Review of Experimental Animal and *In Vitro* Studies and Drinking Water Monitoring Frequency.
- September 14-17, 2010: Re-Evaluation of Human Health Effects of Atrazine: Review of Non-Cancer Effects and Drinking Water Monitoring Frequency
- July 26-29, 2011: Re-Evaluation of Human Health Effects of Atrazine: Review of Cancer Epidemiology, Non-cancer Experimental Animal and *In Vitro* Studies and Drinking Water Monitoring Frequency.

These SAP meetings are discussed in more detail in the following paragraphs.

In December 2007, another SAP was convened to address the potential for community-level effects to aquatic plants in Midwestern streams. In this SAP meeting, EPA discussed the preliminary development of an ecological LOC methodology and presented an analysis of the monitoring program using that methodology (USEPA 2007b). The LOC development included a summary of the underlying approach to relate effects from time variable real world exposure to aquatic plant community effects found in the cosm studies using the Comprehensive Aquatic Systems Model ($CASM_{ATZ1}$). The SAP document included a preliminary sensitivity analysis of $CASM_{ATZ1}$, results of the ecological stream monitoring study conducted from 2004 to 2006, and identification of watersheds that exceeded the $CASM_{ATZ1}$-based LOC with associated population statistics.

Using a stratified, random statistical survey design EPA identified forty watersheds representing high atrazine use locations vulnerable to atrazine runoff (USEPA 2003c). Monitoring was conducted in these watersheds from 2004 to 2006 and is still occurring in select watersheds. During the December 2007 SAP meeting, EPA proposed a GIS-based approach for extrapolating results from these 40 watersheds to all watersheds where atrazine is used (USEPA, 2007b).

The SAP issued its evaluation and recommendations on March 5, 2008 (USEPA, 2007b). In their report, the SAP concurred with the conceptual approach of using a community simulation-based model to relate time variable exposures to community-level effects data represented by the cosm data. The SAP recommended that the $CASM_{ATZ1}$ model should be revised with respect to parameterization, process formulation and functionality to more accurately model second and third order Midwest stream characteristics and that a more comprehensive sensitivity analysis should be conducted (USEPA, 2007b). The SAP also recommended that EPA determine the extent to which atrazine levels in water bodies exceed the aquatic community-level LOCs, including interpretation of the monitoring results and identification of chemographs that exceed the LOC, and the identification of the location of other watersheds with characteristics similar to those that exceeded the LOC in the monitoring study.

In May 2009, EPA again convened an SAP meeting, presenting new evaluations of the applicability of the revised CASM Atrazine model ($CASM_{ATZ2}$) to freshwater atrazine risk assessments for first- and second-order freshwater streams and provided reasons in support of its decision not to proceed with further development and application of this model (USEPA, 2009a). A simpler alternative to the CASM-based approach, the Plant Assemblage Toxicity Index (PATI), was presented for relating atrazine surface water exposures to cosm effects data. Other related topics presented to the SAP included a revised assessment of cosm exposure profiles, a review of the literature and subsequent analysis of single-species toxicity tests to develop statistical estimates of Effects Concentrations (ECs) and Specific Growth Rate (SGR) parameters for major taxonomic groups, an update on the monitoring program results, interpretation of the surface water monitoring results with both the revised $CASM_{ATZ2}$ and the alternate PATI model, identification of the primary watershed characteristics driving atrazine exposure and a preliminary extrapolation of the results to the entire atrazine use area to identify other areas where atrazine exposures may exceed the LOC. The EPA Office of Water also provided an example of statistical alternatives for expressing the model results to develop a numeric aquatic life criterion for atrazine that integrates both concentrations and exposure durations of atrazine.

The Panel agreed with the EPA decision to move to the PATI model for a first tier national approach, but also recommended using a refined $CASM_{ATZ2}$, the Hazard Criterion (HC_5 or HC_{10}) and/or species sensitivity distributions to compare LOC estimates from each (USEPA, 2009). The Panel further suggested that a refined $CASM_{ATZ2}$ could be used to further investigate those watersheds that exceed the LOC; however this would require a significant effort to incorporate site specific parameterization. The Panel concluded that the description of the selection criteria and scoring of the effects (Brock Scores method) in the cosm studies was inadequate and needed to be refined.

EPA also presented to the 2009 SAP its watershed analysis to identify specific characteristics that distinguish watersheds with waters that frequently exceed the aquatic community LOC for atrazine and to use those characteristics to identify other similarly vulnerable watersheds. EPA's analysis identified the presence of soils with shallow, drainage-restrictive layers in areas with atrazine use as a characteristic that distinguished monitoring sites exceeding the aquatic community LOC in multiple years from those sites that did not exceed the LOC during the sampling period (USEPA, 2007b, 2009). The 2009 SAP recommended using an effects index or concentration metric, rather than categorical LOC thresholds (i.e., exceeded the LOC in multiple years vs. did not exceed during the study) in order to take advantage of data from all 40 Atrazine Ecological Exposure Monitoring Program sites (AEEMP[1]; USEPA, 2009). The Panel encouraged the development of a "Cornbelt Watershed Regression for Pesticide (WARP) Model" and recommended considering additional data related to application (planting dates, timing of atrazine application), weather (rainfall intensity and duration), soils and hydrology

[1] Syngenta refers to this monitoring program as the Atrazine Ecological Monitoring Program (AEMP).

(runoff propensity index, composite curve numbers, watershed geometry), and management (riparian buffers/setback areas, tillage, conservation practices, etc.).

In November 2009, EPA initiated a re-evaluation of atrazine to consider new research that had been published, as well as several years of atrazine drinking water monitoring data, to ensure that EPA's regulatory decisions for atrazine continue to protect public health. To that end, EPA convened four SAPs from 2010 to 2011 focusing on the potential effects of atrazine on human health. New hazard and dose-response data reviewed during this re-evaluation suggested that a shorter duration of exposure may be appropriate for the toxicological endpoint of concern. Given the potential for a shorter duration of concern, EPA considered whether weekly or biweekly water monitoring would adequately characterize shorter durations of atrazine exposure in drinking water. In the April and September 2010 FIFRA SAP consultations on atrazine, EPA reviewed methods for designing monitoring studies to capture exposures of concern and approaches for analyzing and interpreting existing monitoring data and characterizing the uncertainties in those estimates for use in human health risk assessments (USEPA, 2010a and 2010b). The SAP commented that the toxicological exposure time frame of interest will define the importance of peaks and determine the most useful approaches both for designing a monitoring study and for evaluating the utility of existing monitoring studies (USEPA, 2010a). However, the SAP noted that, in light of uncertainty over a critical exposure period, ranging from a few days to a few weeks, the best course of action may be attempting "to capture the pattern of atrazine concentrations in the source water of each CWS based on the characteristics of that particular water system, as opposed to a one-size-fits-all approach" (USEPA, 2010b).

Identifying monitoring sites and determining the sampling frequency require careful consideration to avoid underestimating true atrazine concentrations (USEPA, 2010b). A predictive model can be used to target sites that merit the most detailed monitoring and target the time periods when atrazine is most likely to be present (USEPA, 2010a and 2010b). The Panel recommended that the USEPA give thought to "using the simulation models and CWS characterizations as part of the monitoring process. In particular, it is feasible that models will eventually be accurate enough to provide predictions of atrazine concentrations in source waters to a CWS for the coming crop season. Instead of requiring a CWS to collect and analyze water samples in their output stream (drinking water) at some predefined frequency (*e.g.*, daily or weekly in the case of some sites), it should be possible to use the models to facilitate targeting sampling to periods of time most likely to experience an exceedance" (USEPA, 2010b).

To estimate exposures from monitoring data sampled at intervals, particularly where peak concentrations over shorter durations are important, the USEPA needs methods that can predict values that may be greater than those sampled (USEPA, 2010a). The SAP recommended combining regression-based models, such as the United States Geological Survey's (USGS) Watershed Regression on Pesticides (WARP), with either a deterministic model or with a statistical approach using kriging or random-function models (USEPA, 2010b).

In the July 2011 SAP, the EPA conducted an evaluation of methods for characterizing the uncertainties resulting from the existing AMP monitoring study design (USEPA, 2011a). Methods for addressing temporal uncertainty with monitoring data includes bias factors, kriging, conditional simulation, and mass balance modeling. The Panel concluded that temporal uncertainty in monitoring data could be addressed using bias factor or hybrid Pesticide Root Zone Model (PRZM) modeling approaches (USEPA, 2011a).

2.3. Endangered Species Assessments

On August 1, 2003, EPA released an assessment of the potential effects of atrazine to 26 listed Evolutionarily Significant Units (ESUs) of Pacific salmon and steelhead. In addition, as part of the Natural Resources Defense Council settlement agreement and second settlement agreement with the Center for Biological Diversity and Save Our Springs Alliance, several assessments have recently been conducted on the potential for atrazine to affect a number of listed species. These effects determinations, which are available on the web at www.epa.gov/espp, review atrazine's potential direct and indirect effects.

3. Stressor Source and Distribution

Atrazine is mobile and persistent in the environment. The main routes of dissipation are microbial degradation under aerobic conditions, runoff, and leaching. Because of its persistence and mobility, atrazine will move into surface and ground water. This is confirmed by the widespread detections of atrazine in surface water and ground water.

3.1. Herbicidal Mechanism of Action

Triazine herbicides such as atrazine bind with a protein complex of the Photosystem II in chloroplast photosynthetic membranes (Schulz *et al.,* 1990). The result is an inhibition in the transfer of electrons that in turn inhibits the formation and release of oxygen. An evaluation of the mode of action for atrazine in mammals was presented in an April 2010 SAP- "Re-Evaluation of Human Health Effects of Atrazine: Review of Experimental Animals and *In vitro* Studies and Drinking Water Monitoring Frequency" (USEPA, 2010a). Proposed modes of action for reproduction were presented and if conserved across species, may be applicable to other animals.

3.2. Overview of Pesticide Use and Usage

Information on use sites, formulations, application methods, and application timing in this section of the document has been obtained from various EPA sources, including databases such

as OPPIN and the Label Use Information System (LUIS), and confirmed through a review of label information (USEPA, 2012a).

3.2.1. Agricultural Use Sites

Atrazine is a triazine herbicide first registered by USDA in 1958. It is currently registered as a restricted use pesticide only to be applied by certified applicators. Atrazine is registered for use against broadleaf and some grassy weeds in corn, sweet corn, sorghum, soybeans, sugarcane, wheat, oats, macadamia nuts, guava, and range grasses. Because application in wheat is to wheat stubble on fallow land following wheat harvests when the land is not cultivated, wheat is not considered the target crop. Application to range grasses is for the establishment of permanent grass cover on rangelands and pastures under USDA's Conservation Reserve Program (CRP in OK, NE, TX, and OR) and range/pastureland. Most of the atrazine applied to corn and sorghum is applied preemergence.

Formulations:
Atrazine is available in many formulations, including granular, wettable powder, water dispersible granules, emulsifiable concentrate, flowable concentrate, soluble concentrate, ready-to-use solution, and water soluble packs.

Application Methods:
Atrazine may be applied by groundboom sprayer, aircraft, tractor-drawn spreader, rights-of-way sprayer, low pressure handwand, backpack sprayer, lawn handgun, push-type spreader and belly grinder (hand-crank spreader).

Application Timing on Crops with the Highest Use:
Corn: Applications to corn are most often preemergence (mid-April through mid-May in the major corn-growing areas). Postemergence applications are most likely to occur up to the end of
June, until corn reaches 12" in height. There is some variability in timing based on geographical regions.

Sorghum: Applications to sorghum are most often preemergence (mid-May to mid-July in the major sorghum-growing areas). Postemergence applications are most likely to occur up to the end of August. There is some variability in timing based on geographical regions.

Sugarcane: Applications to sugarcane are usually at planting (fall), in the spring after emergence, and an additional postemergence application (often at layby). Since ratoon crops may face heavier weed pressure, additional applications are more likely in sugarcane ratoon crops.

3.2.2. Non-Agricultural Use Sites

Atrazine is registered for use in conifer forests, Christmas tree farms, sod farms, ornamental grasses, ornamental plants, ornamental turf, outdoor residential, lawns mostly confined to Florida and the Southeast, schools, parks, playgrounds, and athletic fields. Atrazine can also be used on roadsides, rights-of-ways, airfields, vacant lots, roadsides, lumber yards, agricultural buildings, industrial sites and storage sites. The amound of atrazine applied to non-agricultural sites is not known.

3.2.3. Agricultural Usage Data

Based on private market survey data from 2000-2010, agricultural usage averaged approximately 72 million pounds of active ingredient for 71 million acres, annually. **Figure 1** presents atrazine agricultural usage in pounds of atrazine applied between 2000 and 2010.

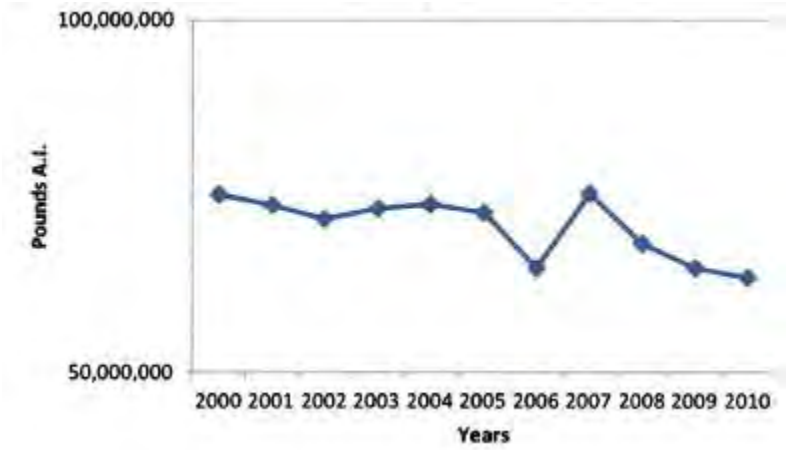


Figure 1. Atrazine Agricultural Usage (lbs ai Applied 2000-2010). Source: Proprietary Data (2000-2010).

Screening Level Usage Analysis Data (2003-2010)

Table 1 provides the most recent Screening Level Usage Analysis (SLUA), which was prepared in November, 2011 (USEPA, 2011b). The SLUA provides available estimates of pesticide usage data for atrazine on agricultural crops in the United States. The reported usage data in the SLUA are obtained from various sources and are merged, averaged and rounded so that the presented information is not proprietary, or business confidential.

SLUA data sources include USDA-NASS (United States Department of Agriculture's National Agricultural Statistics Service) (2003-2010) and Private Pesticide Market Research (2003-2010). These results reflect amalgamated data developed by the EPA and are releasable to the public. Limitations to the data include the following:

- Additional registered uses for certain crops may exist but are not included because the available surveys do not report usage (*e.g.*, small acreage crops).
- Lack of reported usage data for the pesticide on a crop does not imply zero usage.
- Usage data on a particular site may be noted in data sources, but not quantified. In these instances, the site would not be reported in the SLUA.
- Non-agricultural use sites (*e.g.*, turf, post-harvest, mosquito control, etc.) are not reported in the SLUA.

Some sites show use even though they are not on the label. This usage could be due to various factors, including, but not limited to data collection or reporting errors, or application errors.

			Percent Crop Treated (PCT)	
	Crop	**Lbs. A.I.**	**Avg.**	**Max.**
1	Corn	60,000,000	60	70
2	Barley+	7,000	<1	<2.5
3	Fallow	350,000	<2.5	5
4	Pastureland	60,000	<1	<2.5
5	Pecans +	1,000	<1	<2.5
6	Potatoes+	11,000	<2.5	<2.5
7	Sorghum	5,600,000	65	70
8	Soybeans	500,000	<1	<2.5
9	Sugarcane	2,000,000	65	75
10	Sunflowers +	6,000	<1	<2.5
11	Sweet Corn	400,000	70	75
12	Wheat	100,000	<1	<2.5

All numbers rounded.

<2.5 Less than 2.5 percent of crop treated
<1 Less than 1 percent of crop treated
\+ Crops not known to be listed on active end use product registrations when this report was run.

Table 1. Screening-Level Estimates of Agricultural Uses of Atrazine (2003-2010) (USEPA, 2011b)

Typical Use Patterns (2006-2010)

For the more recent timeframe of 2006-2010, usage averaged approximately 66 million pounds a.i. for 67 million acres. Atrazine is typically applied at a rate of 0.3-2.3 lbs a.i./A, depending on the crop as shown in **Table 2**. (Proprietary Data, 2006-2010).

In addition to the average application rate data, a rate distribution was generated to calculate an upper bound rate for each crop. The upper bound rate in this analysis is defined as the rate

at which 90% (or as close to 90% as possible) of the acres treated with atrazine were treated at, or below that rate, as shown in **Table 2**.

Crop	Average Application Rate (lbs ai/A)	Average number of Applications Per Year	Upper bound rate* (lbs ai/A) *percentile in parenthesis*
Corn	1.0	1.2	1.60 (89%); 1.75 (92%)
Sorghum (Milo)	1.0	1.2	1.5 (85%); 1.60 (90%); 1.75 (93%)
Sugarcane	2.3	1.4	3.75 (82%); 4.0 (100%)
Fallow	0.9	1.1	1.25 (79%); 1.45 (85%); 1.5 (95%)
Sweet Corn	0.8	1.1	1.5 (91%)
Wheat, Spring (stubble)	0.3	1.0	0.25 (67%); 0.45 (68%); 0.5 (100%)
Wheat, Winter (stubble)	0.7	1.0	0.75 (83%); 0.90 (91%); 1 (100%)

Source: Proprietary Data, 2006-2010

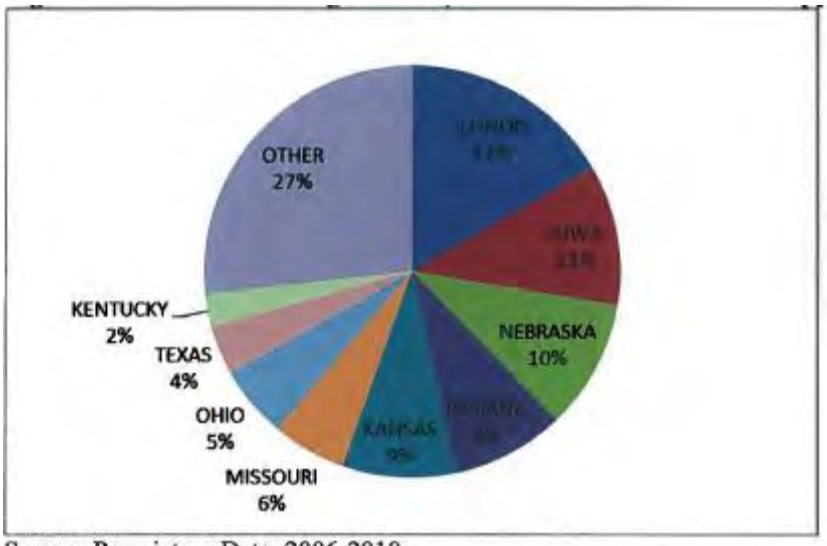
*The upper bound rate (90th percentile) is defined as the rate at which 90% (or as close to 90% as possible) of the acres treated with atrazine were treated at or below that rate. For example, in the above table, for sugarcane, 82 percent of the acres are treated at 3.75 lbs a.i./A or less, while the remaining 18 percent are all treated at 4.0 lbs a.i./A.

Table 2. Typical Use Patterns for Atrazine Used on Selected Crops (2006-2010).

Top Crops and States with Highest Use (2006-2010)

For 2006-2010, the top crop in terms of average annual pounds of active ingredient applied was corn (88%), followed by sorghum (8%), and sugarcane (2%) and sweet corn and fallow (1 % each). Spring and winter wheat stubble accounted for less than one percent of total pounds a.i. used during this period.

As shown in **Figure 2,** between 2006-2010, the states with the most agricultural usage in terms of pounds a.i. applied were Illinois (17%), Iowa (11%), Nebraska (10%), Indiana and Kansas (9% each), followed by Missouri, Ohio, Texas, and Kentucky with less than 6% each. The "other" category includes 29 other states with Minnesota, Michigan and Wisconsin having the most usage among those states.



Source: Proprietary Data, 2006-2010

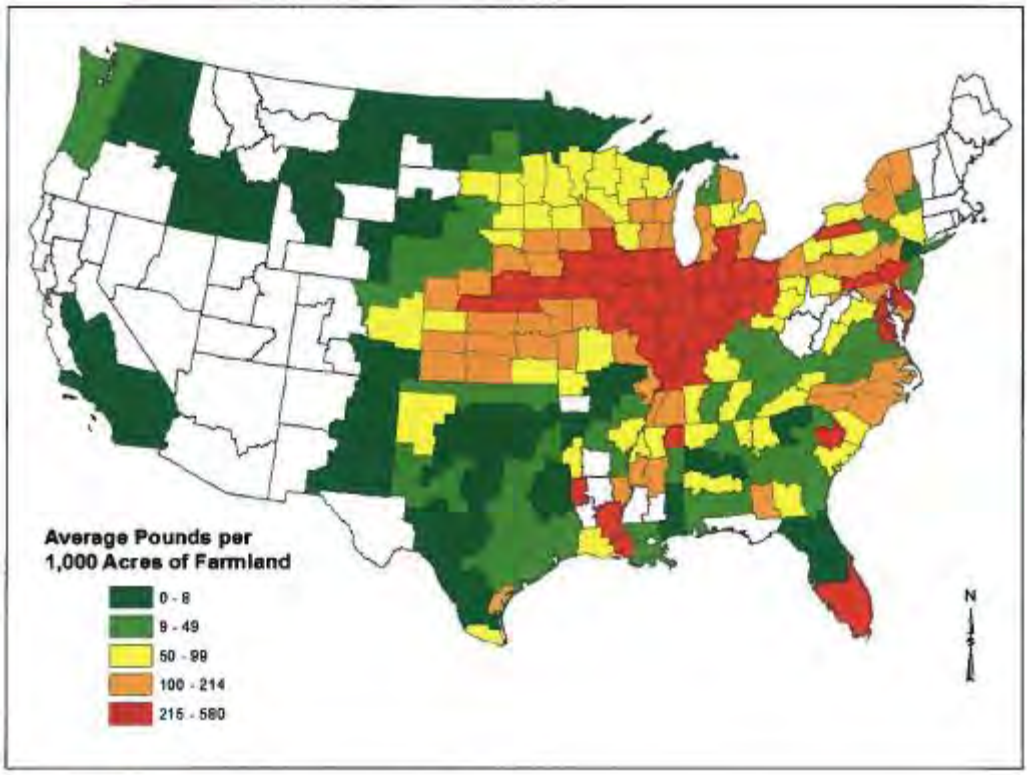
Figure 2. States with the Highest Use (Percent of Total Pounds A.I. Applied) 2006-2010.

National Mapping Data

Another measure of usage is the use intensity. In this analysis, the use intensity is expressed as the pounds a.i. applied per acre of farmland. This differs from the application rate, which is expressed as the pounds a.i. applied per treated acre. **Figure** 3 is a map of agricultural pesticide usage at the Crop Reporting District (CRD) level that spatially represents atrazine use intensity in the US. As shown, areas such as Florida and the corn producing states (colored in red) have the highest use intensity.

CRDs are districts created by USDA NASS which include aggregations of counties (USDA, 2010). Pesticide usage is displayed as average pounds (for the years 2006-2010) per 1,000 acres of farmland in a CRD to normalize for the variation in farmland between CRDs. Farmland acreage was obtained from USDA (2007).

Usage is based on private market surveys of pesticide use in agriculture (Proprietary Data, 2006-
2010). The survey data are limited to the states that represent the top 80-90 percent of acreage for the individual crops; therefore, use may be occurring in regions outside the scope of the survey. CRDs showing no usage of pesticides may be due to either the lack of pesticide use in the region or non-participation in the agricultural surveys. In addition, across the years, there may be variations in the specific crops included in the CRD survey. This may result in a lower annual average for the CRD.



Sources:
Proprietary Data. 2006-2010
USDA, 2006-2010. NASS Crop Reporting Districts.
USDA, 2007. Census of Agriculture.

Figure 3. Atrazine Usage by Crop Reporting District (2006-2010).

3.2.4. *Non-Agricultural Usage*

Information on non-agricultural usage in this section of the document has been obtained from available private market survey data from Kline & Co. The information provided on atrazine use in this section is for select non-agricultural use sites and does not represent all non-agricultural usage since data were not available for all non-agricultural use sites.

Non-agricultural usage data for professional applications to turf and ornamentals are available for 2002, 2004, and 2006 (**Table 3**). Over this time period, there was a notable increase in use by lawn care operators and on golf courses, institutional turf, and turf farms (Kline & Co., 2002, 2004, 2006).

Site	2002	2004	2006
Golf Courses	N/A	3,085	6,431
Institutional Turf	3,493	11,479	152,079
Landscape	593	41,978	204
Lawn Care Operators	211,561	97,095	686,107
Turf Farms	145,013	269,829	302,636
Nursery/Greenhouse	N/A	7,029	7,824
TOTAL Reported (lbs. a.i.)	360,660	430,495	1,155,281

N/A= no atrazine use was reported in the 2002 reports
Source: Kline & Co., 2002, 2004, and 2006

Table 3. Atrazine Select Non-Agricultural Usage (Pounds A.I.) (2002, 2004, 2006).

4. Environmental Fate and Transport

4.1. Physical and Chemical Properties

Atrazine physical and chemical properties are shown in **Table 4.**

Table 4. Physical and Chemical Properties of Atrazine		
Physical/Chemical Property	**Units**	**Value**
CAS Reg. Number	NA	1912-24-9
Chemical Formula	NA	$C_8H_{14}ClN_5$
Physical State	NA	Powder
Color	NA	White
Melting Point	^0C	175-177
Molecular Weight	g/mole	215.69
Water Solubility@20°C	mg/L	33
Vapor Pressure@ 20°C	torr	$3.0x10^{-7}$
Henrys Law Constant (calculated)	atm-m^3 $mole^{-1}$	$2.6x10^{-9}$
K_{ow}	Unitless	501.18

4.1.1. Environmental Fate Summary

Atrazine is mobile and persistent in the environment. The main routes of dissipation are microbial degradation under aerobic conditions, runoff, and leaching. Because of its persistence and mobility, atrazine will move into surface and ground water. This is confirmed by the widespread detections of atrazine in surface water and ground water. Summaries of the environmental fate data are shown in **Appendix A**.

There is no evidence that atrazine degrades by abiotic hydrolysis at pH 5, 7, and 9 (MRID 40431319). Photodegradation studies show a high variability in atrazine photodegradation rates. Atrazine is persistent to direct photodegradation in water ($t_{1/2}$= 335 days) under natural sunlight (MRID 42089904; 40431320). Aqueous photodegradation products of atrazine include 2-chloro-4-isoproylamino-6-amino-s-triazine(DEA),chlordiamino-s-triazine(DACT), and 2-chloro-

6-ethylamino-4-amino-s-triazine (DIA), 2-hydroxy-4-isopropylamino-6-amino-s-triazine (HA), 2-hydroxy-6-ethylamino-4-amino-s-triazine (DIHA), and 2-hydroxy-4-isopropylamino-6-amino-s-triazine (DHEA). (Degradation product structures are presented in Appendix L). Similarly, atrazine is moderately persistent ($t_{1/2=}$ 12 to 45 days) to photodegradation on soil under natural light (MRID 40431320; 42089905). Soil photodegradation products of atrazine include DEA, DACT, and DIA.

Atrazine is persistent ($t_{1/2}$= 146 days) in aerobic mineral soils (MRID 40629303, MRID 40431321, MRID 42089906). Aerobic soil metabolism degradation products include DEA, DACT, DIA, HA, DIHA, DHEA. Atrazine is also persistent in anaerobic aquatic ($t_{1/2}$ = 608 days) and anaerobic soil ($t_{1/2}$= 159 days) environments (MRID 40431323, MRID 40431321, MRID 42089906). Anaerobic degradation products of atrazine include DEA, DACT, DIA, HA, DIHA, DHEA.

Soil sorption coefficients for atrazine are low (K_f= 0.427-2.00 (1/n=0.72-1.06). The average Koc= 100.475 ml/g-OC; N=4) (MRID 40431324), which indicates a FAO mobility classification of mobile in soil.

Field dissipation studies show atrazine dissipation is dependent on microbial-mediated degradation, runoff, and leaching. The half-life of atrazine in six field studies in CA, GA, and MN ranged from 12.75 to 261 days in corn planted soil and 38.52 to 261 days in fallow soil (MRID 42165504; 42165505, 40431336, 42165506, 40431337, 42165507, 40431339, 42165508, 40431339, 42165508, 40431338, 42165509). Microbial degradation is an important route of dissipation in the cited field studies. Although atrazine leaching or runoff is not clearly shown in the field studies, atrazine dissipation is dependent on runoff (Acc. Nos. 00023543, 00027118, 00027124, 00027123, 00027119) and leaching (Spalding *et al*. 1980; Junk *et al.* 1980; Spalding *et al.* 1979). The half-life in four long-term field dissipation studies in MN and CA ranged from 102-402 days (MRID 40431338, MRID 42089909, 40431336, 42089910, 40431339, 42089911, 40431337, 42089912). Degradation products in the studies include HA, DEA, and DIA. Concentrations of atrazine and its degradation products DEA, HA, and DIA were detected with soil depth in long-term field dissipation studies.

In forestry field studies in Oregon, atrazine was detected on leaf surfaces, leaf litter, and soil. The half-life of atrazine was 87 days for exposed soil, 13 days on foliage and 66 days in leaf litter (MRID 40431340, 42041405).

4.1.2. Degradation Products

Degradation products of atrazine are shown in **Table 5**. There are two major types of degradation products for atrazine. The first type of degradation products are formed through dealkylation of the amino groups. The second type of degradation products are formed through substitution of a chlorine by a hydroxy group. These degradation products can be formed

through abiotic and microbial-mediated processes. Two of these degradation products, DIA and DACT, are also degradation products of simazine. In addition, DACT is also a degradation product of cyanazine. Structures of the degradation products are shown in **Appendix L**.

Table 5. Chemical Names for Atrazine Degradation Products

Common Name	Chemical Name	Chemical Formula	CAS Reg No.	Synonyms
Deisopropylatrazine	2-chloro-6-ethylamino-4-amino-s-triazine	$C_5H_8ClN_5$	1002-28-9	CEAT/DIA/G-28279
Deethylatrazine	2-chloro-4-isopropylamino-6-amino-s-triazine	$C_6H_{10}ClN_5$	6190-54	CIAT/DEA/G-30033
Hydroxyatrazine	2-hydroxy-4-isopropylamino-6-ethylamino-s-triazine	$C_8H_{15}N_5O$	2163-80	OIET/HA/G-34048
Diadealkylatrazine	chlordiamino-s-triazine	$C_3H_4ClN_5$	3397-62-4	CAAT/DACT/DDA/ GS-28273
Deisopropylhydroxyatrazine	2-hydroxy-6-ethylamino-4-amino-s-triazine	$C_5H_9N_5O$	7313-54-4	OEAT/DIHA/GS-17792
Deethylhydroxyatrazine	2-hydroxy-4-isopropylamino-6-amino-s-triazine	$C_6H_{11}N_5O$	----------	OIAT/DHEA/GS-17794

Dethyl-atrazine (DEA; G-30033) and deisopropyl-atrazine (DIA; G-28279) were detected in all laboratory and field studies (**Table 6**); hydroxy-atrazine (HA; G-34048) was detected in all studies except for the photodegradation on soil study; and diaminochloro-atrazine (DACT; G-28273) was detected in all studies except for the aquatic metabolism studies. Deethylhydroxy-atrazine (DEHA; GS-17794) and deisopropylhydroxy-atrazine (DIHA; GS-17792) were also detected in the photodegradation in water, aerobic soil metabolism, and anaerobic soil metabolism studies. For studies limited to several months, the relative concentrations of the degradation products in soil were generally DEA>DIA>DACT~HA.

Table 6. Identification of Atrazine Degradation Products in Environmental Fate Studies			
Study	**Degradation Products**	**Maximum Percentage of Applied Atrazine**	**References**
Hydrolysis	None	None	MRID 40431319
Photodegradation in Water	DACT	15	MRID 42089904 MRID 00024328
	DEA	16	
	DIA	5	
	DIHA	0.22	
	HA	1.19	
	DHEA	0.27	
Photodegradation on Soil	DACT	18.3	MRID 40431320 MRID 42089905
	DEA	14.5	
	DIA	7.9	
Aerobic Soil Metabolism	DACT	0.317	MRID 40629303 MRID 42089906
	DEA	4.18	
	DIA	1.61	
	DIHA	0.410	
	HA	4.20	
	DHEA	0.774	
Anaerobic Soil Metabolism	DACT	0.3	MRID 40629303 MRID 42089906
	DEA	2.1	
	DIA	0.74	
	DIHA	0.22	
	HA	1.22	
	DHEA	0.44	
Anaerobic Aquatic Metabolism	DEA	4.7	MRID 40431323 MRID 46338702
	DIA	1.4	
	HA	12.4	
Aerobic Aquatic Metabolism	DEA	2.9	MRID 46338702
	DIA	0.4	
	HA	14.8	

The mobility of atrazine degradation products can range from low to high mobility in soil. The chloro-triazine degradation products are expected to exhibit higher mobility than the hydroxyl-triazine degradation products because they have lower partition coefficients (**Table 7**).

Table 7. Soil Sorption Coefficients for Atrazine Degradation Products				
Degradation Product	**K_f**	**K_d**	**K_{oc}**	**References**
DACT	0.16-1.56	0.108-0.800	30.65-75.96	MRID 41257904 MRID 40431327 MRID 40431333
DIA	0.16-2.7	0.422-6.08	35.1-82.3	MRID 41257906 MRID 40431331 MRID 40431325
DEA	0.06-1.02	0.116-0.963	12.15-44.90	MRID 41257906 MRID 40431334
HA	1.98-389.6	1.643-8.165	38.50-155.34	MRID 41257902 MRID 40431332 MRID 40431326

4.2. Surface Water Monitoring

Characteristics of representative monitoring programs for atrazine from 1975 to 2011 are shown in **Table 8.** Additional atrazine surface water monitoring data prior to 2003 has been previously analyzed in the USEPA IRED (2003b). As expected, the programs vary regarding their objective and monitoring strategy. Several of the programs such as the National Water-Quality Assessment Program (NAWQA), California Surface Water Monitoring Program (CSW), Iowa Ambient Monitoring Program, USGS-EPA Pilot Monitoring Program, Heidelberg University National Center of Water Quality (NCWQR) were developed to assess general pesticide occurrence in ambient surface water. In contrast, other monitoring programs such as the Nebraska State Surface Water Monitoring Program, Kansas State Surface Water Monitoring, Wisconsin State Surface Water Monitoring Program, Minnesota State Monitoring Program, Montana State Monitoring Program, and Syngenta Atrazine Ecological Monitoring Program (AMP), monitoring programs were targeted to atrazine use areas. These monitoring programs provide atrazine occurrence data from 1975-2011 (36 years) across 48 contiguous states. The number of sampling stations varied from six stations in NCWQR monitoring program to 2209 sampling stations in the USGS-NAWQA monitoring program.

Table 8. Characteristics of Representative Monitoring Programs for Atrazine and Its Degradation Products in Surface Water

Study	Number of States	Number of Sampling Stations	Years	Targeted Monitoring	Surface Water Type	Reported LOD (µg/L)	Degradates Analyzed
NAWQA	48	2209	1991-2011	No	Ambient	≤0.16	Yes
CSW	1	474	1991-2011	No	Ambient	≤ 4.76	Yes
Iowa	1	175	2003-2006	No	Ambient	0.05	No
Nebraska	1	232	2001-2006	Yes	Ambient	≤ 0.3	No
Minnesota	1	9	1993-2007	Yes	Ambient	0.05	No
Montana	1	25	2006-2008	Yes	Ambient	0.0022	Yes
Kansas Streams	1	393	1977-2008	Yes	Ambient	<6.3	No
Kansas Lakes	1	284	1975-2008	Yes	Ambient	<6.3	No
Wisconsin	1	8	2008	Yes	Ambient	0[1]	No
USGS-EPA Reservoir	12	20	1999-2000	No	Ambient Finished	<0.009	Yes
NCWQR	1	6	1983-2008	No	Ambient	0[1]	No
AEEMP	12	74	2004-2011	Yes	Ambient	<0.05	No
AMP	13	250-Raw 204-Finish	2003-2011	Yes	Ambient Finished	0.05	Yes
PDP	26	61	2004-2009	No	Ambient Finished	0.0066	Yes

1-LOD was reported as zero in data.

A key difference among the monitoring programs is the annual sampling frequency at each sampling station. **Figure 4** provides a comparison of cumulative probability distributions of site-year sampling frequencies among the monitoring programs. With the exception of the NCWQR

monitoring program, the non-targeted atrazine monitoring programs had lower site-year sampling frequencies than the NCWQR. The 90th percentile annual sampling frequency for the non-targeted monitoring programs ranged from 2 to 147 days. The sampling frequency is an important consideration in quantitative interpretation of the pesticide concentrations from monitoring data as well as the development of reliable chemographs (USEPA, 2011a).

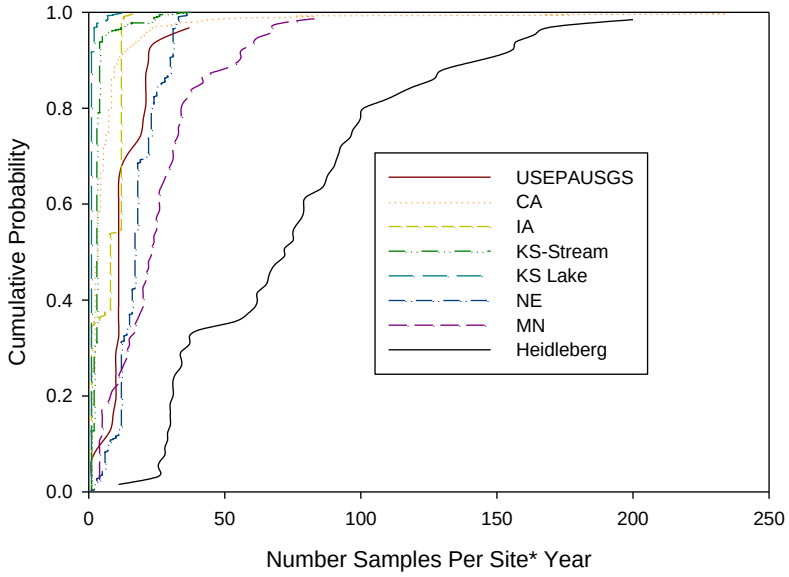


Figure 4. Distribution of Sampling Frequencies for Atrazine Surface Monitoring Programs

4.2.1. Monitoring Data Analysis

The monitoring data for atrazine and its degradation products were analyzed by site-year. This strategy was employed because pesticide occurrence is dependent on the spatially-dependent site conditions including pesticide use, agronomic practices, soil properties, meteorology, *etc*., as well as temporally-dependent conditions, including pesticide application timing and rainfall occurrence. Additionally, the site-year strategy provides a quantitative analysis of the data used in developing annual atrazine chemographs for the PATI model. An annual atrazine chemograph is generated by stair-step imputation between measured values for specific monitoring site-years. The chemograph, therefore, provides an annual daily time series of atrazine concentration, which can be used for calculating time-weighted mean concentrations.

The monitoring data were analyzed using a macro in an Excel spreadsheet. The data were analyzed for site-year statistics, including number of samples, number of non-detections, arithmetic annual average concentration, time-weighted annual mean, maximum 60-day mean concentration, and annual maximum daily concentration. Time-weighted means were derived

from a chemograph constructed on 365-day calendar year. A stair-stepping imputation was used between the measured values. The tails at the beginning and end of the site-year were assumed to equal the lowest detectable concentration in the NAWQA (0.16 µg/L) until the first or last measured value in the year. The lowest detection in NAWQA was used a conservative estimate of detectable concentrations of atrazine. Statistical analyses for atrazine were limited to site-years with 4 or more samples. This restriction was used because it is the minimum sampling frequency for assessing atrazine rolling average concentrations for Human Health Maximum Contaminant Levels. The descriptive statistics for degradation products of atrazine and atrazine residues in groundwater were described using detection frequency and maximum concentrations.

4.2.2. Ambient Surface Water Monitoring Programs

The atrazine monitoring data illustrates that the detection frequencies of atrazine concentrations in ambient water samples range from 0.28% to 94.32% (**Table 9**). For the purpose of the analysis, ambient surface water is defined as surface water from flowing water (rivers and streams), reservoirs, ponds, lakes, and ditches as well as raw surface source water from community water systems (CWS). As expected, the highest detection frequencies are associated with monitoring sites in corn production states such as Iowa, Nebraska, Minnesota, Ohio (NCWQR), and Kansas. The maximum daily concentration and annual average concentration of atrazine from all ambient surface water data are reported at 683.4 µg/L and 31.80 µg/L, respectively. These concentrations are associated with a monitoring site in the Nebraska (Site ID SLB2TLSNDY60).

Table 9. Descriptive Statistics of Atrazine Concentrations In Ambient Surface Water Monitoring Programs

Monitoring Program	Detection Frequency	Maximum Daily Peak	Maximum 60 Day Mean	Maximum Time Weighted Annual Mean
	%	µg/L		
NAWQA	82.51	201	54.3	11.63
CSW	0.28	5.3	1.8	0.3
Iowa	70.25	16.3	12	2.3
Nebraska	64.36	683.4	192	31.8
Minnesota	86.64	32	2.5	1.3
Montana	7.27[a]	0.0022	NA	NA
Kansas	57.71	105	61.8	11.2
Wisconsin	75.12	21.2	19.2	8.8
NCWQR	85.28	54.4	10.9	2.2
USGS-EPA Reservoir	84.77	11.6	6.1	1.9
AEEMP	94.32[a]	237.5	49.3	8.2
AMP	88.21[a]	227	39.9	9.6
PDP	75.57[a]	4.2	2.8	1.1

a-Detection frequencies were not limited to site-years with 4 or more samples.

Occurrence of atrazine degradation products were derived from the NAWQA, Atrazine Monitoring Program (AMP), USGS-EPA, California (CSW), Montana, and USDA Pesticide Data Program (PDP) monitoring programs. Because the AMP, USDA PDP, USGS-EPA monitoring programs are focused on pesticide concentrations in drinking water, raw water samples from these programs are used to represent occurrence patterns of the atrazine degradation products in ambient surface water. The chemical names for the atrazine degradation products are shown in Table 10. The monitoring data shows high detection frequencies of atrazine degradation products (DEA, DIA, DACT, DHEA, HA) in ambient surface water. The lowest detection frequencies of atrazine degradation products were found in the Montana Ambient Monitoring and California Surface Water- Ambient Water (CSW) program. This frequency is probably associated with lower atrazine use in these areas when compared to other monitoring programs. The maximum concentrations of atrazine degradation products decreased in the following order: DDA-DEA> DIA> ACT>OEIT>DHEA. The occurrence pattern is probably associated with the relative mobility of the chlorinated (DEA, DIA, DACT) and non-chlorinated (HA, DHEA) degradation products. The maximum occurrence concentrations of the degradation products are orders of magnitude lower than parent atrazine in ambient surface water.

Table 10. Descriptive Statistics of Atrazine Degradation Products in Ambient Surface Source Water Monitoring Programs

Monitoring Program	Degradation Products	N	Detection Frequency	Maximum Concentration
			%	µg/L
NAWQA-Ambient Water	DEA	31830	69.03	10>
	DIA	4360	38.62	4.44
	DACT	1849	21.03	0.6249
	DHEA	2	100	0.11
	HA	17	11.76	0.3
USGS-EPA Reservoir	DEA	383	78.85	0.577
	HA	381	81.10	2.17
	DIA	383	79.89	0.386
	DACT	383	71.54	0.514
	HA	381	81.10	2.17
AMP – Raw Water	DEA	27354	54.80	8.8
	DIA	27357	37.73	4.79
	DACT	27354	2.66	8.80
Montana-Ambient Water	DEA	55	0	NA
	DIA	55	0	NA
	HA	55	0	NA
CSW-Ambient Water	DEA	72	0	NA
	DIA	751	0.399	0.021
	DACT	466	2.36	0.672
PDP	DEA	2104	66.40	1.078
	DIA	2105	44.09	0.776
	HA	927	68.40	0.48

4.2.3. Finished Surface Source Drinking Water Monitoring Programs

Occurrence of atrazine and its degradation products in finished drinking water were derived from data in the AMP, USGS-EPA, and the USDA PDP monitoring programs (**Table 11**). The atrazine residue concentrations in drinking water are reported without consideration of water treatment processes. Activated carbon has been found to be the best available technology (BAT) to reduce atrazine concentrations in finished drinking water. Although the detection frequencies of atrazine residues in finished water are comparable to occurrence concentrations in ambient surface water, the maximum concentrations of atrazine and its degradation products in finished drinking water are generally lower than in ambient surface waters.

Table 11. Descriptive Statistics of Atrazine and its Degradation Products in Finished Surface Source Water Monitoring Programs

Monitoring Program	Atrazine Residues	N	Detection Frequency %	Maximum Concentration µg/L
USGS-EPA Reservoir	Atrazine	225	82.22	1.94
	DEA	224	74.55	0.267
	DIA	225	64.89	0.178
	DACT	225	62.66	0.0826
	HA	224	81.25	1.79
AMP	Atrazine	34215	76.06	59.57
	DEA	26488	54.80	4.84
	DIA	26488	37.73	2.61
	DACT	20037	2.66	2.23
PDP	Atrazine	2105	76.05	2.733
	DEA	2105	62.57	0.865
	DIA	2105	40.67	0.469
	HA	929	68	0.37

4.2.4. Ground Water Monitoring Programs

Occurrence of atrazine and it degradation products in groundwater were taken from the NAWQA, Montana State monitoring program, and the USDA PDP Program (**Table 12**). Although atrazine and its degradation products were detected in all the monitoring programs, the detection frequencies of atrazine residues in ground water were lower than ambient surface water. Additionally, the maximum concentration of atrazine (16.6 µg/L) in groundwater is substantially lower than maximum concentration detected in surface water (683.4 µg/L). As expected from laboratory mobility data, the more mobile chlorinated atrazine degradation products (DEA, DIA, DACT) were detected more frequently than the less mobile hydroxyl-substituted atrazine degradation products.

Table 12. Descriptive Statistics of Atrazine Concentrations from Representative Ground Water Monitoring Programs				
Monitoring Program	Atrazine Residues	N	Detection Frequency %	Maximum Concentration µg/L
NAWQA	Atrazine	13479	29.95	16.6
	DEA	13410	32.78	2.6
	DIA	2963	12.35	1.11
	DACT	1754	8.20	0.7632
	HA	52	9.62	0.81
	DIHA	15	0	<0.025
PDP	Atrazine	800	9.13	0.231
	DEA	800	12.75	1.55
	DIA	800	4.38	0.202
	HA	800	11.13	0.255
	DIHA	800	9	0.99
MT	Atrazine	2120	13.39	0.98
	DEA	1380	23	0.79
	DIA	1381	7.09	2.2
	DACT	154	35.71	1.3
	DHEA	154	0	NA
	HA	1376	8.21	2.5
	DIHA	154	0	NA

4.3. Atmospheric Monitoring

Atrazine has been detected in air, rain, snow, and fog samples (Majewski and Capel, 1995). Observed concentrations of atrazine range from 0.003 to 40 µg/L in rain, 0.000008 to 0.020 µg/L in air, 0.270 to 0.820 µg/L in fog, and 0.02 to 0.03 µg/L in snow. These detections in air, rain, snow and fog were said to have been associated with atrazine use areas and application timing.

5. Stressors of Concern

The residues of concern in an assessment include the parent compound, and may also include any degradate(s) that are observed at significant levels (>10% by weight relative to parent from available degradation studies), and/or determined to be of toxicological concern. Previous ecological risk assessments for atrazine have evaluated potential degradate(s) for inclusion in the risk assessment (USEPA 2009b). Based on those analyses, parent atrazine will be considered the stressor of concern. This conclusion is based on either available degradate toxicity data indicating that it is less toxic than parent atrazine, or on the proportion of the degradates expected to be in the environment and available for exposure relative to atrazine. In this case, parent atrazine is expected to be protective for non-target organisms that may be exposed to the parent and any of its degradates (USEPA, 2009b).

In its ecological risk assessments, the EPA does not routinely include a quantitative evaluation of mixtures of active ingredients, either those mixtures of multiple active ingredients in product formulations or those in the applicator's tank. In the case of the product formulations of active ingredients (that is, a registered product containing more than one active ingredient), each active ingredient is subject to an individual risk assessment for regulatory decision regarding the active ingredient on a particular use site. Effects data are available for atrazine formulated products that contain more than one active ingredient (**Appendix B;** USEPA 2009b), and as such the data may be used qualitatively or quantitatively in accordance with the EPA's Overview Document and the Services' Evaluation Memorandum (USEPA 2004; USFWS/NMFS 2004). Available toxicity data for environmental mixtures of atrazine with other pesticides will be presented as part of the ecological risk assessment. It is expected that the toxic effect of atrazine, in combination with other pesticides used in the environment, is likely to be a function of many factors, including but not necessarily limited to: (1) the exposed species, (2) the co-contaminants in the mixture, (3) the ratio of atrazine and co-contaminant concentrations, (4) differences in the pattern and duration of exposure among contaminants, and (5) the differential effects of other physical/chemical characteristics of the receiving waters (*e.g.* organic matter present in sediment and suspended water). Quantitatively predicting the combined effects of all these variables on mixture toxicity to any given taxa with confidence is beyond the capabilities of the available data and methodologies. However, a qualitative discussion of implications of the available pesticide mixture effects data on the confidence of risk assessment conclusions will be addressed as part of the uncertainty analysis.

6. Evaluation of Atrazine Toxicity to Specific Taxa

Consistent with the process described in the Overview Document (USEPA 2004), the risk assessment for atrazine relies on a surrogate species approach. Toxicological data generated from surrogate test species, which are intended to be representative of broad taxonomic groups, are used to extrapolate the potential effects on a variety of species included under these taxonomic groupings.

Acute and chronic toxicity data from single-species studies submitted by pesticide registrants along with the available open literature will be used to evaluate the potential direct and indirect effects of atrazine to aquatic and terrestrial species, including sublethal effects that can be directly linked to survival, growth, or fecundity. These data include toxicity on the technical grade active ingredient, degradates, and when available, formulated products (*e.g.*, "Six-Pack" studies). The open literature studies are identified through EPA's ECOTOXicology (ECOTOX 2007c) database, which employs a literature search engine for locating chemical toxicity data for aquatic life, terrestrial plants, and wildlife. The evaluation of both sources of data may also provide insight into the direct and indirect effects of atrazine on biotic communities from loss of species that are sensitive to the chemical and from changes in structure and functional characteristics of the affected communities. Several ECOTOX runs have been conducted over

the years prior to this problem formulation (2003, 2004, 2006, 2007, 2008, and 2011). However, if an additional ECOTOX search is conducted prior to the writing of the atrazine Registration Review risk assessment, this new information will also be evaluated for possible quantitative and/or qualitative data and when appropriate, will be included in the risk assessment in support of Registration Review.

Assessment endpoints are defined as "explicit expressions of the actual environmental value that is to be protected" (USEPA 1992). Selection of the assessment endpoints is based on valued entities (*e.g.,* birds, fish and aquatic plants), organisms important in the life cycle of ecological receptors, the primary constituent elements (PCEs) of designated critical habitat for listed species, the ecosystems potentially at risk (*e.g.,* waterbodies, riparian vegetation, and upland and dispersal habitats), the migration pathways of atrazine (*e.g.,* runoff, spray drift, *etc*.), and the routes by which ecological receptors are exposed to atrazine *(e.g.,* direct contact, *etc.).*

As described in EPA's Overview Document (USEPA, 2004), the assessment endpoints for pesticide risk assessments are growth, reproduction, and survival of species. For this assessment, evaluated taxa include aquatic-phase amphibians, freshwater and saltwater fish, freshwater and saltwater invertebrates, aquatic plants, birds (surrogate for terrestrial-phase amphibians), mammals, terrestrial invertebrates, and terrestrial plants. Acute (short-term exposure) and chronic (long-term exposure) toxicity information is characterized based on registrant-submitted studies and a comprehensive review of the open literature on atrazine and its degradates.

A summary of the data to be used for quantitative and qualitative risk assessment for non-target species and communities exposed to atrazine in aquatic and terrestrial habitats is provided in this section. See **Appendix B** for a complete list of submitted "Acceptable" and "Supplemental" studies).

6.1. Toxicity to Plants

6.1.1. Toxicity to Terrestrial Plants

Plant toxicity data from both registrant-submitted studies and studies in the scientific literature were reviewed for this assessment. Registrant-submitted studies are conducted under conditions and with species defined in EPA toxicity test guidelines. Sub-lethal endpoints such as plant growth, dry weight, and biomass are evaluated for both monocots and dicots, and effects are evaluated effects at both seedling emergence and vegetative life stages. A guideline study generally evaluates toxicity to ten crop species. A drawback to these tests is that they are conducted on herbaceous agricultural crop species only, and extrapolation of effects to other species, such as woody shrubs and trees and wild herbaceous species, contributes uncertainty to risk conclusions. Preliminary data (discussed below) suggests that sensitive woody plant

species exist. However, since atrazine is labeled for use in forestry production effects to many types of trees are not expected at concentrations anticipated in the environment.

Commercial crop species have been selectively bred and may be more or less resistant to particular stressors than wild herbs and forbs. The direction of this uncertainty for specific plants and stressors, including atrazine, is largely unknown. Homogenous test plant seed lots also lack the genetic variation that occurs in natural populations, so the range of effects seen from tests is likely to be smaller than would be expected from wild populations.

Based on the results of the submitted terrestrial plant toxicity 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 carrot and the most sensitive monocot is oat. EC_{25} values, on an equivalent application rate basis, for oats and carrots, which are based on a reduction in dry weight, are 0.003 and 0.004 lb a.i./A, respectively; NOAEC values for both species are 0.0025 lb a.i./A. **Table 13** summarizes the most sensitive Tier II terrestrial plant seedling emergence toxicity data.

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 a.i./A. In contrast, two of the four tested monocots showed no effects from atrazine (corn and ryegrass), while EC_{25} values for oats and onion were 0.61 and 2.4 lb a.i./A, respectively. **Table 14** summarizes the most sensitive terrestrial plant vegetative vigor toxicity data used to derive risk quotients in this assessment.

Table 13. Nontarget Terrestrial Plant Seedling Emergence Toxicity (Tier II). Only endpoints in bold will be used quantitatively.					
Surrogate Species	**% ai**	**EC_{25} / 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	**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 - 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 (*Solanum lycopersicum*)	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 14. Nontarget Terrestrial Plant Vegetative Vigor Toxicity (Tier II). Only endpoints in bold will be used quantitatively.					
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	420414-02 Chetram 1989	Acceptable
Monocot - Oat (*Avena sativa*)	97.7	2.4 / 2.0	red. in dry weight	420414-02 Chetram 1989	Acceptable
Monocot - Onion (*Allium cepa*)	97.7	**0.61 / 0.5**	red. in dry weight	420414-02 Chetram 1989	Acceptable
Monocot - Ryegrass (*Lolium perenne*)	97.7	> 4.0 / > 4.0	No effect	420414-02 Chetram 1989	Acceptable
Dicot - Carrot (*Daucus carota*)	97.7	1.7 / 2.0	red. in plant height	420414-02 Chetram 1989	Acceptable
Dicot - Soybean (*Glycine max*)	97.7	0.026 / 0.02	red. in dry weight	420414-02 Chetram 1989	Acceptable
Dicot - Lettuce (*Lactuca sativa*)	97.7	0.33 / 0.25	red. in dry weight	420414-02 Chetram 1989	Acceptable
Dicot - Cabbage (*Brassica oleracea alba*)	97.7	0.014 / 0.005	red. in dry weight	420414-02 Chetram 1989	Acceptable
Dicot - Tomato (*Solanum lycopersicum*)	97.7	0.72 / 0.5	red. in plant height	420414-02 Chetram 1989	Acceptable
Dicot - Cucumber (*Cucumis sativus*)	97.7	**0.008 / 0.005**	red. in dry weight	420414-02 Chetram 1989	Acceptable

In addition, a report on the toxicity of atrazine to woody plants (Wall *et al.*, 2006; MRID 46870401) was reviewed by the EPA. A total of 35 species were tested at application rates ranging from 1.5 to 4.0 lbs a.i./A. Twenty-eight species exhibited either no or negligible phytotoxicity. Seven of 35 species exhibited >10% phytotoxicity. However, further examination of the data indicates that atrazine application was clearly associated with severe phytotoxicity in one species (Shrubby Althea). These data suggest that, although sensitive woody plants exist, atrazine exposure to most woody plant species at application rates of 1.5 to 4.0 lbs a.i./A is not expected to cause adverse effects. A summary of the available woody plant data is provided in **Appendix B**.

6.1.2. Toxicity to Aquatic Non-Vascular Plants

The following two toxicity sections (6.1.2 and 6.1.3) are organized based on the taxonomic groups shown in **Figure 5** and are representative of closely related taxa. These sections are followed by Section 6.1.4, which describes the toxicity information available from microcosm and mesocosm studies, including the breadth of this diversity in the studies. Sections 6.1.2 and

6.1.3 represent the toxicity to individual species, whereas Section 6.1.4 represents the toxicity of atrazine to the aquatic plant communities found in North America. Although the toxicity information is presented in separate sections (single species tests vs. cosm studies), the data represent the effects of atrazine on aquatic autotrophic species and communities of aquatic plants, and are considered of equal importance in the risk characterization.

The category of "Aquatic Non-Vascular Plants" is representative of a broad diversity of unicellular and multicellular organisms. These include Eubacteria (*e.g.*, blue-green algae), Archaeoplastida (*e.g.*, red algae, glaucophytes, green algae, and aquatic bryophytes), Chromalveolates (*e.g.*, aveolates, cryptomonads, dinoflagellates, diatoms, water molds, and brown algae), Excavates (*e.g.*, euglena), and the Unikonts (*e.g.*, fungi, and collared-flagellates) except the "Animals" lineage.

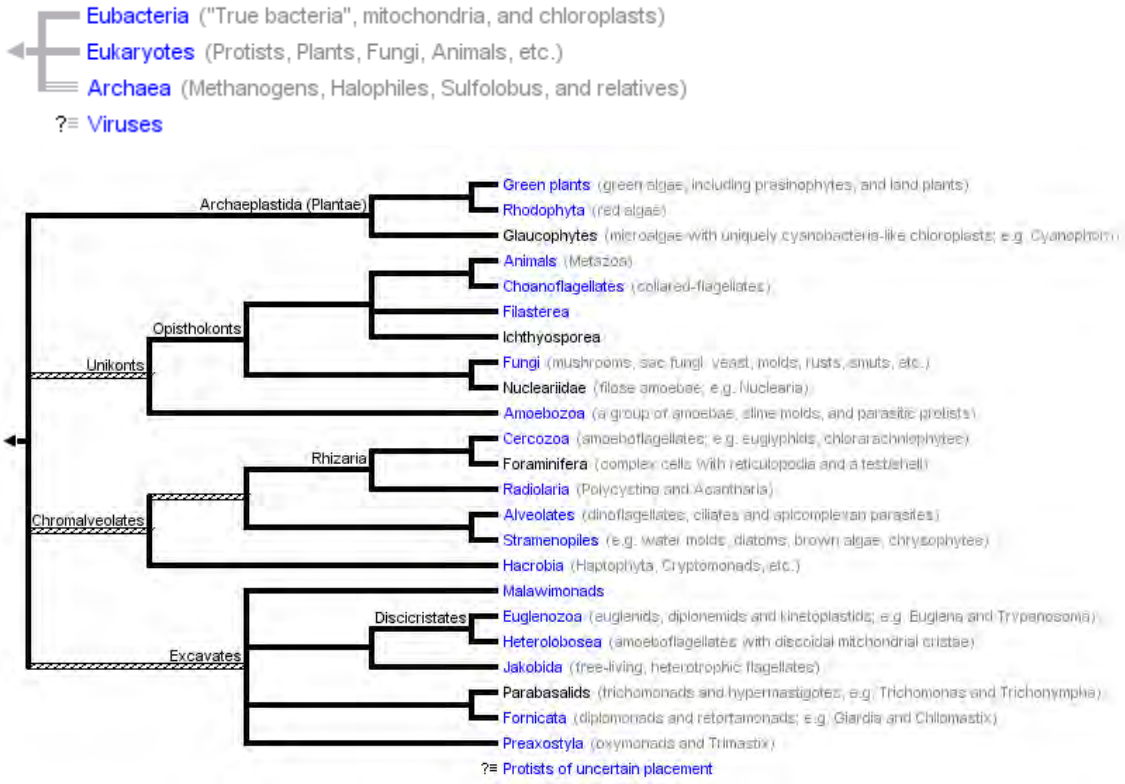


Figure 5. The taxonomy followed in this problem formulation is based on the information available at the Tree of Life Web Project (http://tolweb.org/tree/) and is consistent with current understandings of the relationships between these taxa.

Single-species aquatic plant toxicity studies will be used as the foundation for evaluating whether atrazine may affect primary production and diversity in aquatic ecosystems (see

section 13 for further explaination). Numerous aquatic non-vascular plant toxicity studies have been submitted to EPA and/or have been published in the open literature (**Appendix B**; USEPA 2007c). A summary of the most sensitive endpoints for freshwater non-vascular plants is provided below; **Appendix B** includes a more comprehensive list of the available data. The most sensitive single species data for aquatic non-vascular plants from either supplemental or acceptable studies (**Table 15**) will be used for risk characterization purposes only and will not be used for quantitative purposes in risk quotient calculation.

Table 15. Summary of the most sensitive aquatic non-vascular plant toxicity endpoints available from the registrant submitted studies and the open literature.								
Taxonomic Group		**Number of Families Tested**	**Number of Genera Tested**	**Number of Species Tested**	**Minimum ED/IC/EC_{50} Endpoint[1]**	**FW/SW and Duration[2]**	**Species and Effect Used for Reported Endpoint**	**Citation (MRID)**
EUBACTERIA:	CYANOBACTERIA: (Blue-Green Algae)	5	14	29	EC_{50} <1 µg/L	FW 7 days	*Oscillatoria lutea* 93% reduction of chlorophyll production	Torres and O'Flaherty 1976 (000235-44)
EUKARYOTES:								
ARCHAEOPLASTIDA	**GREEN PLANTS:**							
	EMBRYOPHYTA: (Non-Vascular Land Plants)	1	1	1	EC_{50} < 2 µg/L	FW 24 hrs	*Fontinalis hypnoides* 90% reduction in photosynthesis	Hoffman and Winkler 1990
	EMBRYOPHYTA: (Vascular Land Plants)	21	30	42	EC_{50} = 0.001 µg/L	FW 14 days	*Elodea canadensis* 50% reduction in biomass	McGregor et al. 2008
	CHLOROPHYTA and STREPTOPHYTA[3]: (Green Algae)	16	26	34	EC_{50} < 1 µg/L	FW 7 days	*Stigeoclonium tenue* 67% reduction in chlorophyll production	Torres and O'Flaherty 1976 (000235-44)
	PRASINOPHYTA: (Prasinophytes)	2	3	4	IC_{50} = 14.2 µg/L LOAEC = 1.1 µg/L	SW 4 hours	*Nephroselmis pyriformis* 50% photoinhibition	Magnussun et al. 2010

	RHODOPHYTA: (Red Algae)	2	2	2	EC_{50} = 79 µg/L	SW 72 hrs	*Porphyridium cruentum* 50% reduction in oxygen production	Mayer 1986 (402284-01)
CHROMALVEOLATES	**HACROBIA:**							
	HAPTOPHYTA: (Coccolithophorads)	2	2	2	EC_{50} = 30 µg/L	SW 72 hrs	*Isochrysis galbana* 50% growth inhibition	Debelius et al. 2008
	CRYPTOPHYTA: (Cryptomonads)	2	3	5	EC_{50} = 22.17 µg/L NOAEC < 12.5 µg/L	SW 96 hrs.	*Storeatula major* 50% reduction in abundance	DeLorenzo et al. 2004
	STRAMENOPILES:							
	BACILLARIOPHYTA: (DIATOMS)	17	22	46	EC_{50} = 19.4 µg/L	SW 48 hrs.	*Bellerochea polymorpha* 50% reduction in population growth	Walsh et al. 1988
	PHAEOPHYTA: (Brown Algae)	1	1	2	LOAEC = 10 µg/L NOAEC < 1 µg/L	SW > 18 days	*Laminaria hyperborea* growth reduction	Hopkin and Kain 1978
	CHRYSOPHYTA: (Golden Algae)	3	3	4	EC_{50} = 77 µg/L	SW 1 hr	*Monochrysis lutheri* 50% reduction in oxygen evolution	Hollister and Walsh 1973
	XANTHOPHYTA: (Yellow-Green Algae)	3	3	4	EC_{50} = 185 µg/L	SW 72 hrs	*Nannochloropsis gaditana* 50% total fluorescence inhibition	Debelius et al. 2008
	AVEOLATES:							

	PYRROPHYCOPHYTA (Dinoflagellates):	4	5	5	EC_{50} = 17.19 µg/L NOAEC < 12.5 µg/L	SW 96 hrs.	*Amphidinium operculatum* 50% reduction in total biovolume	DeLorenzo et al. 2004
	CILIOPHORA: (Ciliates)	2	2	2	ED_{50} = 5.83 µg/L	FW 24 hrs	*Tetrahymena pyriformis* 50% reduction in survival	Toth & Tomasovicova 1979
EXCAVATES	**EUGLENOZOA:** (Euglenoids)	1	1	1	496 µg/L	FW 7 days	*Euglena gracilis* 50% inhibition of photosynthesis	Thuillier-Bruston et al. 1996

[1]These endpoints were collected over different exposure periods.
[2]FW= fresh water, SW = salt water
[3]The Embryophytes are treated separately here.

Eubacteria: Toxicity data from studies on Cyanobacteria (Cyanophyaceae) span three orders and include six species: the Oscillatoriales (*Oscillatoria lutea*), the Nostocales (*Anabaena cylindrica; A.inaequalis; A. variabilis; A. flos-aquae*), and the Chroococcales (*Microcystis aeruginosa*). The lower 95% confidence interval on the overall EC_{50} data in the ECOTOX database (as prepared for the Environmental Fate and Effects Division) is 13.6 µg/L. The most sensitive endpoint from these open literature data reports a 93% reduction in chlorophyll production at <1 µg/L in *O. lutea* in a 7-day study (**Table 15**). In another study (Stratton 1984) using *A. inaequalis*, the most sensitive endpoint was reduced biomass (measured as cell count) followed by reduced growth rate and lastly by reduced photosynthesis. This pattern of reduced biomass as the most sensitive measured endpoint was reflected in several other studies on cyanobacteria (**Appendix B).**

Archaeoplasida (Embryophyta): Under the broad category "Non-Vascular Aquatic Plants" the Bryophyta (mosses, liverworts and hornworts) are the only group that is represented (**Table 15**). All other Embryophyta taxa are represented in either aquatic or terrestrial vascular plant sections of this problem formulation. The available toxicity data for bryophyta does not report an EC_{50}; however, an EC_{90} of 2 µg/L is reported for the species *Fontinalis hypnoides* based on reduced photosynthesis (Hoffmann and Winkler 1990). This study also reports morphological effects to the structural composition of chloroplasts and leaf blade cellular structure at 2 and 10 µg/L, respectively.

Archaeoplasida (Chlorophyta and Streptophyta): This group of non-vascular plants is represented by 118 different studies in the toxicity literature, including 16 different families and and 34 species of both marine and freshwater environments (**Table 15**). The lower 95% confidence interval on the overall EC_{50} data in the ECOTOX database is 20.0 µg/L. The most sensitive endpoint for the freshwater toxicity tests is based on a 67% reduction in chlorophyll production in *Stigeoclonium tenue* at <1 µg/L (Torres and O'Flaherty 1976). The authors also tested *Chlorella vulgaris* and report a 50% reduction in chlorophyll production at 1 µg/L. In another study by Kish (2004), the author reports a NOAEC of 0.012 µg/L for *Pithophora oedogonia* based on total chlorophyll. In the saline aquatic systems, this group of Archaeoplasida is less sensitive than their counterparts in freshwater. The most sensitive chlorophyte was reported by DeLorenzo *et al*. (2004), who showed a 50% reduction in chlorophyll production at 11.87 µg/L during a 96 hour test on *Ankistrodesmus sp*. DeLorenzo *et al*. (2011) report that significant reductions in various endpoints occur in *Dunaliella tertiolecta* when exposed to atrazine (a single dose of 100 µg/L) at elevated salinity (40 ppt), higher temperature (35^{o} C), and a combination of these factors than at typical conditions that were used in DeLorenzo *et al.* 2004.

Archaeoplasida (Prasinophyta): This group of non-vascular plants is represented by 5 different studies in the toxicity literature, including 2 different families and 4 species (**Table 15**). The most sensitive endpoint for the freshwater toxicity is EC_{50} of 34.3 µg/L based on reduced photosynthesis (Podola and Melkonian 2005). The most sensitive endpoint for saltwater taxa is an IC_{50} of 14.2 µg/L, based on 50% photoinhibition (Magnussun *et al*. 2010).

Archaeoplasida (Rhodophyta): The Rhodophyta (red algae) are represented in the toxicity literature by the *Porphyridium cruentum* (Bangiophyceae) (**Table 15**), which is the species from which the Japanese seaweed Nori is produced (Mayer 1986). This study reported a reduction in O_2 production, which reflects the decline in photosynthetic activity, at an EC_{50} of 79 µg/L. The data suggest that the red algae may be less sensitive to atrazine than the other Arcaeoplastida groups.

Chromalveolates (Hacrobia): These taxa are represented in the toxicological literature by 4 different families and 7 species (**Table 15**). The most sensitive reported endpoints come from the DeLorenzo *et al*. (2004) study that reported an EC_{50} of 22.17 µg/L based on reduced abundance but also report effects at the lowest concentration tested (12.5 µg/L). Similarly, Debelius *et al*. (2008) reported a 50 % growth inhibition at 30 µg/L.

Chromalveolates (Stramenopiles): The Stramenopiles are a highly diverse lineage of aquatic organisms that include diatoms, brown algae, golden-algae and yellow-green algae. They are represented by studies including 24 different families and 56 species of both marine and freshwater environments (**Table 15**). The available data suggests that these taxa have relatively similar toxicity to atrazine exposure. The most sensitive freshwater taxon, *Phaeodactylum tricornutum*, was reported to have a 50% inhibition of photosynthesis at 33.6 µg/L and effects at the lowest concentration tested, 4.5 µg/L (Magnussun *et al*. 2010). The most sensitive estuarine/marine taxon tested is a diatom with an EC_{50} of 19.4 µg/L based on population growth reduction (Walsh *et al*. 1988). In a study of atrazine toxicity to brown algae by Hopkin and Kain (1978), reproductive and sporophyte growth effects were reported at all concentrations tested (NOAEC < 1 µg/L).

Chromalveolates (Aveolates): This diverse lineage of aquatic microorganisms is represented in the toxicological literature by 4 different families and 5 species (Table 4.2). The most sensitive reported EC_{50} is 17.19 µg/L (NOAEC < 12.5 µg/L) based on reduction of total biovolume (DeLorenzo *et al*. 2004).

Chromalveolates (Ciliophora): Ciliates are represented in the toxicological literature by 5 studies on two species from different families (**Table 15**). The most sensitive reported endpoints come from the Toth and Tomasovicova (1979) study that reported ~ 50 % reduction in survival at 5.83 µg/L.

Excavates (Euglenozoa): The euglenoids are represented in the toxicological literature by only one study on *Euglena gracilis* (**Table 15**; Thuillier-Bruston *et al.* 1996). The authors report a 50% inhibition of photosynthesis at 496 µg/L.

Unikonts (Amebozoa): The Unikonts are a lineage that includes fungi, amoebae, collared-flagellates and animals (**Table 15**). There are a great number of studies on animals, which are discussed in **Section 6.2 o**f this problem formulation; however, only one aquatic single species test is available from the remainder of the Unikonts. This study on an amoeba reported an LD_{50} greater than 100 µg/L (Prescott and Olson 1977).

6.1.3. Toxicity to Aquatic Vascular Plants

Archaeoplasida (Embryophyta): Single-species aquatic plant toxicity studies will be used as one of the measures of effect to evaluate whether atrazine may affect primary production and diversity in aquatic ecosystems. Numerous aquatic vascular plant toxicity studies have been submitted to EPA and/or have been published in the open literature. **Appendix B** includes a more comprehensive list of the available data.

Freshwater vascular plants are as sensitive or more sensitive to atrazine as freshwater non-vascular plants, with the most sensitive vascular plant EC_{50} value of 0.001 µg/L, based on biomass reduction in *Elodea* (McGregor et al. 2008) (**Table 15**). The available estuarine/marine toxicity data for aquatic vascular plants show less sensitivity than from fresh water studies, with 50% mortality of *Vallisneria Americana* at 12 µg/L from a 47-day study (Correll & Wu 1982).

The most sensitive single species data for aquatic vascular plants from either supplemental or acceptable studies (**Table 15**) will be used for risk characterization purposes only and will not be used for quantitative purposes such as use in risk quotient calculation.

6.1.4. Toxicity to Aquatic Plant Communities

While reviewing this section, please consider the charge question below.

SAP Question:

- The cosms were comprised of natural communities of periphyton/phytoplankton; in some cases, vascular plants, invertebrates and vertebrates present in those communities were included in the study (Chapter I, Section 6.1.4). These sources were generally described as streams, lakes, reservoirs, and springs, and are considered to be representative of the structure and function of aquatic plant communities in such water bodies. Given the diversity of sources and the described communities, please comment on the extent to which these cosm studies taken together provide useful and reasonable physical models of the natural aquatic plant communities exposed to atrazine in the U.S.

In addition to reviewing the toxicity data for individual species, the toxicity of atrazine to aquatic plant communities is evaluated. Concentrations of atrazine that affect plant productivity and community structure typically occur at levels lower than those that directly intoxicate fish and aquatic invertebrates. This focus is required to ensure that the atrazine concentrations in watersheds do not cause significant changes in aquatic plant community structure and productivity and thus put at risk the food chain and entire ecosystem integrity. In

this approach single-species plant toxicity data and cosm studies (**Appendix D**) will be used to determine what atrazine exposure patterns and concentrations are likely to result in adverse effects to aquatic plant communities. From these data, a LOC will be developed, which together with monitoring data can be used to identify watersheds where atrazine levels need to be mitigated consistent with the 2003 Memorandum of Agreement (USEPA 2003b) signed by the EPA and Syngenta. While the LOC is based on effects to aquatic plant communities by ensuring protection of primary producers, it is intended to provide protection for the entire aquatic ecosystem including fish, invertebrates, and amphibians.

Potential effects of atrazine on plant communities have been evaluated using available cosm studies (**Appendix D**). Cosm studies conducted with atrazine provide measurements of primary productivity that incorporate the aggregate responses of multiple species in aquatic plant communities. Because plant species vary widely in their sensitivity to atrazine, the overall response of the plant community may be different from the responses of the individual species measured in laboratory toxicity tests. Cosm studies allow observation of population and community recovery from atrazine effects and of indirect effects on higher trophic levels. In addition, cosm studies, especially those conducted in outdoor systems, incorporate partitioning, degradation, and dissipation, factors that are not usually accounted for in laboratory toxicity studies, but that may influence the magnitude of ecological effects.

The review of the cosm studies (**Appendix D**) included the establishment of criteria for selection. First, all studies were prescreened. The screen requires that: (1) treatments were exposed to only atrazine, and not mixtures or multi-active ingredients, (2) exposure concentrations were reported, (3) measured effects were specific to aquatic plant communities (defined as two or more species), and (4) the study was written in English. If any of these four criteria were not met the study was no longer considered for use.

Studies that met the basic elements of the prescreen criteria were further screened using additional quality criteria. Criteria included basic elements such as use of controls and use of at least two replicates per treatment group. The accepted studies were then used as the basis for deriving the initial atrazine Level of Concern (LOC) (See **Chapter III** for complete details on the LOC Methodology). The acceptance criteria presented in Appendix D are intended to identify studies with confounding study design and performance elements to allow greater confidence in the study results. The criteria were derived using peer reviewed sources from U.S. EPA, SETAC (Society of Environmental Toxicology and Chemistry), and OECD (Organization for Economic Co-operation and Development) (Giddings *et al*. 1999; OECD, 2004; U.S. EPA, 2004).

A total of 35 cosm studies were originally included in the 2003 IRED (USEPA 2003c), and an additional 38 cosm studies were identified in the May 2009 SAP for a total of 73 studies. After the prescreening and acceptance criteria were applied to the 73 studies, 31 of the original 35 studies passed the screen and were presented in the IRED and 15 of the 38 studies recommended for consideration in the May 2009 SAP report (46 total studies). Citations of all 73 cosm studies considered can be found in **Appendix D**.

A total of 87 endpoints were used in the analysis to develop the level of concern for atrazine. These endpoints came from the 46 studies that passed the prescreening and acceptance criteria. Effects observed in the cosm studies included changes in aquatic plant biomass, chlorophyll *a* concentration, photosynthesis rate (^{14}C uptake, oxygen production), and shifts in aquatic plant community structure (e.g. species composition and diversity) relative to a control. The durations of these studies ranged from a few weeks to several years at constant or variable and declining exposure concentrations ranging from 0.1 µg/L to 10,000 µg/L. Most of the studies focused on atrazine effects on phytoplankton, periphyton, and macrophytes; however, some also included measurements on animals. Although most studies did not provide the identity of the phytoplankton, periphyton or zooplankton, those that did report it showed that a great diversity of taxa were tested (**Table 16**). The numbers provided in **Table 16** only reflect a subset of the microorganism diversity tested. Estimates from some studies suggest that there were 150-200 microorganism species present in a single mesocosm sourced from lake water (*e.g.,* Pratt *et al.* 1988). It is assumed that these studies represent natural communities and the breadth of diversity found in North American freshwater environments. A summary of all cosm endpoints used in the analysis is presented in **Appendix D**.

Table 16: The taxonomic distribution of reported species in COSM studies. See **Figure 5** and discussion in **Section 6.1.2** for representatives of these taxonomic groups and relationships between them. These numbers represent only approximations of those taxa that were identified to genera and/or species. **Appendix G.f.** contains details on which COSM studies contained these taxa.

Taxonomic Group		Genera	Species
EUBACTERIA:	CYANOBACTERIA: (Blue-Green Algae)	14	27
EUKARYOTES			
ARCHAEOPLASTIDA	**GREEN PLANTS:**		
	EMBRYOPHYTA: (Non-Vascular Land Plants)	-	-
	EMBRYOPHYTA: (Vascular Land Plants)	11	20
	CHLOROPHYTA and STREPTOPHYTA: (Green Algae)	43	86
	PRASINOPHYTA: (Prasinophytes)	1	1
CHROMALVEOLATES	**HACROBIA:**		
	HAPTOPHYTA: (Coccolithophorads)	2	4
	CRYPTOPHYTA: (Cryptomonads)	4	13
	STRAMENOPILES:		
	BACILLARIOPHYTA: (DIATOMS)	24	67
	CHRYSOPHYTA: (Golden Algae)	7	12
	XANTHOPHYTA: (Yellow-Green Algae)	2	2
	AVEOLATES:		
	PYRROPHYCOPHYTA (Dinoflagellates):	4	4
EXCAVATES	**EUGLENOZOA:** (Euglenoids)	1	1
UNIKONTS	**FUNGI:**	2	2
	CHOANOFLAGELLIDA:	3	3
	ANIMALS:		
	VERTEBRATES:	9	15
	INVERTEBRATES:	137	196

Effects in the cosm studies were scored using a binary effect/no effect score. In previous analyses, the cosm studies were assigned Brock scores, which is a 5-point effects scoring system. A Brock score of 1 was assigned to studies that did not produce an effect and a Brock score of 5 was assigned to studies that produced clear effects without recovery for 56 days or more. Studies with Brock scores of 1 (no effect) or 2 (slight or transient effect) were distinguished from studies assigned 3 (clear effect with recovery) or higher for the LOC analysis. Functionally, the binary effects scoring is identical to the manner in which Brock scores were used (Brock scores of 1 and 2 were considered no effects and Brock scores of 3 or higher were considered to be effects). However, in response to recommendations by the 2009 SAP (USEPA 2009a), all Brock scores were re-evaluated to ensure that each endpoint was categorized into the appropriate "effect" or "no effect" group. A binary effect/no effect system was considered to be more clear and transparent, which is the reason for adopting it for this analysis.

Recovery from the effects of atrazine and the development of resistance to the effects of atrazine in some vascular and non-vascular aquatic plant species have been reported in both single species studies and cosm experiments and may add uncertainty to these findings. However, reports of recovery are often based on differing interpretations. For the purposes of this assessment, recovery is defined as a return to pre-exposure levels for the *affected individual, population or community*, not for a replacement population or community of more tolerant species.

6.1.5. Biological Relevance: The Importance of Biodiversity and Plant Communities

"Biological diversity can be defined as the variety of life and its processes. This definition encompasses genetic, species, assemblage, ecosystem and landscape levels of biological organization and it has structural, compositional and functional components" (Hughes & Noss 1992). A recently published review of North American phytoplankton species richness (Stomp *et al.* 2011), based on the total number of species collected during surveys from 1973-1975 for the EPA National Eutrophication Survey, shows that phytoplankton diversity is greatest (**Figure 6**) throughout the southeastern and isolated areas of the Midwest. This survey, while pointing out that there is higher phytoplankton diversity in the regions with atrazine use, does not answer critical questions regarding community structure, function and food web stability, nor does it address if the sampled lakes were previously exposed to atrazine or if the species present are known to be tolerant to atrazine.

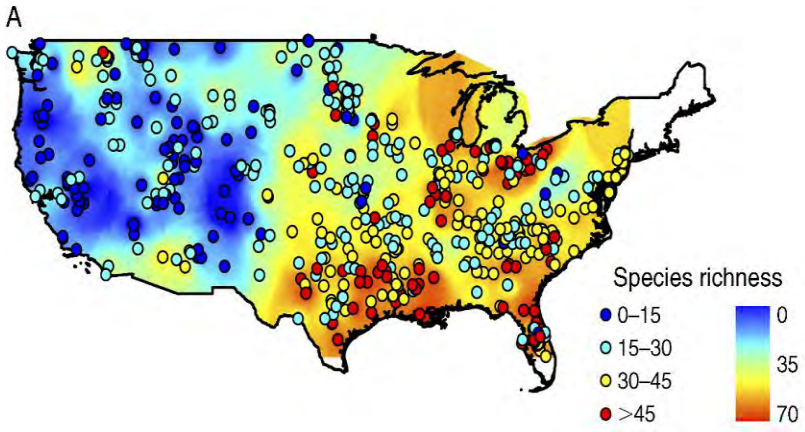


Figure 6. Geographical distribution of phytoplankton species richness across the continental United States (Stomp et al. 2011, reproduced with permission).

The complexity of species coexistence in phytoplankton communities is thought to be directly related to primary productivity (Leibold 1996), light availability (Huisman *et al.* 2004; Steinman 1992; Hill *et al.* 1995), and nutrient supply ratios (*e.g.*, Nitrogen:Phosphorus ratios, Tilman 1982). Atrazine has the potential to affect all three of these factors and thus have a negative impact on the primary producers, their associated food webs, and overall ecosystem function and integrity. Negative impacts leading to instability or harm to the aquatic plant communities would impact aquatic and terrestrial species, which are supported by the aquatic plant communities for their growth, survival, and reproduction in a complex network of interactions.

Decreased diversity may lead to increased nutrient and toxicant outflow to larger streams and lakes.

Cardinale (2011) reported that in stream systems niche partitioning among species of algae can increase the uptake and storage of nitrate. His research also showed that more than 80% of increased cell densities in cultures were driven by niche complementarity in microcosms. Cardinale's research provides "direct evidence that communities with more species take greater advantage of the niche opportunities in an environment and this allows diverse systems to capture a greater proportion of biologically available resources". Cardinale also showed that when niche partitioning was removed or reduced to minimal levels, the periphyton communities collapsed to single species dominance. Cardinale attributes the niche partitioning in streams to specific algal characteristics that make them "best adapted" to specific habitats within the stream. "These adaptations were expressed only when environmental conditions were dynamic in space and or time and when heterogeneity provided ecological opportunities for species to coexist." Some of the adaptations included sheer resistance for high flowing water, large filamentous algal dominance in slow moving waters, and increasing growth rate with habitat disturbance.

This concept was also tested by Villeneueve *et al.* (2011) who concluded that increased turbulence led to more diversity in periphyton communities. Villeneueve *et al.* also found that the higher diversity periphyton communities were more sensitive to the tested pesticides (diuron and azoxystrobin) than less diverse communities. This is likely due to the interconnectivity of diversity and niche partitioning causing a more dramatic loss of species richness in higher diversity systems. The second factor to consider regarding low diversity systems is that they may be comprised of highly tolerant species that are less likely to be affected by the pesticide exposure, while a high diversity system would contain a much greater proportion of sensitive species. In low diversity systems made up of sensitive species, the effect could be great as well. These response phenomena have a potential to greatly impact food web stability.

Food web stability is directly linked to diversity, number of trophic levels, and both top-down and bottom-up pressures.

The aquatic food web is centered around and dependent upon aquatic plant communities (including all autotrophic organisms). These communities are the primary producers and provide sugar energy, lipids, as well as macro- and micronutrients to the herbivore taxa (*e.g.*, insects, snails, fish, tadpoles, and waterfowl). In highly diverse and productive aquatic plant communities, high quality food is usually abundant, whereas in productive low diversity systems, there may be limited high quality food resources available to herbivores.

The population sizes of the taxa comprising the primary producer community are greatly dependent on abiotic conditions (bottom-up pressure; *e.g.*, light and nutrients) and the size and condition of the herbivore and predator communities (top-down pressure; *e.g.*, snail populations). Steinman (1992) found that limitation of periphyton photosynthesis could be mitigated by increasing the levels of light. The effect of light limitation on productivity is well documented. For example, seasonal decreases in available light have been shown to lead to reduced productivity (Triska *et al.* 1983; Hill and Harvey 1990; Hill *et al.* 2001). The algae in the Hill *et al.* (1995) study were reported to adapt to the low light condition over time, but productivity was 4 times greater in high light conditions. The control of autotrophic communities by grazing pressure has also been a focus of research, and several studies show that the top-down pressures are most apparent in short food webs (*i.e.*, producers and a single consumer; *e.g.,* Steinman *et al.*, 1987; McQueen *et al.*, 1989; Steinman, 1992; Kurle and Cardinale, 2011). Steinman (1992) found that herbivore control was so complete that autotroph biomass could not respond to increases in the levels of light and that when grazing pressure was released, the controlling factors shifted back to abiotic factors (*i.e.*, seasonality and light).

Hill *et al.* (2001) found that nutrient concentrations (nitrate and phosphate) increased in streams after overstory leaf emergence, which they attributed to a “cascade of shade effects” through the reduction of primary producer communities, resulting in additional abiotic components available to the other portions of the ecosystem. Because herbivore growth increased almost linearly with increased light, reflecting food supply limitation at low light, the cascade of shade effects ultimately led to decreased herbivore densities. The effects of low-

light on the primary productivity of the system can be synergistic with the effects of herbivore grazing pressure. Higher grazing pressure also reduces algal communities and diversity and is more pronounced in low light conditions (*e.g*., Steinman, 1992; Hill *et al.*, 1995), especially after shifts from higher light to lower light (*i.e*., herbivore populations would be higher due to increased food resources in the high light condition, and would demand the same energy input from the lower productivity of the low light condition).

Other impacts on the food web come from another type of top-down pressure, the predator-herbivore interaction. Kurle and Cardinale (2011) report that higher diversity and production-to-biomass ratios in the autotrophic communities reduce the strength of trophic cascades. Therefore, in systems with high algal diversity, herbivores have a greater ability to evade or defend against predators, so that herbivore pressure on the primary producers is more even over time. However, low diversity systems have a propensity to have increased top-down pressure and would be more erratic in behavior and more prone to collapse when stressed (Steinman, 1992; Kurle and Cardinale, 2011).

In addition to food, shelter, and reproduction, there are documented symbiotic relationships between algae and invertebrate and vertebrate species (*e.g*., Douglas, 2010; Oliver and Moon, 2010; and Kerney, 2011). These symbiotic relationships present additional uncertainty regarding the impact of atrazine on these relationships.

Importance of the Biological integrity of headwater streams, lakes, wetlands and estuaries:

Meyer *et al.* (2007) summarize the importance of small streams and springs to the entire river system and discuss the ways they enhance the biological diversity of the entire river system. Headwater streams play a critical role in the export of food (e.g., drifting insects; benthic organisms, and emerging insects), they provide a filtration process which increases dissolved oxygen through photosynthetic output, reduces particulate matter (macrophytes), and provides critical nutrient transformations which increase downstream water quality. In addition to these exports, the larger river, lake, and reservoir organisms also depend on the headwaters for refuge (e.g., high flow events, thermal events, predation and competition) and rich feeding sites for spawning and nursery habitat (Meyer *et al*., 2007). While the study by Meyer *et al.* (2007) was focused on the headwater stream, these same exports and downstream dependencies are common to lakes, wetlands and estuaries.

The focus of the assessment endpoints presented in **Section 6.1.4** is required to ensure that the atrazine concentrations in watersheds do not cause significant changes to the freshwater aquatic plant community (used as a surrogate for estuarine/marine plant communities) structure and productivity and thus put at risk the food chain and entire ecosystem health.

6.2. Effects to Animals

6.2.1. Toxicity to Terrestrial Animals

6.2.1.1. Toxicity to Birds, Reptiles and Terrestrial Phase Amphibians

Effects data for acute and chronic bird, terrestrial-phase amphibian, and reptile data, including data published in the open literature, are summarized in the following sections. Additional studies and details on the studies summarized below are included in **Appendix B.** As specified in the Overview Document, EPA uses birds as a surrogate for terrestrial-phase amphibians and reptiles when sufficient toxicity data for each specific taxonomic group are not available (U.S. EPA, 2004).

6.2.1.1.a. Birds: Acute Exposure (Mortality) Studies

The available data in birds suggest that atrazine is slightly toxic to avian species on an acute oral exposure basis. For parent atrazine, the lowest reported acute oral LD_{50} is 783 mg/kg-bw (bobwhite quail, *Colinus virginianus*) (MRID 00024721). The previous Data Evaluation Record (DER), which reported the study authors' LD_{50} result, was recalculated using the current EFED methodology. In addition, as this study was conducted using 14-day old birds as oppose to typically adult birds. For an atrazine formulation in which the resulting LD_{50} values were >2,000 mg/kg-bw (1520 mg a.i./kg), signs of poisoning in mallards (*Anas platyrhynchos*) first appeared 1 hour after treatment and persisted up to 11 days ,and in ring-necked pheasants, (*Phasianus colchicus*), remission of signs of intoxication occurred by 5 days after treatment (U.S. EPA, 2003a; MRID 001600-00). Signs of poisoning included weakness, hyper-excitability, ataxia, and tremors; weight loss also occurred in mallards.

An acute oral toxicity study with passerines is not available for atrazine.

Because all subacute avian LC_{50} values are greater than 5,000 mg/kg-diet, atrazine is categorized as practically non-toxic to avian species on a subacute dietary basis. In the subacute dietary study in mallard ducks (*A. platyrhynchos*), 30% mortality was observed at the highest test concentration of 5,000 mg/kg-diet (MRID 00022923); one mortality was observed in the Japanese quail (*Coturnix japonica*) study at 5,000 mg/kg-diet. The time to death was Day 3 for the one Japanese quail (*C. japonica*) and Day 5 for three mallard ducks (U.S. EPA, 2003a; MRID 00022923 and 0002292; J. Spann at Patuxent Wildlife Center, 1999, personal communication). Four species of birds were tested in Hill *et al.*, (1975) (MRID 00022923) study; however, control performance for the tests was not reported. In addition, the treated feed was not analyzed for stability.

6.2.1.1.b. Birds: Chronic Exposure (Growth, Reproduction) Studies

Reproduction studies in birds have reported effects at atrazine concentrations of 75 mg a.i./kg-diet and higher. Both northern bobwhite quail (*C. virginianus*) and mallard duck (*A. platyrhnchos*) reproduction studies were conducted using atrazine. Stability or homogeneity in the test feed was not analyzed for either study and therefore, there is uncertainty in the dietary exposure concentration. In the northern bobwhite study, the following endpoints were affected at 675 mg a.i./kg-diet: egg production and embryo viability, and a reduction in weight gain in the males (MRID 42547102). The number of cracked eggs in the control was about three times the typical value reported in the 850.2300 guideline. The NOAEC in the bobwhite study was 225 mg a.i./kg-diet. In the mallard study, at a concentration of ≥225 mg a.i./kg-diet, there were effects on egg production; hatchability, male weight gain and food consumption were affected at 675 mg a.i./kg-diet (MRID 42527101). Hatchling weight was significant at all concentrations tested, 7.5-13% decrease at 75 to 675 mg a.i./kg-diet.

6.2.1.1.c. Birds: Sublethal Effects

Japanese quail (*C. japonica*) body weights (absolute) were reduced at atrazine concentrations of 25 mg/kg-bw after receiving an oral daily dose of 35% (w/w) atrazine for 45 days (Hussain *et al*., 2011; E153875). Feed consumption was reduced at concentrations of 50 mg/kg-bw. The name and type of formulation used in the study was not reported.

6.2.1.2. Reptiles

Limited data are available for reptiles, and there were no available data for terrestrial phase amphibians.

Atrazine was tested on eggs of the red-eared slider turtle (*Trachemys scripta 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. 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) (MRID 455453-03 and 455453-02).

Two additional open literature studies in which snapping turtle (*Chelydra serpentine*) and alligator eggs (*Alligator mississippiensis*) were exposed to atrazine either via direct application or incubation in soil treated with atrazine were available (De Solla *et al*., 2006 and Crain et al., 1999). In the snapping turtle study some males with testicular oocytes and females were produced in the atrazine-treated groups (3.3 – 3.7%), but not in the control group; however, no statistical differences were found among the treatment and control groups. For the alligator study, no differences in gonadal and reproductive tract histology or hepatic aromatase activity

were observed in any of the atrazine-treated or control alligators. These studies are described further in **Appendix B.**

Toxicity data for terrestrial-phase amphibians are discussed in the amphibian section (Section 7).

6.2.1.3. Toxicity to Mammals

Atrazine acute and chronic toxicity values for mammals are presented below.
The mammalian LOAEL in reproduction toxicity studies was 500 mg/kg-diet based on significant reductions in adult rat body weight and adult food consumption (NOAEL 50 mg/kg-diet) (U.S. EPA, 2003a; MRID 40431303).

6.2.1.3.a. Mammals: Acute Exposure (Mortality) Studies

The acute oral LD_{50} value for parent atrazine in the rat (*Rattus norvegicus*) is 1,869 mg/kg-bw (MRID 00024709).

6.2.1.3.b. Mammals: Reproduction Toxicity Studies

Typically a 2-generation reproduction study is used to evaluate chronic toxicity to wild mammals and the study conducted using the rat (*Rattus norvegicus*) (MRID 40431303) described below has been used in previous evaluations; however, additional reproduction/developmental toxicity data are now available for atrazine (U.S. EPA, 2011a). The reviews for this available data will be evaluated to determine if there is additional relevant reproduction data for evaluating chronic risk to mammals. In the 2-generation reproduction study (MRID 40431303), technical grade atrazine was administered to rats (*Rattus norvegicus*) 30/sex/dose) in the diet at concentrations of 0, 10, 50, and 500 mg/kg-diet. Parental body weights, body weight gain, and food consumption were statistically significantly reduced at the 500 mg/kg-diet dose in both sexes and both generations throughout the study. Compared to controls, body weights for F_0 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, occurring 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 mg/kg-diet (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 mg/kg-diet (3.8 mg/kg/day in males, and 3.7 mg/kg/day in females).

6.2.1.4. Toxicity to Terrestrial Invertebrates

Atrazine is practically non-toxic to honey bees (*Apis mellifera L.*); the reported LD_{50} value is >97 µg/bee with 5% mortality reported at the highest dose tested (MRID 00036935). Atrazine also did not cause adverse effects in fruit flies (*Drosophila melanogaster*), houseflies (*Musca domestica*), and mosquito larvae (*Aedes aegypti*) exposed to 15 µg/fly (Lichtenstein *et al*., 1973). LC_{50} values in earthworms ranged from 273 to 926 ppm soil (Mosleh *et a*l., 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 for these studies may be found in **Appendix B.**

6.2.2. Effects to Aquatic Animals

A brief summary of submitted and open literature data considered relevant to the ecological risk assessment is presented below. Additional information is provided in **Appendix B.**

6.2.2.1. Toxicity to Fish

A summary of acute and chronic fish data, including data from the open literature, is provided in the following sections. Additional information is included in **Appendix B.**

6.2.2.2. Acute Exposure (Mortality) Studies

Atrazine toxicity has been evaluated in numerous fish species, and the results of these studies demonstrate a wide range of sensitivity. LC_{50} values range from 2,000 to 60,000 µg/L (2 mg/L to 60 mg/L (See **Appendix B** for additional details on these studies). Therefore, atrazine is classified as moderately to slightly toxic to fish on an acute basis.

Parent Atrazine

Freshwater Fish

Acute toxicity data for freshwater fish are available for at least 8 different species. The most sensitive freshwater fish acute study is the rainbow trout with an 96-hour LC_{50} of 5,300 µg a.i./L, which appears to be based on nominal concentrations (MRID 43344901). The test solutions were renewed every 24 hours and the loading was calculated as 0.37 g/L. In this study, the fish exhibited a dose-response change in coloration (darkening) for 48 hours after test initiation (data not reported). Other than temperature, water quality parameters such as dissolved oxygen were not reported in this static study; the dilution water was aerated prior to dosing and was renewed every 24 hours.

Estuarine/marine Fish

Atrazine toxicity data have been submitted for two estuarine/marine fish species: sheepshead minnow and spot (*Leiostomus xanthurus*). A sheepshead study (MRID 45208303; 45227711; LC_{50} = 2,000 µg a.i./L) and the spot study (MRID 45202920; LC_{50} = 8,500 µg a.i./L) only reported the LC_{50} value with no summary mortality data reported. In addition, in the sheepshead study the fish were fed at 48-hours which the reviewer indicated that the fish were *ca.* 48 hours old at test initiation and withholding food for 96 hours was not appropriate. Another sheepshead minnow acute study reported an 96-hour LC_{50} of 13,400 µg a.i./L, based on measured concentrations (MRID 00024716). At concentrations of 4,600 µg a.i./L and greater, fish in this study exhibited sublethal effects such as loss of equilibrium, surfacing and extended abdomen.

Atrazine Formulations

Toxicity studies using atrazine formulations are available for freshwater fish. The acute LC_{50} values range from 12,600 to 42,000 µg a.i./L and are classified as slightly toxic. 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. Acute studies with atrazine formulations for estuarine/marine fish were not available.

6.2.2.3. Chronic Exposure (Growth/Reproduction) Studies

Chronic freshwater fish toxicity studies will be used to assess potential effects to fish and aquatic phase amphibians via potential effects to growth and reproduction. Freshwater fish early life-stage and life-cycle studies, as well as early-life stage studies for estuarine/marine fish for atrazine are available and summarized in **Appendix B.**

Freshwater Fish

Several early life-stage (ELS) and life-cycle studies were available for freshwater fish using parent atrazine as well as ELS studies with atrazine formulations. For two of the ELS studies with rainbow trout (*Oncorhynchus mykiss*) and channel catfish (*Ictalurus punctatus*) the studies did examined an early life-stage however the study durations were too short to capture chronic exposure, with test durations of 27-d (4-d post hatch) and 8-d (4-d post hatch), respectively (MRID 45202902). Another ELS study was conducted with rainbow trout (86-d duration) with a reported NOAEC and LOAEC of 410 and 1,100 µg/L, respectively, based on delayed hatching and reduced weight (MRID 45208304). However, this study was conducted with the solvent dimethylsufoxide (DMSO) which can promote movement of chemicals across membranes. It also appears that only one replicate tank was used per concentration and if more were used, then variability within a treatment group was not reported. In the last available ELS study, zebrafish (*Brachydanio rerio)* were exposed for 35 days, and the reported NOAEC and LOAEC was 300 and 1,300 µg/L, respectively (MRID 45202908); raw data was not available.

In addition to the ELS studies, there were several life-cycle fish studies conducted with atrazine. A 44-week study using brook trout (*Salvelinus fontinalis*) resulted in the most sensitive NOAEC, 65 µg a.i./L based on growth (MRID 00024377). Upon reexamining this study for the problem formulation, several concerns with this study were identified: 1) it appears the study did not use a solvent control although DMSO was used in the atrazine concentrations; therefore, potential solvent effects could not be evaluated; 2) the use of DMSO is discouraged as it can promote movement across membranes; 3) following distribution to the test chambers, the fish were treated with malachite green and formalin (25 µg/L of formalin containing 3.7 g/L malachite green) to prevent further disease (disease was observed prior to distribution to the tanks when the fish were treated at that time); and 4) according to the authors, variability in the reproduction endpoint was highly variable which precluded the ability to ascribe statistical significance to treatment groups that appeared to have reduced values. This variability may have been potentially enhanced by the use of only two replicates during the reproduction phase. The other chronic studies reported in MRID 00024377, bluegill sunfish (*Lepomis macrochirus*) and fathead minnow (*Pimephales promelas*), also appeared to use DMSO without a solvent control. In the bluegill study, the percent survival in the F1 generation was 22% after 30 days, and according to the authors the spawning was too sporadic to be conclusive. Control survival in the fathead minnow (*Pimephales promelas*)) after 30 days was 47-60%. Another chronic fathead minnow life-cycle study, MRID 42547103, resulted in a non-definitive NOAEC (<150 µ/L) as growth in the F1 generation was significantly lower in all treatment groups compared to the negative control.

Short-term reproduction studies for freshwater fish were also available in which reproduction in adult fish was monitored for several weeks. These studies were conducted using mature actively-spawning fish to evaluate reproduction and did not capture exposure to embryo or larval stages. Adult fathead minnows (Pimephales promelas) were exposed to atrazine (5 and 50µg/L) or 21-day with estradiol as a positive control (Brignole et al. 2004). While not

statistically significant, the study authors reported a dose-dependent trend for percent embryos fertilized, male gonad-somatic index (GSI) and testicular maturity.

A 30-d short-term reproduction study with fathead minnow using atrazine reported effects on reproduction (total number of eggs, and number of spawns) as well as alterations in ovarian maturation compared to the control (Tillitt *et al*., 2010). These adverse effects were reported at a concentration of ≥0.5 µg/L; however, an apparent threshold response was observed as similar results were obtained at 0.5 and 5.0 µg/L. The raw data for this study was provided, and this study will be evaluated for possible inclusion in the atrazine risk assessment.

Estuarine/marine fish

Two chronic toxicity tests with sheepshead minnow were available. The study with the most sensitive NOAEC was a 28 days post hatch early life-cycle study with a NOAEC value of 1,100 µg a.i./L based on growth; this study was classified as acceptable (MRID 46648203 and 46952604).

6.2.2.3.a. Sublethal Effects and Additional Open Literature Information

In addition to registrant-submitted studies, data from the open literature that reported sublethal effect levels to freshwater fish were also evaluated. A number of open literature studies were reviewed as part of the 2003 IRED. The results of these studies, which showed sublethal effects to olfaction, behavior, kidney histology, and tissue growth at atrazine concentrations ranging from 0.1 to 3,000 µg/L (**Appendix B**). In addition, the risk assessment for the California red-legged frog (CRLF) (U.S. EPA, 2009b) also identified open literature studies reporting sublethal effects. Prior to conducting the atrazine risk assessment for registration review, another open literature search may be completed (via ECOTOX) and additional studies reporting sublethal effects may be reviewed at that time. A summary of studies reviewed in CRLF assessment is presented below.

The reported sublethal effects included a change in plasma vitellogenin in male and female rainbow trout and plasma testosterone in males at atrazine concentrations of ca. 50 µg/L (MRID 45622304). Effects on fish behavior, including preference for the dark part of aquarium (MRID 45204910), grouping behavior (MRID 45202914), as well as alterations in trout kidney histology (MRID 45202907) have been reported at atrazine concentrations of 5 µg/L. In salmon (*Salmo salar*), smolt gill physiology, represented by changes in Na-K-ATPase activity was altered at 2 µg/L (Waring and Moore, 2004) with similar effects observed at 0.5 µg/L (Moore *et al*., 2007). Survival was evaluated after transfer to full salinity sea water (33 ‰) in Waring and Moore (2004). Atrazine exposure for 5 to 7 days in freshwater followed by transfer to full salinity sea water resulted in higher mortality at atrazine concentrations of 14 µg/L (14 % mortality) and higher mortality in one study at 1 µg/L (15 % mortality) and higher mortality in a separate experiment presented in the publication (no controls died; statistical significance was not indicated). The salinity used by Waring and Moore (2004) simulated full strength seawater (33 %).

Tierney *et al*. (2007) studied the effect of 30-minute exposure to atrazine on behavioral and neurophysiological responses of juvenile rainbow trout to an amino acid odorant (L-histidine at 10^{-7} M, which had been shown to elicit an avoidance response in salmonids). Although the study authors concluded that L-histidine preference behavior was altered by atrazine at exposures $\geq$ 1 µg/L, no significant decreases in preference behavior were observed at 1 µg/L, nor was a dose response relationship observed. Hyperactivity (measured as the number of times fish crossed the center line of the tank) was observed in trout exposed to 1 and 10 µg/L atrazine. In the study measuring neurophysiological responses following atrazine exposure, electro-olfactogram (EOG) response was significantly reduced (EOG measured changes in nasal epithelial voltage due to response of olfactory sensory neurons).

Although these studies raise questions about the effects of atrazine on plasma steroid levels, behavior modifications, gill physiology, and endocrine-mediated functions in freshwater and anadromous fish, it is not possible to quantitatively link these sublethal effects to the selected assessment endpoints for the assessed species (*i.e*., survival, growth, and reproduction of individuals). Also, effects on survival, growth, or reproduction were not observed in the available life-cycle studies at concentrations that induced these reported sublethal effects. Further details on sublethal effects are provided in **Appendix** B.

6.2.2.4. Toxicity to Aquatic Invertebrates

A summary of acute and chronic freshwater invertebrate data, including data published in the open literature, is provided below in the following sections.

6.2.2.4.a. Acute Studies

Atrazine toxicity has been evaluated in numerous aquatic invertebrate species, and the results of these studies demonstrate a wide range of sensitivity. Definitive EC/LC_{50} values range from 48 to 30,000 µg/L (0.048 mg/L to 30 mg/L), with several other studies reporting non-definitive EC/LC_{50} values >4,900 to >100,000 µg/L (see **Appendix** B for additional details on these studies). Therefore, atrazine is classified as highly to slightly toxic to aquatic invertebrates on an acute basis.

Parent Atrazine

Freshwater Invertebrates

There are many acute toxicity studies using atrazine for freshwater invertebrate species with a range of toxicity values. The acute LC/EC_{50} values range from 720 to greater than 30,000 µg a.i./L. For the available studies, while acute LC/EC_{50} values are reported, summary data for the controls and individual treatment groups are not reported. Therefore, verification of the

reported LC/EC_{50} values could not be determined. Also, for some studies, details on the test design and/or environmental conditions were not well documented. The most sensitive value is for the midge, *Chironomus tentans*, with a 48-hour LC_{50} value of 720 µg a.i./L (MRID 00024377).

A sediment toxicity test with whole sediment was available for *Chironomus tentans* (MRID 45904002). The study was a 10-day static-renewal study using spiked sediment. The NOAEC and LOAEC, based on dry weight, was 24 and 60 mg a.i./kg based on mean measured sediment concentrations, respectively, and 4.0 and 21.5 mg a.i./L based on mean measured pore-water concentrations.

Estuarine/marine Invertebrates

As with the freshwater invertebrates, there are many acute toxicity tests available for estuarine/marine invertebrates, and like the freshwater invertebrate studies, the studies primarily only report LC/EC_{50} values with no documentation of test concentration toxicity data. The reported range of acute LC/EC_{50} values for estuarine-marine organisms range from 48 to 13,300 µg/L, with several non-definitive endpoints. The most sensitive organism tested was the juvenile estuarine/marine shrimp, *Neomysis integer* (LC_{50} of 48 µg/L; Noppe et al. 2007); only the LC_{50} value was reported, so the results could not be confirmed and control mortality was unknown.

A 10-d sediment toxicity test with the clam, *Mercenaria mercenaria*, was available in the open literature (Lawton *et al*. 2006; E89627). This study reported no effects on survival, mass and size at atrazine concentrations of ≤20,000 µg/kg, the highest concentration tested.

Formulations

Freshwater Invertebrates

Two 48-hour acute toxicity studies with *Daphnia* for atrazine formulations (80WP and 40.8 4L) are available with acute LC_{50} values ranging from 36,500 to 49,000 µg/L and >31,000 µg a.i./L (MRID 42041401;45227712). These studies were conducted above the limit of solubility for atrazine (33 mg/L). Another study with *Daphnia magna* reported a 48-hour LC_{50} of >485,000 µg a.i./L using atrazine 500 (48.5% a.i.); the same study reported a 96-hour LC_{50} of 16,000 µg a.i./L for *Hyallela azteca* (Wan *et al.,* 2006). An acute study with glochidia and juvenile stage freshwater mussels, *Lampsilis siliquoidea*, was conducted using Aatrex 4L (40.8% a.i.) (Bringolf *et al.,* 2007; E99469). The reported 96-hour LC_{50} value for both stages was >30,000 µg/L (12,200 µg a.i./L). The freshwater mussel, *Utterbackia imbecillis*, was tested using the formulation Atrazine 4L SA-50 (41% atrazine) under static conditions for 24 hours. The LC_{50} was estimated at 241,000 µg/L (Conners and Black 2004; E74236) which is greater than the solubility of atrazine.

Estuarine-marine Invertebrates

There were several acute toxicity studies conducted with atrazine formulations for estuarine/marine invertebrates including eastern oyster (*Crassostrea virginica*), Pacific oyster (Crassostrea gigas), fiddler crab (*Uca pugilator*), and European brown shrimp (*Crangon crangon*) and cockle (*Cardium edule*). Several studies resulted in non-definitive values, LC_{50} >100 to >100,000 µg a.i./L (MRID 00024720; 45227728), while others resulted in definitive LC_{50} values, 10,000 to 239,000 µg a.i./L (MRID 45227728; 00024395), of which some are above the solubility of atrazine.

6.2.2.4.a. Chronic Exposure Studies

Freshwater Invertebrates

There are several chronic toxicity tests for freshwater invertebrates. The most sensitive chronic endpoint for freshwater invertebrates was based on a 30-day flow-through study on the scud, *Gammarus fasciatus*, with a NOAEC of 60 µg/L, based on growth of the second generation (MRID 00024377). As with the chronic freshwater fish, this study appeared to be conducted using the solvent DMSO with no concurrently tested solvent control. In addition, the control survival after 30 days was64-74%, and only one of the two replicates in the control reproduced. Results were available for freshwater invertebrate species (*D. magna, C. tentans*) from the same document, MRID 00024377; however, they all also appeared to use DMSO with no concurrent solvent control. The reported NOAEC and LOAEC for *D. magna* and *C. tentans* was 140 and 250 µg a.i./L (based on reproduction and survival) and 120 and 230 µg a.i./L (based on reduced pupating and emergence), respectively. In the *Daphnia magna* test, control performance was an issue with only 61% survival after 15 days for the parental generation. Several other chronic toxicity studies were also available with NOAECs ranging from 200 to 5,000 µg a.i./L, but toxicity data and/or methods were not reported; therefore the results could not be verified.

Estuarine-marine Invertebrates

The most sensitive chronic bioassay in estuarine-marine species was a 28-day study in mysid shrimp (*Americamysis bahia*) that reported a NOAEC of 80 µg/L based on a reduction in survival (MRID 45202920). However, toxicity data were not available for endpoint verification Thus, endpoint values are presented in a table with only the mean value and no standard error or deviations. In addition, while the report stated that the assay was conducted according to Nimmo *et al*. (1977), no explicate test duration was reported. Another mysid shrimp life-cycle study was available (MRID 46648202) with a reported NOAEC of 260 µg a.i./L, based on growth.

CHAPTER II. THE EVALUATION OF AMPHIBIAN TOXICITY DATA

While reviewing this section, please consider the charge questions below.

SAP Questions:

- Is the SAP aware of any other laboratory-based or field-based studies not included in this White Paper that should be considered?

EPA identified test design elements that could potentially confound the ability of a study to discern a causal relationship between exposure to atrazine and an effect on amphibians (Section 7.2). Based on consideration of those test design elements, EPA then evaluated the available amphibian data and assigned a classification (*e.g.*, Quantitative, Qualitative (high, medium, and low level of confidence), and Invalid) to each study indicating EPA's confidence in the study's conclusions (Section 7.3 and Appendix C). The confidence in each study was based on an evaluation of the identified test design elements and resulting level of uncertainty in determining a direct causal relationship between atrazine and potential effects to amphibians.

- Please comment on the completeness of EPA's list of pertinent test design elements. Also, please comment on the degree to which these test design elements, singularly or in combination, would be expected to contribute towards confounding the test results.

- Please comment on EPA's conclusions about the level of confidence placed on each study's results.

After evaluating all the available amphibian studies, one study was found to have accounted for all the identified test design elements (Question #2) and determined to be suitable for quantitative use in risk assessment for the endpoints of survival, growth and development (Section 7.3 and Appendix C). This study was required by an EPA Data Call-In (DCI) Notice following the recommendations from the 2003 SAP on atrazine and amphibians. The resulting study examined the effects of atrazine on *Xenopus laevis* at concentrations of 0.01 to 100 µg/L at two different laboratories. Based on the 2007 SAP, the conclusion was, and there was agreement by the Panel, that the data from this study were robust and sufficient to conclude that exposure to atrazine at concentrations ranging from 0.01 to 100 µg/L had no effect on *X. laevis* development (which included survival, growth, metamorphosis and sexual development).

- Please comment on whether any new information has become available that leads to a different conclusion from the one which EPA reached in that the results of the DCI study were adequate to evaluate potential effects of atrazine exposure to amphibians.

- If such information is now available, please comment on how a threshold determination (a concentration that is expected to cause no effect) may be accomplished using the identified studies.

After evaluation of the available amphibian toxicity data, EPA concluded that the DCI study mentioned above was appropriate for quantitative use in a risk assessment for survival, growth and development. While the 2007 SAP Panel agreed that atrazine appeared to have no effect on *X. laevis* development at atrazine concentrations ranging from 0.01 to 100 µg/L, they expressed concerns about the suitability of *X. laevis* as a surrogate for native species. Review of the available toxicity data utilizing indigenous species suggests that suitable protocols, including adequate husbandry methods in particular, that would enable EPA to quantify a toxicity endpoint representative of a clear and consistent response from atrazine for native species, may not exist.

- Please comment on whether there are suitable methods for testing native amphibians with particular regard to husbandry and laboratory culturing conditions, consistent with the design elements recommended by the 2003 SAP.

A number of studies report the potential for atrazine to modify immune function and infection susceptibility in amphibians (**Appendix C**). EPA believes the research on these different hypotheses does not provide sufficient data to establish causal linkages among different levels of biological organization to result in adverse effects. Therefore, EPA concluded that a mode of action or adverse outcome pathway leading to effects on amphibian survival, growth or development cannot be established at this time.

- Please comment on whether the data in the existing database reasonably supports the hypotheses, or demonstrates that atrazine affects immune function and/or infection susceptibility leading to adverse effects on survival, growth or development; i.e., are there sufficient data to establish an adverse outcome pathway for atrazine effects on immune function? Please provide a rationale for the Panel's position and discuss the associated strengths and weakness with the data supporting the rationale.

- If the Panel concludes that the existing data are sufficient to formulate hypotheses that atrazine adversely affects immune function and infection susceptibility, but are not sufficient to test the hypotheses (refute or confirm), then please comment on specific study protocols that can be used to test these hypotheses with sufficient rigor to identify effects that can be directly and quantitatively attributed to adverse impacts on amphibian reproduction, growth and/or survival.

7. Toxicity Data to Amphibians (aquatic-phase and terrestrial)

If acute and chronic toxicity data are not available for aquatic-phase amphibians, the EPA relies on freshwater fish acute and chronic toxicity data as surrogates for aquatic-phase amphibians (U.S. EPA, 2004). Additionally, birds are used as surrogates for terrestrial-phase amphibians. To evaluate the potential for atrazine to affect amphibians in the environment, the Agency evaluated the available amphibian toxicity dataset.

7.1. History of Previous Amphibian SAPs

A large number of studies are available examining the potential effects of atrazine on amphibians. Many of these studies have been previously reviewed by OPP and reported in endangered species assessments for the compound and in ecological risk assessments written in support of registration decisions. Reviews of more recent literature on amphibians are contained in **Appendix C**. The current reviews were conducted on open literature papers that passed the U.S. EPA ECOTOX and OPP acceptability screening criteria (**Appendix N**). Previously, reviews of studies specific to the potential effects of atrazine on amphibian gonadal development were written in support of consultations with the FIFRA SAP. For the 2003 SAP on potential developmental effects of atrazine on amphibians, the Agency determined that existing data were sufficient to warrant further examination of atrazine effects on development (U.S. EPA, 2003d). The SAP concurred with a tiered testing approach proposed by EPA using the African clawed frog (*Xenopus laevis*) and indicated that *X. laevis* was a suitable surrogate test species for amphibians. In 2004, EPA issued a data call-in (DCI) to registrants for the study outlined in the first tier and at a 2007 SAP on the potential for atrazine to affect amphibian gonadal development, the results of the DCI study and evaluation of open literature data to date were presented (U.S. EPA, 2007a). The EPA concluded that based on available data, atrazine did not appear to produce consistent effects on amphibian development and that based on the tiered testing approach reviewed by the 2003 SAP, no further testing was needed. However, EPA indicated in 2007 that it would continue to monitor information as it becomes available. The SAP agreed that the study conducted in response to the DCI (which was comprised of two studies conducted in parallel) showed no effect to *X. laevis* on development from exposure to atrazine concentrations ranging from 0.01-100 ppb. The 2007 SAP Panel did, however, express concerns about the use of *X. laevis* to represent native species and the sensitivity of the strain of *X. laevis* used in the study.

7.2. Test Design Elements

Subsequent to the 2007 SAP, toxicity data on effects of atrazine for a variety of amphibian endpoints (*e.g.*, metamorphosis, sexual development, immune response and infection susceptibility) have continued to be published in the open literature. At this time, attributing any clear dose-response for potential effects of atrazine on native species and terrestrial-phase/reproductively mature amphibians remain as uncertainties. After a review of the available data for native species, adequate test methods, culturing in particular, have not been

identified to reliably test hypotheses. Some of these uncertainties arise from confounding factors present with the available data in terms of the reported test design. As indicated in previous SAPs on atrazine and amphibians, there are many study design elements that if followed and reported, help to reduce uncertainty and allow for greater confidence in the ability to discern a cause and effect relationship with regards to atrazine exposures. These test design elements include:

1. Sufficient replication to allow for adequate power to detect the desired difference from the control;
2. Screening for potential contaminants/interferences in food and water sources;
3. If the use of a solvent is required, the use of a recommended solvent at no more than the recommended maximum rate. Current ASTM and U.S. EPA OCSPP guidelines recommend that in chronic testing not to use more than 0.1mL solvent/L solution; dimethylsulfoxide (DMSO) is not currently a recommended solvent for chronic testing. In addition, the test would contain both a negative and a solvent control concurrently with the treatment groups, as previous historical knowledge and response to a solvent may not be indicative or representative of the response in the current study. This is particularly important for studies for which there is no standard, validated protocol or guideline;
4. Measurement of the test chemical in both the control(s) and the treatment groups to relate potential effects to an exposure concentration and to check for potential contamination in the control(s);
5. Test equipment (*e.g.*, test vessels) that is constructed of recommended materials and avoids the use of materials (*i.e.*, plastics) that may leach contaminants that may interfere with developmental endpoints;
6. Loading that adheres to recommended rates to help ensure adequate water quality and development in addition to reporting the measured water quality parameters. If conducting a study under static or static-renewal conditions, it is particularly important to measure and report water quality elements such as ammonia levels to better ensure adequate environmental conditions. The renewal period may need to be shortened and/or the biological loading rate decreased in an effort to maintain water quality;
7. The use of organisms from controlled environments in an effort to understand prior exposure history. This may include using laboratory-raised organisms (preferred) or organisms from outdoor sources in which information about potential contaminants is known;

In addition to the test design elements described above, the resulting reported data need to be sufficient to enable the reviewer to understand and substantiate the study results or conclusions. Furthermore, care should be taken to avoid aberrant effects in controls that confound the ability to discern treatment related effects (*e.g.*, high control mortality or high incidence of intersex especially when evaluating developmental endpoints).

Overall, similar to findings from open literature reviews conducted in 2003 and again in 2007, many of the studies reported in the open literature since the 2007 SAP contain uncertainties

and deficiencies in their methods and/or results and the results of any single study need to be considered within the context of its uncertainties and deficiencies. Common deficiencies or uncertainties reported in the current and previous SAP reviews of the open literature include, but are not limited to, lack of prior exposure history of the test organisms, potential interferences in food sources, biological loading rate, test vessel material, and solvent choice and concentration. These uncertainties can affect the environmental conditions and husbandry requirements of the test species and can confound the ability of a study to discriminate whether exposure to a compound results in a clear and reproducible response that is concentration dependent. In previous SAP white papers for evaluating effects of atrazine on amphibian development, each of the studies was evaluated with regard to the following parameters:

1. experimental design, protocols and data quality assurance;
2. strength of cause-effect and/or dose-response relationships;
3. mechanistic plausibility; and,
4. ecological relevancy of measured endpoints.

In an effort to understand what uncertainties or potential confounding effects may be present in the current compendium of studies, test design elements that were expected to potentially confound test results were identified, many of which were used in the evaluations for the previous SAPs. In addition, uncertainties related to reporting of data/statistical results and aberrant effects in the control were also identified. Certain elements (*e.g.*, control mortality) were deemed more influential in reducing the ability to evaluate causality, and, therefore, if a study contained one of these elements the study was classified as invalid. Each of the available studies was reviewed and a record of the critical design elements was maintained for each study. For field studies, excluding micro- or mesocosms, the body of available data was primarily evaluated in support of the 2003 and 2007 SAPs, of which the many reported uncertainties in those studies precluded the ability to provid conclusive evidence of an effect that can be consistently reproduced. Therefore, the focus of this current evaluation is on the laboratory and cosm studies.

The identified test elements were as follows:

A. A study was rejected as invalid, if the study had <u>any</u> of the following elements:

1. Atrazine concentrations were not reported. Minimally, nominal test concentrations must be reported; ideally, the amount of test material applied and the exposure concentration in the water column should have been measured analytically at the start of exposure (t=0) and periodically throughout the study.
2. The study did not provide sufficient replication (n=2). At least two replicates per treatment and controls must be included regardless of the statistical method used (e.g., regression, hypothesis testing).

3. A control was not run concurrently with treatments. If the study used a solvent then either a solvent or a negative control must be run concurrently.
4. Test chemical contamination of controls.
5. Control mortality was greater than 30%.
6. The use of an appropriate solvent control was greater than 0.05% (500 µL/L), which is the current maximum for acute static and static-renewal testing for ASTM and U.S. EPA OSCPP 890 and 850 guidelines.
7. Sufficient data were not provided to enable the reviewer to understand/substantiate study results/conclusions. Statistical analysis of the data (univariate and/or multivariate), including numerical and/or graphical presentation of the results were not clearly described and presented.
8. The presence of other stressors (physical, chemical, biological) in controls/treatments was at levels that would be expected to compromise the interpretation of atrazine (degradates) as the causal stressor.
9. Aberrant effects in controls that confound ability to discern treatment related effects (*e.g*., high incidence of intersex or skewed sex ratio).

B. **If a study passed the screen above, further evaluation of the study was conducted. While the following test design elements would not immediately invalidate a study, one or more of these elements raises concerns about the ability to discern a cause-effect relationship with regards to atrazine.**

1. When a solvent was used, both a negative and solvent control group was not used in the study.
2. If both solvent and negative controls were used and there was a significant difference between the control groups for an endpoint, *i.e*., there is a statistically significant solvent effect.
3. The concentration of an appropriate solvent control was greater than 0.01% (100 µL/L), which is the current recommended level for chronic aquatic testing for ASTM and U.S. EPA OSCP 890 and 850 guidelines.
4. DMSO was used as a solvent (DMSO can promote movement of chemicals across membranes).
5. Plastic test vessels were used in the study (potential leaching of chemicals which may interfere with study).
6. The study was not conducted using technical atrazine (*e.g*., a formulation (which may or may not contain more than one active ingredient) or an effluent). Exposure to the additional chemicals may influence the response and confound the ability to discern potential effects from exposure to atrazine alone.

7. The loading rate was higher than recommended which may or may not be coupled with unreported or limited reporting of water quality parameters. One tadpole/L/day was the recommended rate discussed at 2007 SAP (also see Figure 3 (pg 95) in 2003 SAP white paper for graph depicting the relationship between developmental stage and weight with further discussion on pg. 67); 1 g/L/day is the ASTM recommended loading rate for acute studies under flow-through conditions and is 0.5-0.8 g/L for acute static and renewal tests. For example, high loading rate (>one tadpole/L/day), coupled with an infrequent test solution renewal period (>48 hours), along with no reporting of adequately maintained water quality raises uncertainties in the adequacy of environmental conditions in the study. However, the reviewer may be able to infer enough information about adequate water quality if certain design elements are reported and deemed appropriate.
8. The study used organisms that were collected in the field where a complete prior exposure history is not known. While a study may indicate that organisms were collected from an area not known for agricultural use or that the area had been analyzed for some chemicals, it is difficult to account for all possible contaminants.

After the test design elements were catalogued, an overall classification was assigned to each of the studies. If a study was not classified as invalid, it was classified as either "Qualitative" or "Quantitative" based on the screening process described above. There were three levels of Qualitative: lower, medium and higher levels of confidence. These subcategories were based on the number and type of uncertainties identified. For example, a combination of three elements that was evaluated included loading, reported water quality, and test solution renewal period. If a study contained limited information on water quality with a higher than recommended loading (*i.e.*, >1 tadpole/L) along with a renewal period of greater than 48 hours then there may be a high level of uncertainty in the environmental water quality which could lead to significant confounding effects in the study, especially for growth and development. However, defining combinations where potential confounding effects could occur is not concrete and can vary for each test design. All possible combinations or situations in which compromised water quality may occur are difficult to predict; however, the combination described above was deemed to be one possible scenario.

Overall, the test design elements described above were the basis for classifying a study. However, all the test design elements and results of each study were evaluated as a whole to determine the studies adequacies for inclusion into the evaluation of atrazine exposure and the potential for effects to amphibians. For example, if a study contained one or more elements that would introduce uncertainties about the plausibility of a cause-effect relationship, then the overall level of uncertainty of the study and its results was evaluated for its inclusion in a risk assessment. While the EPA recognizes that the overall classification of each study is subject to interpretation, the basis for the current classifications were general test design elements that are referenced in ASTM and EPA guidelines as well as from the previous amphibian SAPs that

evaluated peer-reviewed amphibian studies. The test design elements identified above provide the foundation for a study in terms of potentially accounting for factors that may influence the study results.

7.3. Results of Evaluating Amphibian Test Design Elements

Of the 75 open literature studies reviewed, several uncertainties were observed in multiple studies. Measurement of atrazine in control solutions was not reported for 44 studies (59%) with uncertainty in seven others due to ambiguous wording regarding analytical measurements. Therefore, the failure to report/measure atrazine concentrations in control solutions is considered a substantial deficiency in a relatively large proportion of the amphibian studies. Because of the large number of studies that did not report this endpoint, this evaluation assumed that the test solutions were prepared and maintained correctly without cross contamination and that the source water did not contain atrazine as it can be filtered out using carbon. However, this is a significant assumption.

Biological loading is a critical factor that can impact water quality, with dissolved oxygen and ammonia concentration being two important elements that are influenced by test organism loading (Hoke and Ankley, 2005). Biological loading rate in the available studies was not always reported in a mass/volume unit, but rather more commonly as number of tadpoles per volume or number per test vessel. In several studies (30 with 4 possibles) that tested tadpoles/larvae for longer than a week, the reported loading was higher than the value recommended by the 2007 SAP (*i.e.,* one tadpole/L). In addition, most of these studies had reported limited water quality information. Although temperature was the primary water quality parameter reported, few studies included information on other parameters such as ammonia. Therefore, there is uncertainty regarding whether there was adequate water quality for these studies especially for chronic exposures (early tadpole/larvae stage through metamorphosis).

Finally, the collection of native amphibian species from the environment was common among the available studies. While some studies indicate that the organisms were collected from an area that is not known to be influenced or has low influence from agricultural activity (Williams and Semlitsch, 2010), other study authors did indicate that organisms were collected in areas that were presumed to have been influenced by agricultural activity (Rohr *et al.,* 2003) or that they do not know the prior exposure history of the organisms (Brodkin *et al.,* 2007). Nonetheless, it is difficult to account for all types of potential stressors, both chemical and biological, for wild-caught organisms.

7.4. Categorizing Amphibian Endpoints

Different categories of endpoints have been reported in the amphibian data with major endpoints being: survival, growth (mass and length), metamorphosis and development, immune response and infection, behavior modifications (*e.g.*, feeding, locomotion, mating),

biochemical or molecular alterations, and sexual development. These studies encompassed several different species of frogs, toads and salamanders, across different life stages. The majority of the studies evaluated the larval stage (aquatic phase) of the organism for some duration or until complete metamorphosis.

In an effort to compare endpoints and study conditions, no observed adverse effect concentrations (NOAEC) and lowest observed adverse effect concentrations (LOAEC) were extracted from the available studies. These extracted endpoints were assigned to one of the categories defined above. Test concentrations above the reported LOAEC were not extracted as well as concentrations below the NOAEC (the next test level below the LOAEC). Only statistically significant endpoints were included in the review; therefore, near significant and/or trend data were excluded from consideration since there was no way to determine whether such measurements were significantly different from untreated animals. For several of the available laboratory studies, the number of atrazine concentrations tested was limited to one or two, thereby reducing the ability to definitively determine a NOAEC and LOAEC, or establish a dose-response relationship. Generally, chronic testing is not typically designed to establish a regression-based (*e.g.*, EC_x) toxicity value; for example, the test concentrations in a chronic test may be spaced far apart (such as a 10X factor as in the case of many of the available amphibian studies); therefore, the reported NOAEC value may not reflect the actual toxicity threshold for that organism. In addition, previous SAP white papers and other reviews of open literature data have acknowledged that for some of the published amphibian data, a non-monotonic response was observed (U.S. EPA, 2003d; U.S. EPA, 2007a; Rohr and McCoy 2010). Therefore, sometimes defining a NOAEC and LOAEC was confounded by an apparent lack of dose-response (*i.e.*, significant effect reported at 10 µg/L but not at 25 µg/L).

Studies that examined mixtures of more than one active ingredient chemical, of which atrazine was one of, are not considered in this evaluation as the ability to discriminate the influence from atrazine exposure alone could be confounded. However, if atrazine was tested as a formulation (with no other active ingredients), it was included in the analysis; although, it is recognized that one or more of the other ingredients (e.g., "inert" ingredients such as solvents or surfactants) in the formulation could also potentially influence the response(s) of the amphibians. Furthermore, nitrate was also added in some studies as an additional stressor and the results from these tests were also included (Sullivan and Spence, 2003). If more than one species was used in the study, individual effects for each species were identified and recorded. If more than one specific effect was observed for a general endpoint category (*e.g.*, mass and snout-vent-length, and both growth endpoints were affected at 10 µg/L, then only one effect at 10 µg/L for growth was recorded.) For the cosm studies, given that there was the potential for many different types of interactions that could occur simultaneously among test organisms that may result in direct or indirect effects, the potential variability within and between these cosm studies was considered to confound the ability to evaluate the relationship between reported results and test design elements when compared to laboratory studies.

The assigned data were collated within a category in an effort to determine if there were patterns of observed effects across studies. Within a category, different species and life stages

were combined, but could be further filtered to evaluate effects to a specific genera/species or life stage, if desired. Once studies were identified around a range of concentrations, study design elements and associated uncertainties within each study were then identified in an effort to determine if commonalities existed around similar atrazine concentrations (e.g., <100 µg/L).

The inclusion of all the studies, regardless of classification, may potentially mask the ability to observe a relationship between effects and test design elements. Therefore, studies classified as "Invalid" or "Qualitative with a lower level of confidence" were removed from the analysis. The remaining studies are presented in Table 17.

These studies represent a small subset of the available studies, and some of the endpoints in the subset of studies were classified as "Invalid" for various reasons. As discussed above, the limited reported water quality coupled with higher than recommended loading and a longer renewal rate presents a high uncertainty in regards to the environmental conditions of the assays which may confound the results. Unfortunately, many of the available studies fit that description, along with other possible uncertainties, and therefore limit their utility in examining potential effects from atrazine. It should be noted that for seven of the 10 studies listed below, analytical measurements of atrazine in the control(s) and test vessel material were not explicitly reported. The results of the subset of studies are described below.

In three studies, there was no effect on growth or time to metamorphosis at 25 µg/L and 100 µg/L, and no effect on rate of development or growth at 30 µg/L, the highest concentrations tested (Choung *et al.,* 2011; Kloas *et al.,* 2009; Spolyarich *et al.,* 2010). Two of those studies were tested using Australian species of frogs, *Litoria raniformis* (Choung *et al.,* 2011) and *Limnodynates tasmaniensis* (Spolyarich *et al.* 2010) with the other species being *Xenopus laevis* (Kloas *et al.,* 2009). The relative sensitivity of the Australian species is not known, as a positive control, such as 17-β estradiol, was not conducted with either study. Another study report effects on metamorphosis with *X. laevis* with a decrease in metamorphic stage after a certain study duration (3-5 weeks) at 100 µg/L, the lowest concentration tested (Freeman and Rayburn, 2005); no significant effect on mass was reported in the study. However, as time to metamorphosis was not determined in this study, the overall effect on metamorphosis is uncertain. In addition, since the study was terminated prior to metamorphosis, with presumably organisms at different developmental stages, it is unknown how each stage was accounted for when comparing tadpole mass between groups. Sex ratio and gonadal development were also reported to not be affected at 30 and 100 µg/L for *L. tasmaniensis* (Spolyarich *et al.,* 2010) and *X. laevis* (Kloas *et al.,* 2009); the other studies did not evaluate sex ratio or gonadal development. However, observed effects on sex ratio need to be considered with respect to when the effect was measured in the life cycle of the organism as rate of gonadal development may not in sync with somatic development rate (metamorphosis).

An effect on flow cytometric analysis (nuclei-whole body homogenized) meant to illustrate development stage was reported at exposure concentrations of 800 µg/L; however, this effect was not observed in all trials conducted at 800 µg/L (Freeman and Rayburn, 2005).

For *Bufo americanus,* behavior (expressed as fear cues and overall activity) was not modified at 196 µg/L (Rohr *et al.,* 2009) and the toads did not have a preference for soil treated with atrazine or not at atrazine soil concentrations of 1430 µg/kg (Storrs Mendez *et al.,* 2009).

There were also four studies that examined acute toxicity to amphibian embryos and larvae from atrazine exposure (Birge *et al*. 1980; Howe *et al*. 1998; Morgan *et al*. 1996; Wan *et al*. 2006). The resulting LC/EC_{50}s were at relatively high atrazine concentrations; LC_{50} (mortality) concentrations were >7,000 µg/L. The lowest reported LC_{50} value was 410 µg/L for *R. catesbeiana* in which the LC_{50} was calculated using observed mortalities as well as abnormalities that were expected to result in mortality under natural conditions (Birge *et al*. 1980). With the exception of Kloas et al. 2009, tatistical analyses for survival for the other studieswere not provided. Rather, an overall mortality/survival was generally reported as survival was generally high.

Even though several studies were classified as Qualitative with a higher or medium level of confidence, there is only one study in which all identified test design elements were accounted for and reported, which was the *X. laevis* study, which was submitted by the registrant after the 2003 SAP. The 2007 SAP concurred that there was no effect for *X. laevis* at concentrations of 0.01-100 µg/L. While the SAP expressed concerns about the use of *X. laevis* as surrogate species, an evaluation of the available native species data suggests that husbandry issues may persist with native species as the overwhelming majority of available data reports using field collected organisms.

Table 17. Study Design Elements and Results for Laboratory Amphibian Studies Conducted Using Atrazine, Classified as Quantitative or Qualitative (with lower level of uncertainty)

Citation / ECOTOX(E)/ MRID	Species / Source / Exposure Period (stages & test duration)[2]	TS[3] / % ai	Solvent (%)[4] / NC/SC (Y/N); MA in CNT (Y/N/NS)[5]	Test vessel material[6]	Loading; Renewal Period[7]	Reported Results[8]				Indentified Uncertainties / Concerns and Classification
						Endpoint	NOEC (µg/L)	LOEC (µg/L)	Effect	
Birge *et al*. 1980 / E6187	*Rana. catesbeiana, R. pipiens, R. palustris, Bufo americanus* / NS (purchased) / E for 4 dph	F / 80	NS / (Y unsure if NC or SC); NS	glass	50 -103 E / 0.5L	**Surv 4 dph LC50**	--	--	*R. catesbeiana* = 410 µg/L *R. pipiens* = 7680 *R. palustris* = 17960 *B. americanus* >48000	LC_{50}s were not based on mortality per se, but on abnormalities that would reportedly preclude survival under natural conditions; solvent (if used) was not identified as tested formulation suspect not; formulation used so uncertainty in source of toxicity Qualitative – Higher level of confidence; however LC_{50} values are combination of both lethality and abnormal effects and formulation used
Choung *et al*. 2011a / E153858	*Litoria raniformis* / Lab / TP (G26) for 10 wks	T/ >98	NA / (Y/NA); NS	glass	10 TP /10L; 2X/wk	**Metam** **Gro** **Surv**	**25 (N)** **25** **--**	**>25** **>25** **--**	Time to metamorphosis Mass; SVL Study indicated survival >97.5% for study; not explicitly reported in terms of NOEC/LOEC	Limited reporting of potential contaminants in food and water; number of egg masses used not specified; measured ammonium (not NH_3) Qualitative-Higher level of confidence; however there is a lack of reported measurements in food

Bold values were classified as 'quantitative' or 'qualitative – medium or higher level of confidence'

[2] TP = tadpole; LV = larvae; M= metamorphorsis; E= embryo; G = gosner stage; NF=Nieuwkoop and Faber; SVL=snout-vent-length; A = adults; H = hatch; J = juvenile; if reported in study, developmental stage or some other identifier at test initiation is given, if not reported than designated as NS (not specified); dph = days post hatch

[3] TS = Test substance; T = technical; F = formulation

[4] NA = not applicable; NS = not specified; ACTN = acetone; EtOH= ethanol; DMSO=dimethylsulfoxide; MeOH=methanol; IPA = isopropyl alcohol

[5] Negative Control (NC) and/or Solvent Control (SC) included in study design; MA in CNT = Measurable atrazine in control treatment group(s)- Yes (Y) or No (N) or Not Specified (NS)

[6] The values reported represent loading reported as mass (g) or number of larvae/tadpoles in a volume of water (L). If loading was reported as number of larvae/tadpoles in a certain test vessel size but test solution volume was not reported, then this is presented # organisms in tank (volume reported if available) as actual loading could not be determined ; PE = polyethylene

[7] Water

[8] Metam = metamorphosis; Gro = growth; Imm/Inf = immune system or infection; Bio/Mol = biochemical/Molecular; Sexdev = sexual development; Behav=behavioral; Surv = survival; N = nominal conc.; M = measured conc; NDR = not dose responsive (no significance at higher dose).

Citation / ECOTOX(E)/ MRID	Species / Source / Exposure Period (stages & test duration)[2]	TS[3] / % ai	Solvent (%)[4] / NC/SC (Y/N); MA in CNT (Y/N/NS)[5]	Test vessel material[6]	Loading; Renewal Period[7]	Reported Results[8]				Indentified Uncertainties / Concerns and Classification
						Endpoint	NOEC (µg/L)	LOEC (µg/L)	Effect	
										water and number of egg masses
Freeman & Rayburn 2005 / E81459	*X. laevis* / Nasco/Xenopus Express / TP (varied NF40 to 54) for 3-5 wks	T / 98	NA / (Y/NA); NS[9]	NS	NS; weekly	**Metam** Gro **Bio/Mol** Surv	**<100 (N)** 800 **Varied** --	**100** >800 **800** --	↓ metamorphic stage Mass ↑ flow cytometric analysis meant to illustrate metemorphosis and development); results varied based on test % Mortality (0-16.7%) for different assays, reported as occurring randomly in treatments; mortality in terms of NOEC/LOEC not reported	Stats for flow cytometric conducted on CVs; uncertainty in weight analysis with regards to accounting for different stages of organisms; fill volume of test vessel (3L) not reported however water quality appears to be acceptable Qualitative- Medium level of confidence for molecular endpoint due to analysis using CV and other reasons described for metamorphosis; Invalid for weight due to potential confounding factor; Qualitative –Higher –for metamorphosis stage; however, lack of analysis of atrazine in control
Howe *et al* 1998 / MRID 45202910	*R. pipiens, B. americanus* / F / TP (G29 & 40) for 96 h	F / 40.8	NA (Y/NA); N	glass	20 TP / 15L	**Surv 96 hr LC50**	--	--	*R. pipiens* (early stage) = 47600 *R. pipiens* (late stage) = 14500 *B. americanus* (early) = 26500 *B. americanus* (late) = 10700	Control survival not reported; no raw data; tested above water solubility Qualitative – Medium level of confidence based on lack of control data and only LC_{50} values reported and testing above water solubility
Kloas 2009a / E112914	*X. laevis* / Xenopus I / TP (8 dpf) for 83 d	T / NS	NA / (Y/NA); Y[10]	glass	<1 g/L/day; 7X/day	**Metam** **Gro** **Sexdev** **Surv**	**100 (N)** **100** **100** **100**	**>100** **>100** **>100** **>100**	Time to metamorphosis Mass; SVL>100[11] Sex ratio; gonadal development % Survival	Atrazine contamination of controls; marked fluctuations in water quality parameters; histological endpoint comparisons not made relative to amphibian reference Quantitative based on fact that although deficiencies, study design and reporting were robust enough to properly evaluate results
Morgan 1996 / E63246	*X. laevis* / Xenopus I (adults) / E for	F / 40.8	NA / (Y/NA); NS[12]	plastic	NS; 24 hr	**Metam** **Surv**	--	11000 or 1100 (4488	LC_{50} = 100000 or 126000 (40800 or51408 ai) EC_{50} (abnormalities) = 33000 or	Limited water quality reported, test solution fill volume not reported; conducted above water solubility; formulation used so source of toxicity

[9] Study reports that solutions measured from experimental tanks and atrazine concentrations were within expected range; however unsure if this included the control

[10] Atrazine detected in one out of 4 blocks of negative control tanks in one of the studies; it was excluded from analysis

[11] Significant decrease in female weight and SVL observed at 0.01, 1 and 25 for females only but not at 0.1 and 100 ppb in one of the two reported studies; No effect on wt and SVL reported in other study; EFED does not consider the differences in wt and SVL to be a concentration-dependent effect

[12] Test concentrations measured, but do not report results for control; unsure if analyzed

Citation / ECOTOX(E)/ MRID	Species / Source / Exposure Period (stages & test duration)[2]	TS[3] / % ai	Solvent (%)[4] / NC/SC (Y/N); MA in CNT (Y/N/NS)[5]	Test vessel material[6]	Loading; Renewal Period[7]	Reported Results[8]				Indentified Uncertainties / Concerns and Classification
						Endpoint	NOEC (µg/L)	LOEC (µg/L)	Effect	
	96 hr							or 448 ai)	<8000 (13464 or 3264 ai)	unknown Qualitative – Medium level of confidence based on limited water quality reported
Rohr *et al*. 2009 / E117315	*B. americanus* / Field / LV (G25-27) for 4 d	T/ 99	ACTN (0.0002) / (N/Y); NS	NS	6 TP/0.5L (96hr) then 1/3.5L; static	**Behav** Surv	**196 (M)** --	**>196** --	Fear cues (predator and parasite); activity Reported no effect on mortality with nearly 100% survival	No negative control (authors report no effect from solvent based on previous data); limited water quality reported Qualitative-Higher level of confidence, however no negative control and limited water quality
Spolyarich *et al*. 2010 / E153638	*L.tasmaniensis* / Field / TP (G28) to G42 (4 wks)	T/ 98	NA / (Y/NA); N	glass	10 TP/10L; 2X/wk	**Metam** **Gro** **Sexdev** Surv	**30 (N)** **30** **30** --	**>30** **>30** **>30** --	Rate of development Length Sex ratio; gonad development Reported 2.3% mortality in test overall; mortality not reported in terms of NOAEC/LOAEC	Sex determination based on gross morphology; histology on male gonads only; Metamorphosis and length data analysis appear to be based using the individual frog as the experimental unit and not the tank as data in figures appears to be on pooled results of the three trials Qualitative-Higher level of confidence as loading seemed within recommended levels, however, ammonia and nitrate concentrations not reported with a 2X/wk renewal period and field collected organisms (Sydney Australia suburb) so prior exposure history is unknown
Storrs Mendez *et al*. 2009/ E118898	*B. americanus* / Field / Metamorphs for 2 d	T/ 99.2	ACTN (NS)/ (NS/NS); NS	NS	NA; NA	**Behav** Surv	**1430** µg/kg(N) NS	**>1430** NS	Soil choice preference (ATZ or no ATZ)	Limited reporting on control group preparation-uncertainty regarding whether control contained solvent; uncertainty in measured conc. due to less than nominal and long storage time Qualitative-Medium level of confidence based on limited reporting for control preparation
Wan *et al*. 2006 / E89626	*R. catesbeiana* /Ward's Natural Science / TP (10cm) for 96 hr	T / 98 & F / 48.5	ACNT (NS); (Y/Y); NS (dilution water analysed)	glass	0.8 g/L; static	**Surv**	--	--	Technical 96 hr LC_{50} > 16000 Formulation 96 hr LC_{50} > 480000 (232800 ai)	Solvent concentration not reported; raw data not provided, control mortality not reported Qualitative – Medium level of confidence based on lack of summary data and no reported solvent concentration

Given the small number of studies available after filtering the studies with a classification of "Invalid" or "Qualitative –Lower level of confidence", a comparison of test design elements within an endpoint category is not really informative. Therefore, an effort was made to evaluate the breadth of toxicity values across all of the available studies identified in Appendix C regardless of whether further review of the study reported uncertainties that resulted in an "Quantitative", "Qualitative (almost all studies were in this category) or "Invalid" classification. The results of this broader analysis are discussed below.

Survival

Available acute data for amphibians indicate that they are relatively insensitive to technical grade atrazine with acute LC_{50} values > 10,000 µg/L for juveniles and embryos (e.g., Howe *et al*.,1998). Teratogenic effects were also evaluated for amphibian embryos with EC_{50} values ≥2,100 µg/L (Fort *et al*., 2004). The lowest acute value was reported by Birge *et al*. (1980) in which the reported 4 days post hatch LC_{50} for *R. catesbeiana* was 410 µg/L; this value represents both lethality as well as observed abnormalities expected to result in mortality under natural conditions.

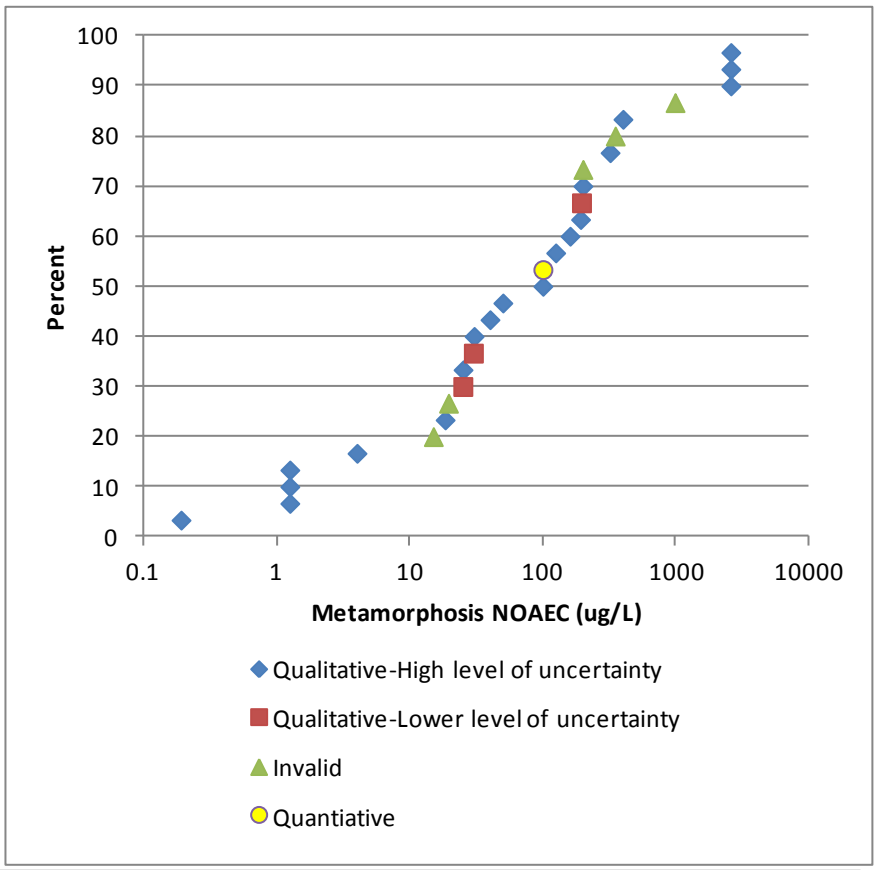
A wide range of sub-chronic or chronic survival results are available. No effects on survival have been reported at atrazine concentrations of up to and around 200 µg/L and above (400 µg/L) for several studies and species: *X. laevis* (Allran and Karasov, 2000 and Hayes *et al*., 2002); *Hyla versicolor* (LaFiandra *et al*.,2008); and *A. barbouri* (Rohr *et al*., 2003). Long term carry-over effects on survival for *A. barbouri* were reported at 4 µg/L, lowest concentration tested (Rohr *et al*.,(2006). In addition, chronic (32 days) atrazine exposure 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). For spring peepers, American toads and green frogs, survival was significant at the lowest concentration tested, 2.84 µg/L; however, survival was not always significant at higher concentrations (25 and 64 µg/L). In this study, atrazine was tested as a formulation (85.5% atrazine), therefore, there is uncertainty in if, and how, the inert ingredients may have influenced the toxicity.

Metamorphosis

Effects on metamorphosis were reported in 29 laboratory studies at concentrations ranging from 3 to 400 µg/L; acute studies such as those evaluated under the FETAX (frog embryo teratogenesis assay- *Xenopus*) protocol were not included. The distributions of the reported metamorphic NOAECs and LOAECs for the available studies along with their classification are presented in **Figure 7** (distribution plotted used the Weibull method). The graphs are meant to serve as a qualitative representation of the range of reported effects and the corresponding

study classification; the graphs are not intended to obtain a quantifiable percent value using the curve (*e.g*., 50% of studies are below this concentration). Some studies examined effects on more than one species concurrently using the same atrazine concentrations which resulted in the same reported NOAEC or LOAEC values for all or some of the tested species. The ability to visually discern each species on the distribution graph was considered desirable, so each species with the same NOAEC/LOAEC was ranked which skewed the percent distribution slightly. In addition, not every study reported had a definitive NOAEC and LOAEC (due to observed effects at all or none of the tested concentrations or use of only one test concentration); therefore, there is not an even number of data points between the NOAEC and LOAEC graphs. Seven studies reported effects at 100 µg/L or less: Koprivnikar, 2010; Coady *et al.,* 2004; Rohr *et al.,* 2004; Larson *et al.,* 1998; Olivier and Moon, 2010; Brodeur *et al*., 2009 and Freeman and Rayburn, 2005. Of the seven studies reporting effects on metamorphosis at 100 µg/L or less, two were conducted with ranids (*Rana pipiens* and *R. clamitans*) and three were conducted with salamanders (*Ambystoma tigrimum, A. barbouri, and A. maculatum*); the other two were conducted with the frogs *Rhinella arernarum* and *X. laevis*. Effects included: 1) reduced developmental stage at test termination at 3 µg/L, only concentration available for analysis (Koprivnikar, 2010); 2) longer time to metamorphosis (Coady *et al.,* 2004) at 11 but not 28 µg/L; 3) decrease in time (day) of metamorphosis (presented as year standardized means) at 40 µg/L but not at 4 µg/L (Rohr *et al.,* 2004); 4) delayed metamorphosis at 81.8 µg/L (Larson *et al.,* 1998); 5) decrease in time to metamorphic stage at 100 µg/L (Brodeur *et al.,* 2009; Freeman and Rayburn, 2005); and 6) stage before death or hatch at 100 µg/L (Olivier and Moon, 2010).

One reported common design element for these four studies is the use of a co-solvent. Ethanol was used in both of the ranid studies, and DMSO or acetone was used in the other studies. However, co-solvents such as ethanol and acetone were also used in other studies that did not report effects on metamorphosis at atrazine concentrations similar to the studies described above (Hayes *et al.,* 2006b- NOAEC = 0.19 µg/L for *R. pipiens*; Storrs and Semlitsch, 2008-NOAEC = 30.4 µg/L for *R. sphenocphala*).



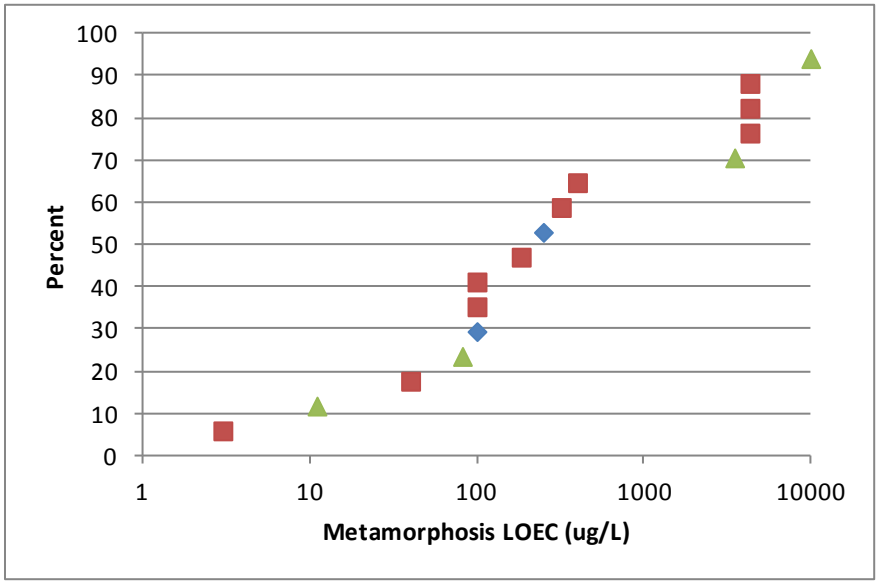


Figure 7. Distribution of Metamorphic NOAECs and LOAECs and Study Classification

Growth

Growth endpoints (*e.g.*, mass and snout-vent-length (SVL)) were examined in 27 of the available laboratory studies. Many of the studies reported effects at or below 400 µg/L and examined both metamorphosis and growth (**Figure 8**). Both of these endpoints are frequently linked together (U.S. EPA, 2007a; Rohr and McCoy, 2010) as growth is reported in the context of metamorphosis. For the laboratory studies, adverse effects on growth were reported from 0.19 µg/L to 800 µg/L (one study at 800 µg/L) with three laboratory studies reporting effects at less than 100 µg/L. In Hayes, *et al*. (2006b) an effect on *R. pipiens* growth (decreased mass and SVL), but not metamorphosis, was reported at 0.19 µg/L; however, Koprivnkiar (2010) reported both an effect on *R. pipiens* growth and metamorphosis at 3 µg/L (*ca*. 100% mortality reported at 300 µg/L). Reported decrease in mass for *X. laevis* at 20 µg/L (lowest concentration tested) was lower than the reported effect on metamorphosis (increase in time to metamorphosis) (LOEC >320 µg/L) for the same study (Sullivan and Spence, 2003). Studies also reported no effects on growth or metamorphosis at atrazine concentrations of 30 µg/L or less: 1.25 µg a.i./L for *B. americanus*, *H. versicolor*, and *P. triseriata* (Williams and Semlitsch, 2010). Several studies have reported no effects for growth around 20-30 µg/L, although they may have reported effects at higher concentrations, for example: Choung *et al*. (2011) reported a NOEC for growth and metamorphosis at 25 µg/L in *Litoria raniformis*; Zaya *et al*. (2011) reported a NOAEC for *X. laevis* growth at 25 µg/L with LOEC at 200 µg/L.

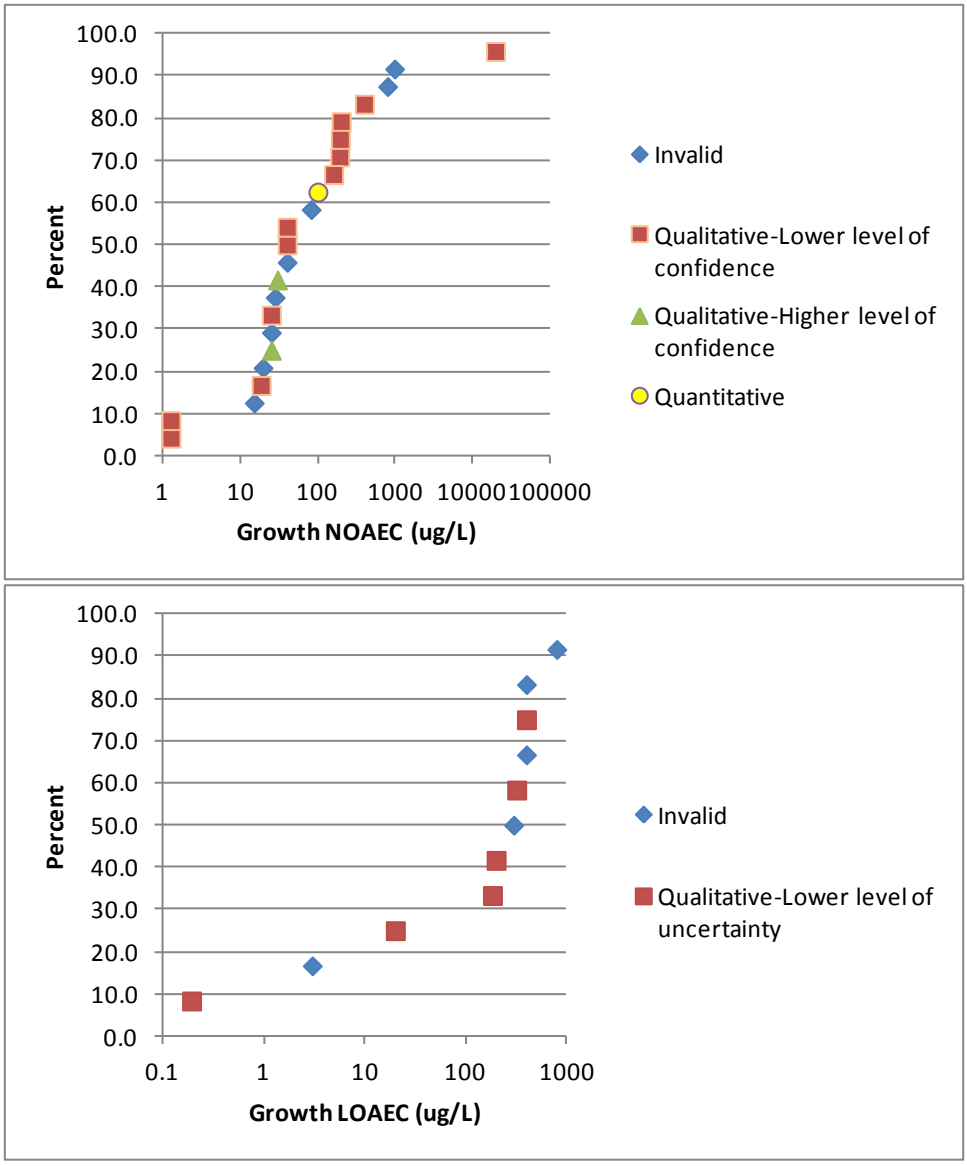


Figure 8. Distribution of Growth NOAECs and LOAECs and Study Classification

Sexual Development

Many studies have been conducted that evaluate different aspects of sexual development (e.g., sex ratio, gonad development, other organs involved in reproduction) in amphibians. Effects on sexual development (*i.e.*, change in sex ratio, increase in gonadal malformations (ovotestes), changes in larynxgeal muscles) were reported at atrazine concentrations of 50 µg/L or lower.

Seven out of eleven of these studies report effects on sex ratio and gonadal malformations at concentrations of 15 µg/L atrazine or lower.

Effects on sex ratio were reported for atrazine concentrations ranging from 0.92 to 124 µg/L (**Figure 9**). However, observed effects on sex ratio need to be considered with respect to when the effect was measured in the life cycle of the organism. Many of the studies were conducted to metamorphosis; however, there is evidence that somatic development (metamorphosis) and gonadal maturation do not necessarily coincide, *i.e*., gonadal maturation occurs later in the life cycle (Storrs and Semlitsch, 2008) but this cited studies has uncertainties including test design and lack of reported water quality.

Effects on gonadal development and morphological changes were examined in several studies. Gonadal effects such as observation of ovotestes, changes in testicular morphology, effects on gonadal somatic index compared to the controls were reported as well as changes in other organs used for reproduction or mating (*e.g*., larynx) were reported at atrazine concentrations ranging from 0.1 to 25 µg/L atrazine. Several of these studies were evaluated in the 2003 and 2007 SAPs: Hayes *et al*. 2002, 2003, 2006a, 2010a; Tavera-Mendoza *et al*. 2002a and 2002b, Goleman *et al*. 2003; and Hecker *et al.,* 2005b. While ethanol was used in only about half (5 out of 11) of the studies discussed above, these studies comprise the low end of the effects curve with effects ≤10 µg/L. However, no effect on sexual development were also reported at concentrations greater than the adverse effect concentrations described above (Spolyarich *et al*., 2010; Kloas *et al*., 2009a; LaFiandra *et al*., 2008).

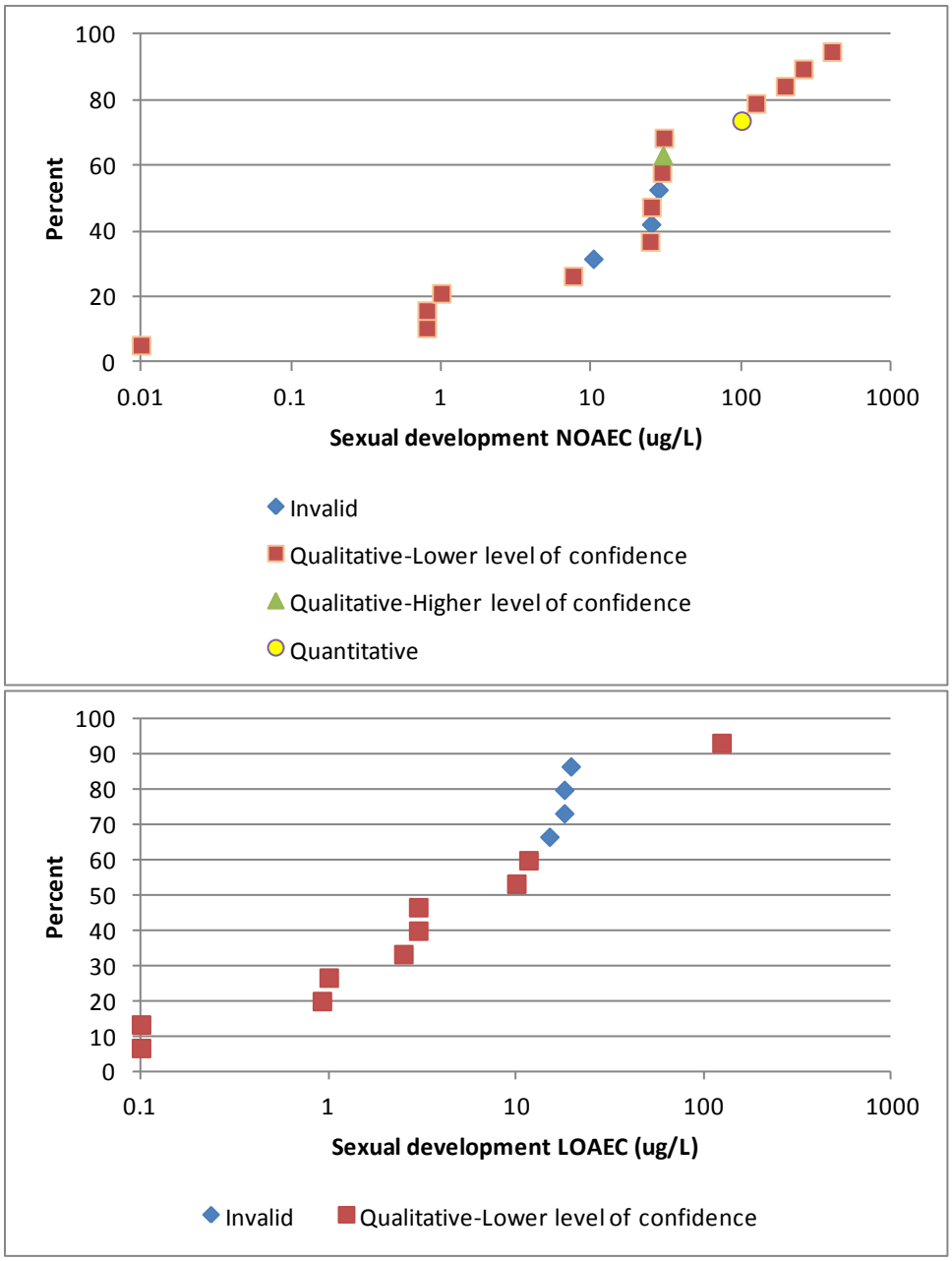


Figure 9. Distribution of Sexual Development NOAECs and LOAECs and Study Classification

Biochemical and Molecular Endpoints

Studies evaluating biochemical and/or molecular endpoints reported effects primarily at concentrations ≤ 500 µg/L. Many of the studies examined a diverse array of endocrine-related endpoints (*e.g*., aromatase, estradiol, and testosterone). Studies reported changes in a variety of biochemical endpoints (*e.g*., testosterone, estradiol, corticosteroid and thyroxine) at concentrations less than 100 µg/L: Coady *et al.,* 2005; Hayes *et al*., 2010a, Hayes *et al*., 2002; and Larson *et al*., 1998. No effect on biochemical endpoints at concentrations of 25 µg/L and above were also reported (Kloas *et al*., 2009a; Oka *et al*., 2008; Hecker *et al*., 2005). However, there are uncertainties with all of these cited studies, except Kloas *et al.* 2009a, which limit the ability evaluate these endpoints.

Again, cosolvent (ethanol or isopropyl alcohol) was used in several of the studies where effects ≤25 µg/L were observed; other studies (*e.g*., Coady *et al*., 2005; Hecker *et al.,* 2005a and 2005b; Villeanue *et al*., 2003) reported no effect on biochemical endpoints (*e.g*., estradiol, aromatase, testosterone) where no cosolvent was used. As there were diverse biochemical and molecular endpoints discussed and evaluated, a graph illustrating the NOAECs and LOAECs may not be very informative and misleading due to the varied endpoints. This is similar for the discussion on the immune system and behavior modifications.

Immune System and Infection

Several papers evaluated the potential effects of atrazine on the immune system and susceptibility to infection; several different immune response endpoints were examined in addition to susceptibility to infection. The majority of the studies evaluating the immune system report effects on ranids at or below 200 µg/L. Several studies reported effects at 30 µg/L or less: Brodkin *et al,.* 2007 (decrease in number of phagocytic cells (at 0.01 µg/L) and a decrease in white blood cells and mean percentage of peritoneal phagocytic cells at 21 µg/L in adult *R. pipien* frogs); Houck and Sessions (2006) (reduction in the number of plaques representing antibody-secreting cells at 1 µg/L for adult *R. pipiens*); Forson and Stofer (2006) (decreased leukocyte levels (16 and 160 µg/L) and increased infections of *Ambystoma tigrinum* virus (ATV) at 16 µg/L in tiger salamanders, *Ambystoma tigrinum* with a NOAEC of 1.6 µg/L reported; and Koprivnikar *et al.,* 2007 (increase in intensity of infection in *R. clamitans* tadpoles at 30 µg/L). An increase in activated caspase3 immunopositive cells was reported at 400 µg/L, NOAEC of 200 µg/L in *X. laevis* (Zaya *et al*., 2011a). No effects on viral load was reported at 200 µg/L in *A. tigrinum* (Kerby *et al*., 2009);

Behavioral Modification

There were some studies that evaluated behavioral aspects (*e.g.*, feeding, locomotion, avoidance) of amphibians when exposed to atrazine concentrations of ≤400 µg/L. Suppressed mating behavior was reported at an atrazine concentration of 2.5µg/L in *X. laevis* (Hayes *et. al*. 2010). In Goleman *et al*. (2003) abnormal swimming was observed in *X. laevis* tadpoles at 19.5 µg/L atrazine with a reported NOAEC of 10.3 µg/L. Increased activity with decreased water conserving behavior (*i.e*., huddled, against side of dish, inactivity) was observed at 40 µg/L, but not at 4 µg/L, in adult *A. barbouri* salamanders (Rohr and Palmer, 2005). In Rohr et al. 2003 and 2004, adverse behavior modification (increased activity after tapping on glass tank, and reduced shelter use was reported at 400 µg/L, but not 40 µg/L, for *A. baarbouri*. No effect on fear cues were reported for B. americanus at 196 µg/L (Rohr *et al*., 2009).

One observation that occurred during the evaluation of all the studies was that the use of a co-solvent appeared to be a commonality. Therefore, an effort was made to determine whether there may be a relationship between the use of a co-solvent and whether a significant effect was reported. The numbers of laboratory studies that reported an effect on any endpoint as well as the number of studies that reported no observed effects were tallied relative to whether the study did or did not use a co-solvent ($p>0.05$, Chi-square test) (**Figure 10**). The results do not suggest that the use of a co-solvent is associated with whether or not the study demonstrated a significant effect. These results include observed effects that range from 0.1 µg/L to 10,000 µg/L.

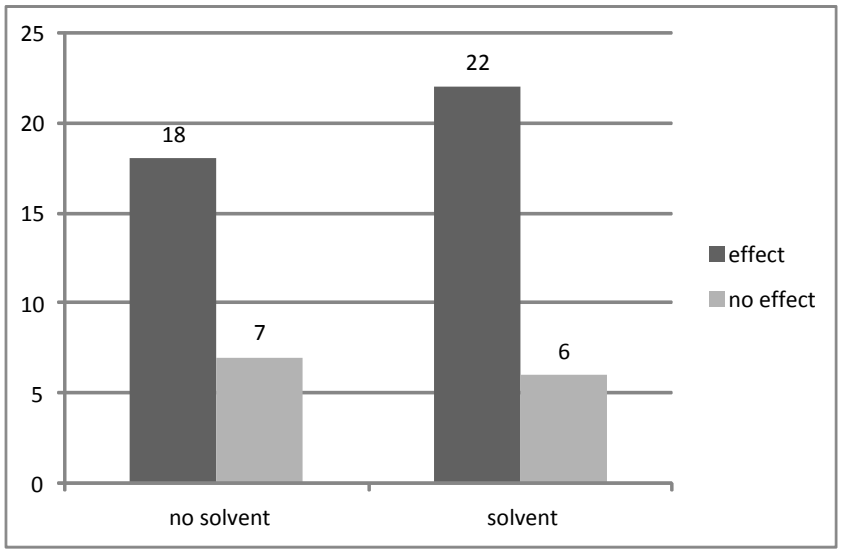


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Figure 10. Number of studies that reported an overall effect or not along with use of a solvent

Cosm Studies

While not included in the analysis described above, several cosm (micro and meso) studies that examined effects of atrazine on amphibians were available. Effects on the endpoints discussed above at concentrations of 100 µg/L or below, are discussed here. A summary of the cosm studies and the identified uncertainties associated with each study are presented in Appendix C. Metamorphosis was examined in the cosm studies. One cosm study reported reduced number of animals reaching metamorphosis at 0.1 µg/L; however, growth and age at metamorphosis were not affected (Langlois *et al.* 2010). No effect on metamorphosis was also reported for other cosm studies at concentrations from 6.4 to 197 µg/L (Relyea *et al,.* 2009; Rohr and Crumrine, 2005; and Du Preez *et al.,* 2008). In the cosm studies, growth effects were observed around 200 µg/L for *R. sphenocephala* and *B. americanus* (Boone and James, 2003; Diana *et al.,* 2000) except for Relyea (2009) who reported increased body weight for gray tree frogs (*H. versicolor*) treated with 6.4 µg/L, although time to metamorphosis was not affected.

For behavioral modifications, the cosm study by Rohr and Crumrine (2005) reported a decrease in the percentage of *R. sylvatic* tadpole hiding and an increase in tadpole activity compared to the control at 50 µg/L. In another cosm study, Rohr *et al.* (2008) reported a decrease in melanomacrophages in *R. pipiens* and eosinophils with an increase in trematode cysts in *R. palustris* at an atrazine concentration of 117 µg/L. Survival was reported as significantly lower for *R. pipiens* but not for *R. palustris,* compared to control. A cosm study by Langlois *et al.* (2010) reported a change in brain and tail biochemistry (changes in estrogen receptor-α and tail dio3 enzyme (involved in thyroid conversion)) in *R. pipiens* at an atrazine concentration of 1.8 µg/L. In the cosm study by Langlois *et al.* (2010) a significant change in sex ratio was observed at 1.8 µg/L for *R. pipiens.*

7.5. Evaluation of Overall Test Design Elements

After compiling studies within one of the identified endpoints above, visually comparing specific study design elements (e.g., solvent use, tank vessel material) between the studies did not reveal any obvious relationship. Also, there does not appear to be a clear dose-response for any endpoint across species. Similar to the situation and range of uncertainties that the SAP was asked to evaluate in 2003, EPA has been unable to find any clear and consistent effects correlated to atrazine exposures across amphibian species, in spite of the large number of studies that purport that such effects exist. A major deficiency with the available amphibian data is the lack of an adequately standardized protocol for chronic amphibian testing, especially in regards to husbandry for native species. The available amphibian data represent a variety of methodologies that have various uncertainties, which limit the ability to discern effects from atrazine without confounding factors. The one study in which all of the identified test design elements were accounted for remains the one that was conducted in response to the DCI in 2004 (which was comprised of two studies conducted in parallel at two different laboratories).

This study did not demonstrate any consistent, concentration-dependent effects of atrazine on sexual development, metamorphosis, growth and survival of *X. laevis* at atrazine concentrations of 0.01 to 100 µg/L (referenced in 2007 SAP and in Kloas *et al*., 2009). The SAP in 2007 expressed concern regarding the extent to which *Xenopus* can be considered representative of native species, while the SAP in 2003 could not identify any compelling reason why the species could not serve as a surrogate. Although a range of effects are noted in the open literature studies, the studies have internal inconsistencies (*e.g*., absence of a dose response, conflicting results) that make it difficult to generalize about any trends across studies. Previously identified uncertainties regarding the potential effects of atrazine on native species (*e.g*., *R. pipiens*) and potential effects of the compound on sexually mature adults or immune response have not been resolved through the available open literature. Therefore, at this time, EPA does not have additional data on amphibians that is sufficient and robust which alters its quantitative understanding of the potential toxicity of atrazine to this taxon.

7.6. Other Evaluations – Published Literature Reviews

The EPA is aware of previous attempts to investigate a relationship between atrazine exposure and adverse effects on amphibians as well as other taxa since the 2007 SAP (Rohr and McCoy, 2010; Hayes *et al.*, 2011; Solomon *et al*., 2008; Mann *et al*., 2009; Vandenberg *et al.,* 2012; Bernanke and Köhler, 2008; Hayes *et al*., 2010). For an open literature paper to be considered for potential inclusion in a risk assessment, the paper is the primary source of the data (US EPA, 2011). Therefore, while the references in the literature review paper may be extracted for screening for further potential review, the literature review papers themselves are typically not considered for further review.

In the paper by Rohr and McCoy (2010), a similar binning exercise as the one described above was conducted. The authors made determinations about the inclusion/exclusion of data in their evaluation. Primary reasons for exclusion of this paper include inadequate reporting of statistical parameters or inappropriate analyses, and control contamination. These factors are very important in being able to infer causality. Their analysis included studies that showed trends and studies in which compounds other than atrazine were present (*e.g*., mixtures and agricultural sites). Evaluation of potential effects in this paper was done by tallying the number of studies that reported an effect and those that did not. This process gave equal weight to each represented study regardless of potential confounding factors beyond those that were considered in their analysis. The authors stated that for survival endpoints, their general conclusions from the studies are consistent with other reviews (Giddings *et al.*. 2005; Huber 1993 and Solomon *et al.* 1996, 2008) in that there is no consistent published evidence that atrazine (at environmentally relevant concentrations) is directly toxic to fish or amphibians with some important exceptions (*e.g*., Alvarex and Fuiman 2005; Rohr *et al.*. 2006b, 2008c, Storrs and Kiesecker 2004). The study authors conclude that while there is much left to learn about

atrazine effects, they identified several consistent effects of atrazine that must be considered when conducting a cost-benefit analysis.

The review by Hayes *et al.*(2011) evaluated atrazine effects on demasculinization and feminization of male gonads across vertebrate classes including amphibians. This review examines the effects of atrazine on sexual development for different vertebrate classes applying the nine "Hill criteria." The authors identify studies in which they believe support each of the nine criteria. The study authors state that the situation of atrazine as an endocrine disruptor which demasculinizes and feminizes male vertebrates meets all nine of the "Hill criteria".

In the review by Solomon *et al*. (2008), a similar effort has described above was used in which the authors evaluated laboratory and field studies and assessed causality using procedures derived from Koch's postulates and the Bradford-Hill guidelines. The authors state that they identified strengths and uncertainties, and some studies were omitted from their summary tables due to concerns about data quality. The authors report that on a weight of evidence analysis, the theory that atrazine at environmentally-relevant concentrations affect reproduction and/or reproductive development in fish, amphibians and reptiles is not supported by vast majority of observations. They further state that this conclusion holds for other theories (e.g., effects on biochemical endpoints, immune function, or parasitism).

An examination of amphibians and agricultural chemicals was presented by Mann *et al*. (2009). Effects on amphibians, in addition to potential mechanisms of toxicity, from chemicals such as atrazine among others were discussed. Similar to the other reviews, the study authors identified studies that reported effects as well as reported no effects for various endpoints such as sexual development, metamorphosis, growth and immune response. The study authors argue that more emphasis needs to be placed on examining pesticide mixtures.

A review by Vandenberg *et al.* (2012) on low-dose effects and nonmonotonic dose response included a discussion on atrazine exposure and sexual development. The study authors cite studies in which effects on sexual development were reported as well as studies that reported no effects. For amphibians, based on a weight-of-evidence (reported as taking together the results from the studies that reported effects along with one negative study), the study authors conclude that low-dose atrazine adversely affects sexual differentiation.

A paper (Bernanke and Köhler 2008) on the impact to wildlife vertebrates from environmental chemicals included a discussion about atrazine. As before, the study authors discuss the impact of pesticides and cite studies which report effects to amphibians from atrazine exposure for several different endpoints such as survival, metamorphosis, behavior modifications, and sexual development.

A paper on potential causes for amphibian declines (Hayes *et al*. 2010) cites studies that report effects from atrazine exposure on sexual development and behaviors, metamorphosis, uptake of atrazine and immune/infection response.

Studies which serve as a foundation for the authors' analysis/discussions have been previously reviewed by both EPA and FIFRA SAPs and determined to have limited confidence in the reported results. Similar to the analysis discussed in this document, these studies cannot reasonably be used to make inferences to refute or confirm the various hypotheses that have been developed regarding the potential effects of atrazine on amphibians.

While some of the literature reviews discussed above comment on the exclusion of studies based on various factors, the EPA does not think that the extent of the exclusion criteria that appeared to be used in these reviews is robust enough to allow for equal comparison among the remaining filtered studies. It appears that the overall process (weight of evidence approach) used to evaluate the potential for atrazine to exert adverse effects on amphibians, after their exclusion process, is a tally system. Given that EPA has differing levels of confidence in these studies, EPA does not believe those procedures are adequate to capture the complex nature of the data and reach a weight-of-the-evidence determination about the hazards to amphibians.

7.7. Evalution of Amphibian Studies and Adverse Outcome Pathways

The available amphibian data suggest that the range of effects reported for amphibians exposed to atrazine vary considerably between species and that the majority of these measurement endpoints do not appear to exhibit a monotonic dose response. Effects on metamorphosis, growth and development as well as sexual development have been reported. Some of these endpoints are linked, such as size in regards to time to metamorphosis, and therefore significant differences for one endpoint may be autocorrelated to another effect endpoint. Many uncertainties and concerns in the conduct and results of the available amphibian data have been identified. Therefore, it is difficult to make definitive conclusions about the impact of atrazine at a given concentration. At this time, there is insufficient information or data on atrazine to make inferences about molecular initiating events that ultimately lead to an adverse outcome, *i.e*., capable of affecting the survival, growth and reproduction of amphibians, which is readily replicated and of sufficient rigor to enable its use in risk assessments. However, the EPA will continue to review data as they become available.

CHAPTER III. ANALYSIS PLAN

8. Conceptual Model

For a pesticide to pose an ecological risk, it must reach ecological receptors in biologically significant concentrations. An exposure pathway is the means by which a pesticide moves in the environment from a source to an ecological receptor. For an ecological pathway to be complete, it must have a source, a release mechanism, an environmental transport medium, a point of exposure for ecological receptors, and a feasible route of exposure.

The conceptual model for atrazine provides a written description and visual representation of the predicted relationships between atrazine, potential routes of exposure, and the predicted effects for the assessment endpoint. A conceptual model consists of two major components: risk hypothesis and a conceptual diagram (USEPA, 1998).

Based on the submitted environmental fate data, atrazine is expected to leach to ground water and move to surface water through runoff and spray drift.

Based on previous ecological risk assessments for atrazine, there is the potential for risk for federally listed threatened/endangered (hereafter referred to as "listed") and non-listed birds, mammals, plants and aquatic species from labeled atrazine uses. Because of the potential risk for direct effects to taxa (both listed and non-listed) described above and in the previous assessments, listed species in all taxa may potentially be affected indirectly due to alterations in their habitat and prey items (*e.g.*, food sources, shelter, and areas to reproduce). These preliminary conclusions are used to derive the risk hypothesis and conceptual diagram discussed below.

8.1. Risk Hypothesis

A risk hypothesis describes the predicted relationship among the stressor, exposure, and assessment endpoint response along with the rationale for their selection. For atrazine, the following ecological risk hypothesis is being employed for this ecological risk assessment:

> *Based on the application methods, mode of action, fate and transport, and the sensitivity of non-target aquatic and terrestrial species, atrazine has the potential to reduce survival, reproduction, and/or growth in non-target terrestrial and aquatic organisms as well as negatively affect the structure, productivity, and function of aquatic plant communities when used in accordance with the current labels. These non-target organisms include listed and non-listed species.*

8.2. Conceptual Diagram

The environmental fate properties of atrazine indicate that runoff, leaching, spray drift and direct spray represent potential transport mechanisms to aquatic and terrestrial habitats where non-target organisms may be exposed. Additional pathways are considered for the evaluation to identify other potential routes of exposure that may be of concern. These transport mechanisms (*i.e.*, sources) are depicted in the conceptual diagrams below (**Figure 11, Figure 12**, and **Figure 13**) along with the receptors of concern and the potential attribute changes in the receptors from exposures to atrazine.

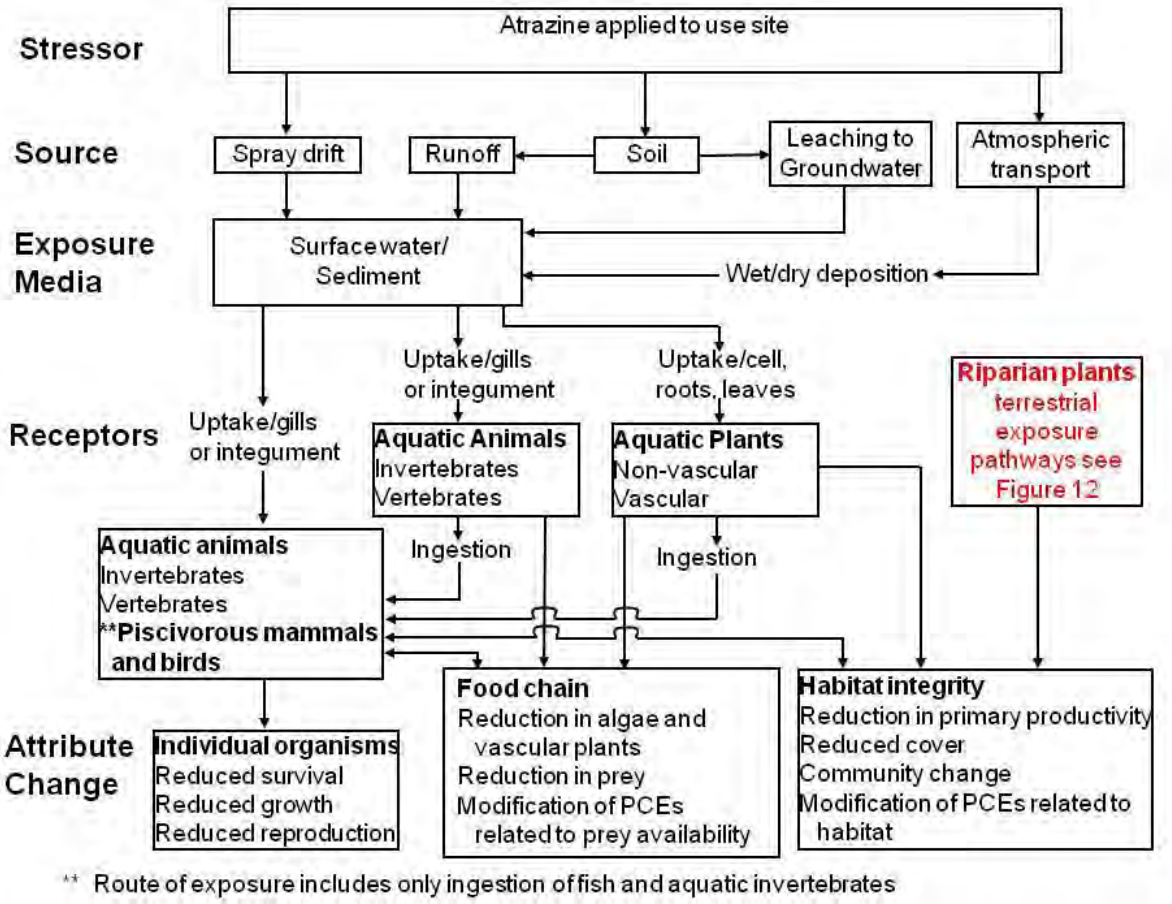


Figure 11. Conceptual Model for Atrazine Effects on Aquatic Organisms.

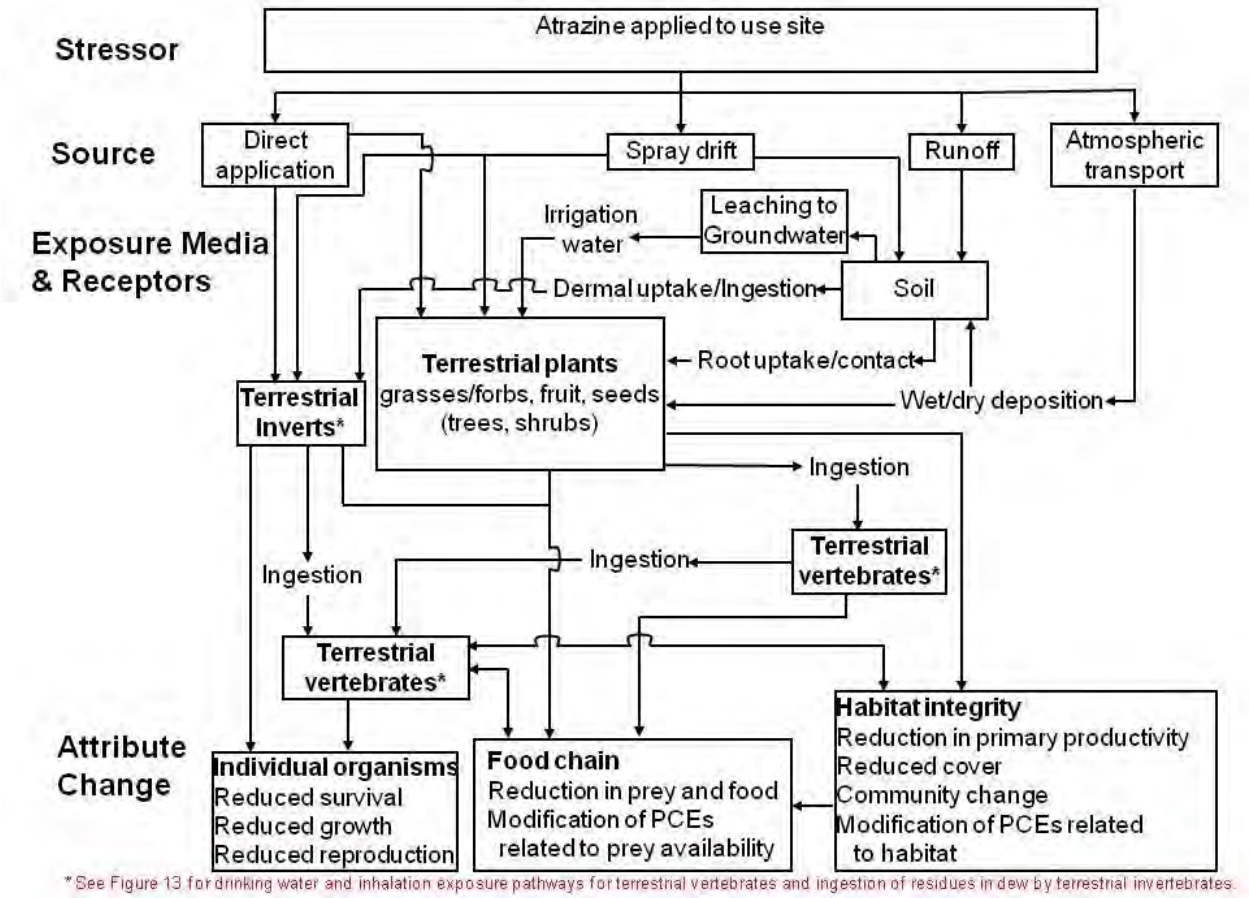


Figure 12. Conceptual Model for Atrazine Effects on Terrestrial Organisms**.**

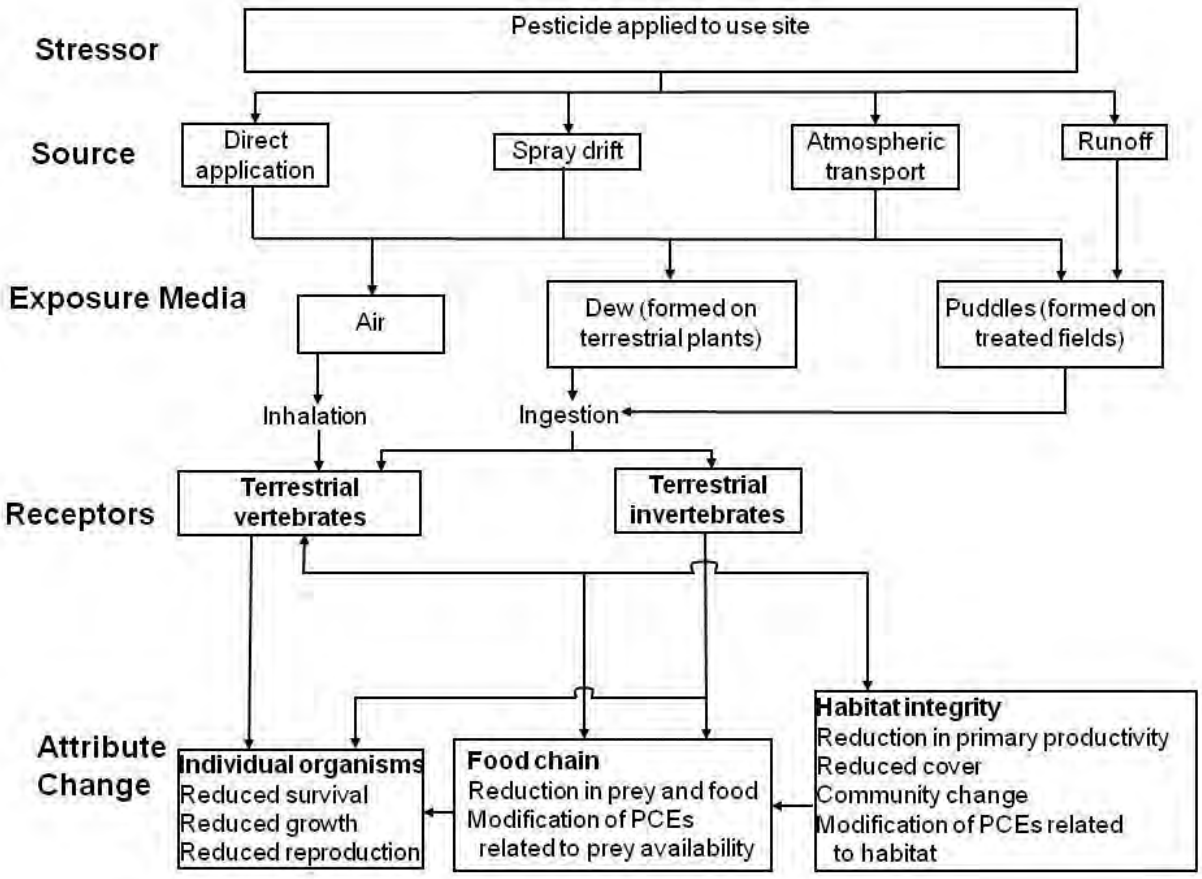


Figure 13. Conceptual Model for Atrazine Routes of Exposure for Terrestrial Animals**.**

In order to address the risk hypothesis, the potential for adverse effects on the environment will be estimated. The use, environmental fate, and ecological effects of atrazine will be characterized and integrated to assess the risks. There are two distinct components to the effects analysis: one for risk to aquatic plants and aquatic plant communities, and the other to all other organisms.

For all taxa not considered to be aquatic plants, in a screening level ecological risk assessment, risk characterization is based on a deterministic approach using the risk quotient (RQ) method which compares exposure over toxicity (USEPA 2004). For the toxicity value component, the lowest toxicity value (*e.g.*, LC_{50} or NOAEC) that is deemed appropriate for quantitative use is chosen from the available atrazine toxicity dataset.

The EPA's process to determine the level of concern (LOC) for atrazine in aquatic ecosystems to protect aquatic organisms and the methodology used to identify watersheds that exceed this LOC are described below. A different treatment is given to aquatic plant effects than other taxa

(*i.e.,* the RQs) because, although aquatic plants are generally more sensitive than fish and aquatic invertebrates, the risk assessment endpoint is community structure/function rather than growth, reproduction, and survival of an individual species. The LOC methodology uses single-species plant toxicity data and microcosm/mesocosm (cosm) studies to determine what atrazine exposure patterns and concentrations can cause adverse effects on aquatic plant communities. Before these cosm effects can be applied, there is a need for a quantitative measure of the relative severity of different exposure time series needs to be developed to compare effects among different experimental ecosystem exposure and to extrapolate these to the field.

The aquatic plant community LOC is derived to ensure that the atrazine concentrations in watersheds do not cause detrimental changes in aquatic plant community structure and productivity. While the LOC is based on effects to aquatic plant communities, by ensuring protection of primary producers, it is intended to provide protection for the entire aquatic ecosystem, including fish, invertebrates, and amphibians.

This analysis plan will be revisited and may be revised depending upon a full review of the data available in the open literature and the information submitted by the public in response to the opening of the Registration Review docket.

9. Measures of Exposure

In order to estimate risks of atrazine exposures in aquatic and terrestrial environments, all exposure modeling and resulting risk conclusions will be made based on maximum application rates for the currently registered uses as discussed in **Section** 3. Measures of exposure are based on aquatic and terrestrial models that estimate environmental concentrations of atrazine using maximum labeled application rates and application methods that have the greatest potential for off-site transport of the chemical. The models used to generate aquatic estimated environmental concentrations (EEC) are the Pesticide Root Zone Model (PRZM) coupled with the EXposure Analysis Model System (EXAMS). Model input values will be consistent with the most recent version of the Input Parameter guidance (current version 2.1). Additionally, measure of exposures, when possible, will be evaluated using monitoring data (Chapter IV).

PRZM (current version 3.12.3, June 2006) and EXAMS (current version 2.98.04.06, April 2005) are simulation models coupled with the graphical user interface, PE5 (PRZM EXAMS Model Shell; v5.0, November 2006), which incorporates the standard scenarios developed by EFED. The models generate daily exposures and 1-in-10-year EECs of atrazine that may occur over a 30-year period in surface water bodies adjacent to pesticide application sites. PRZM simulates application, movement, and transformation of a pesticide on an agricultural field and the resultant pesticide loadings to a receiving water body via runoff, erosion, spray drift, and leaching. EXAMS simulates the fate of the pesticide and resulting concentrations in the

receiving water body. Additional information on these models can be found at: http://www.epa.gov/oppefed1/models/water/models4.htm#surface_water

The standard watershed geometry used for ecological pesticide assessments assumes application to a 10-hectare agricultural field that drains into an adjacent 1-hectare water body that is 2 meters deep (20,000 m^3 volume) with no outlet. The composite model PRZM/EXAMS is used to estimate screening-level exposure of aquatic organisms to atrazine. The measure of exposure for aquatic species is the 1-in-10-year peak or rolling mean concentration. The 1-in-10-year peak is used for estimating acute exposures of direct effects to aquatic organisms. The 1-in-10-year 60-day mean is used for assessing the effects to fish and aquatic-phase amphibians from chronic exposure. The 1-in-10-year 21-day mean is used for assessing the effects on aquatic invertebrates from chronic exposure. Surface water monitoring data will also be considered in aquatic exposure assessment.

The model used to produce terrestrial EECs on food items is T-REX, while the model used to derive EECs relevant to terrestrial and wetland plants is TerrPlant. Detailed information about the models T-REX and TerrPlant, can be found on the EPA's website at http://www.epa.gov/pesticides/science/models_pg.htm#terrestrial. The AgDRIFT spray drift model (v2.01; May 2001; http://www.agdrift.com/AgDRIFt2/DownloadAgDrift2_0.htm) is used to assess exposures of organisms to atrazine deposited on terrestrial or aquatic habitats by spray drift.

The Screening Imbibition Program (SIP v.1.0, Released June 15, 2010) was used to calculate an upper bound estimate of exposure to wildlife via drinking water using atrazine's aquatic solubility limit (33 mg/L), and the most sensitive acute and chronic avian toxicity endpoints. Drinking water exposure alone was determined to be a potential pathway of concern for avian or mammalian species on a chronic basis but not on an acute basis. This pathway will be explored further with the development of SIP v.2.0 in the Ecological Risk Assessment for atrazine. Detailed information about the SIP v.1.0, as well as the tool, can be found on the EPA's website at http://www.epa.gov/pesticides/science/models_pg.htm#terrestrial.

The Screening Tool for Inhalation Risk (STIR v.1.0, November 19, 2010) was used to calculate an upper bound estimate of exposure to atrazine through inhalation. This calculation used atrazine's vapor pressure (2.89×10^{-7} torr) and molecular weight (215.69 g/mole) for vapor phase exposure, the maximum application rate (4 lbs a.i./acre) and method of application for spray drift, and acute and chronic avian and mammalian toxicity values. Results of the model run indicated that inhalation exposure via spray drift and/or vapor-phase of atrazine alone did not appear to be a concern. Detailed information about STIR v.1.0, as well as the tool, can be found on the EPA's website at: http://www.epa.gov/pesticides/science/models_pg.htm#terrestrial.

10. Measures of Effect

Ecological effects data are used as measures of direct and indirect effects to biological receptors. Data are obtained from registrant-submitted studies or from literature studies identified by ECOTOX (USEPA 2007c). The ECOTOX database provides more ecological effects data in an attempt to bridge existing data gaps, and is a source for locating single chemical toxicity data and potential chemical mixture toxicity data for aquatic life, terrestrial plants, and wildlife. ECOTOX was created and is maintained by the USEPA, Office of Research and Development, and the National Health and Environmental Effects Research Laboratory's Mid-Continent Ecology Division.

Information on the potential effects of atrazine on non-target animals is also collected from the Ecological Incident Information System (EIIS; USEPA 2007d). The EIIS is a database containing adverse effects (typically mortality) reports on non-target organisms where such effects have been associated with the use of pesticides.

Incidents reported in the aggregate incident reports and the Avian Incident Monitoring System (AIMS) will also be searched. AIMS is a database administered by the American Bird Conservancy (it was partially funded by the EPA). It contains publicly available data on reported avian incidents involving pesticides
http://www.abcbirds.org/abcprograms/policy/toxins/aims/aims/login.cfm?CFID=139273599&CFTOKEN=96183257

Where available, sub-lethal effects observed in both registrant-submitted and open literature studies will be evaluated qualitatively. Such effects may include behavioral changes such as lethargy and changes in coloration. Quantitative assessments of risks, though, are limited to those endpoints that can be directly linked to the EPA's assessment endpoints of impaired survival, growth, and reproduction.

11. Integration of Exposure and Effects

Risk characterization is the integration of exposure and ecological effect characterizations to determine the potential ecological risk from the use of atrazine and the likelihood of direct and indirect effects to non-target organisms in aquatic and terrestrial habitats. The exposure and effects data are integrated in order to evaluate potential adverse ecological effects on non-target species. For the assessment of atrazine risks, the risk quotient (RQ) method is used to compare estimated exposure and measured single-species toxicity values. Acute and chronic EECs from are divided by acute and chronic single-species toxicity values. The resulting RQs are then compared to the EPA's Levels of Concern (LOC) (USEPA 2004). In addition, the Agency will assess atrazine risk to aquatic plant communities, and thus all aquatic organisms, with an Agency developed method for determining the aquatic plant community LOC. EPA's method is

described below in **Chapter IV**. These criteria are used to indicate when atrazine's use, as directed on the labels, has the potential to cause adverse direct or indirect effects to non-target organisms and communities. In addition, incident data from EIIS, aggregate incident reports, and AIMS will be considered as part of the risk characterization.

Chapter IV. METHODOLOGY FOR DETERMINING THE LEVELS OF CONCERN FOR ATRAZINE.

12. The Risk Quotient Method and Levels of Concern for Terrestrial Plants and Terrestrial and Aquatic Animals.

The Risk Quotient Method is used to integrate the results of exposure and ecotoxicity data. For this method, Risk Quotients (RQs) are calculated by dividing exposure estimates by the acute and chronic ecotoxicity values (i.e., RQ = EXPOSURE/TOXICITY). These RQs are then compared to OPP's levels of concern (LOCs). These LOCs are criteria used by OPP to indicate potential risk to non-target organisms and the need to consider regulatory action. EFED has defined LOCs for acute risk, acute restricted use classification, acute and chronic risk to endangered species. Risk presumptions, along with the corresponding RQs and LOCs are summarized in **Table 18.**

Table 18. Risk Presumptions and LOCs			
Risk Presumption		**RQ**	**LOC**
Birds[1]			
	Acute Risk	EEC/LC_{50} or LD_{50}/sqft or LD_{50}/day	0.5
	Acute Restricted Use	EEC/LC_{50} or LD_{50}/sqft or LD_{50}/day (or LD_{50} < 50 mg/kg)	0.2
	Acute Endangered Species	EEC/LC_{50} or LD_{50}/sqft or LD_{50}/day	0.1
	Chronic Risk	EEC/NOEC	1
Wild Mammals[1]			
	Acute Risk	EEC/LC_{50} or LD_{50}/sqft or LD_{50}/day	0.5
	Acute Restricted Use	EEC/LC_{50} or LD_{50}/sqft or LD_{50}/day (or LD_{50} < 50 mg/kg)	0.2
	Acute Endangered Species	EEC/LC_{50} or LD_{50}/sqft or LD_{50}/day	0.1
	Chronic Risk	EEC/NOEC	1
Aquatic Animals[2]			
	Acute Risk	EEC/LC_{50} or EC_{50}	0.5
	Acute Restricted Use	EEC/LC_{50} or EC_{50}	0.1
	Acute Endangered Species	EEC/LC_{50} or EC_{50}	0.05
	Chronic Risk	EEC/NOEC	1
Terrestrial and Semi-Aquatic Plants			
	Acute Risk	EEC/EC_{25}	1
	Acute Endangered Species	EEC/EC_{05} or NOEC	1

[1] LD_{50}/sqft = (mg/sqft) / (LD_{50} * wt. of animal)
LD_{50}/day = (mg of toxicant consumed/day) / (LD_{50} * wt. of animal)
[2] EEC = (ppm or ppb) in water

13. The Method for Determining the Level of Concern for Aquatic Plant Communities

While reviewing this section, please consider the charge questions below.

SAP Questions:

- The Aquatic Plant Community CE-LOC methodology for atrazine is a four stage process that uses single-species plant toxicity data and cosm studies to discern atrazine concentrations and exposure durations that may cause adverse effects on aquatic plant communities. As a result, a CE-LOC for atrazine is developed which, together with monitoring data, can be used to identify watersheds where concentrations may result in adverse effects to aquatic plant community structure, function, and/or productivity. Please comment on the methodology EPA has used to derive the atrazine CE-LOC for aquatic plant communities, and in particular on EPA's characterization of the uncertainties and assumptions in this methodology (Chapter IV, Sections 13 & 14).

- The 2009 SAP recommended using an effects index or concentration metric, rather than categorical LOC thresholds in order to take advantage of data from Syngenta's Atrazine Ecological Exposure Monitoring Program (AEEMP). At that time the LOC threshold for atrazine effects to plant communities was established at 10 µg/L for a 60-day rolling average. The current analysis using the Plant Assemblage Toxicity Index (PATI) indicates the CE-LOC can range from 4 to 7 µg/L (Chapter IV, Section 14.3 & 14.4). Please comment on this CE-LOC and whether it reasonably represents a range below which permanent or irreversible change in aquatic plant community structure, function, and/or productivity due to atrazine exposure would not be expected.

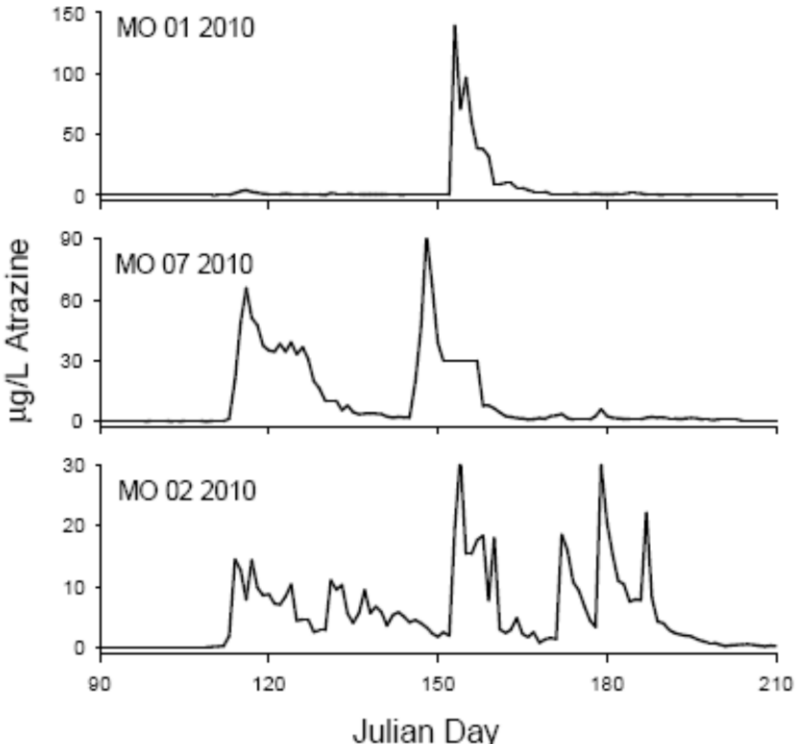
- Based on previous analyses of the available ecotoxicity data, EPA concluded for atrazine that the level of concern for effects on aquatic plant communities (CE-LOC) was lower than the atrazine concentrations observed to produce significant direct or indirect effects on invertebrates, fish and amphibians. Given the current analysis of the ecotoxicity data (Chapter I, Section 6) and the Aquatic Plant Community LOC methodology, EPA continues to believe the original conclusion still holds true. Please comment on how well the available database supports EPA's conclusion that the CE-LOC is lower than exposures that result in significant effects on the growth, survival and reproduction of aquatic animals.

The Aquatic Plant Community LOC Methodology.

The focus of this methodology is to determine a level of concern at which atrazine concentrations would negatively affect the productivity and composition of aquatic plant communities. LOC calculations are typically based on laboratory toxicity studies of individual species and calculated based on the RQs (See Section 12). With atrazine, the concern is the effect of atrazine on the individual species as well as effects to the whole community. Atrazine has been the subject of various microcosm and mesocosm (cosm) studies in which such effects have been documented (**Appendix D**). These studies serve as the foundation for identifying atrazine exposures that are detrimental to aquatic plant communities. However, the concentration and length of exposure varied markedly among these cosm studies. The lengths of the studies varied from one week to one year, and the concentrations remained constant or steadily declined over the exposure period. These studies demonstrate that there is a need to relate the concentration and length of exposure across all cosm studies and the effects they have on the cosm.

The issue of comparing effects across different exposure time-series becomes even more important when trying to relate observed effects in cosms to expected effects in natural systems. Atrazine enters lakes, streams, and rivers primarily as a result of rainfall-driven runoff. This results in highly variable and episodic exposures that can be linked to rainfall distribution, atrazine application patterns, and geology (e.g. topography, and soil properties). **Figure 14** provides examples of atrazine chemographs (graphs showing exposure levels over time, note different y-axis scales) measured in streams in the Midwestern U.S. (raw data available to the public at: EPA-HQ-OPP-2003-0367-0178, EPA-HQ-OPP-2003-0367-0205, and EPA-HQ-OPP-2003-0367-0206). These highly variable exposures are markedly different from the exposures typical of laboratory toxicity tests and cosm studies, which have a defined duration (typically between 6 and 60 days) and relatively constant or steadily declining concentrations. They also differ from the exposures expected in lakes and reservoirs, which tend to be more steady over time. There is thus a need for a method to quantify the relative toxic severity of different exposure time series in order to relate effects between different cosm exposures and to extrapolate effects from cosm exposures to field exposures.

Figure 14. Examples of atrazine exposure time-series for natural freshwater systems.

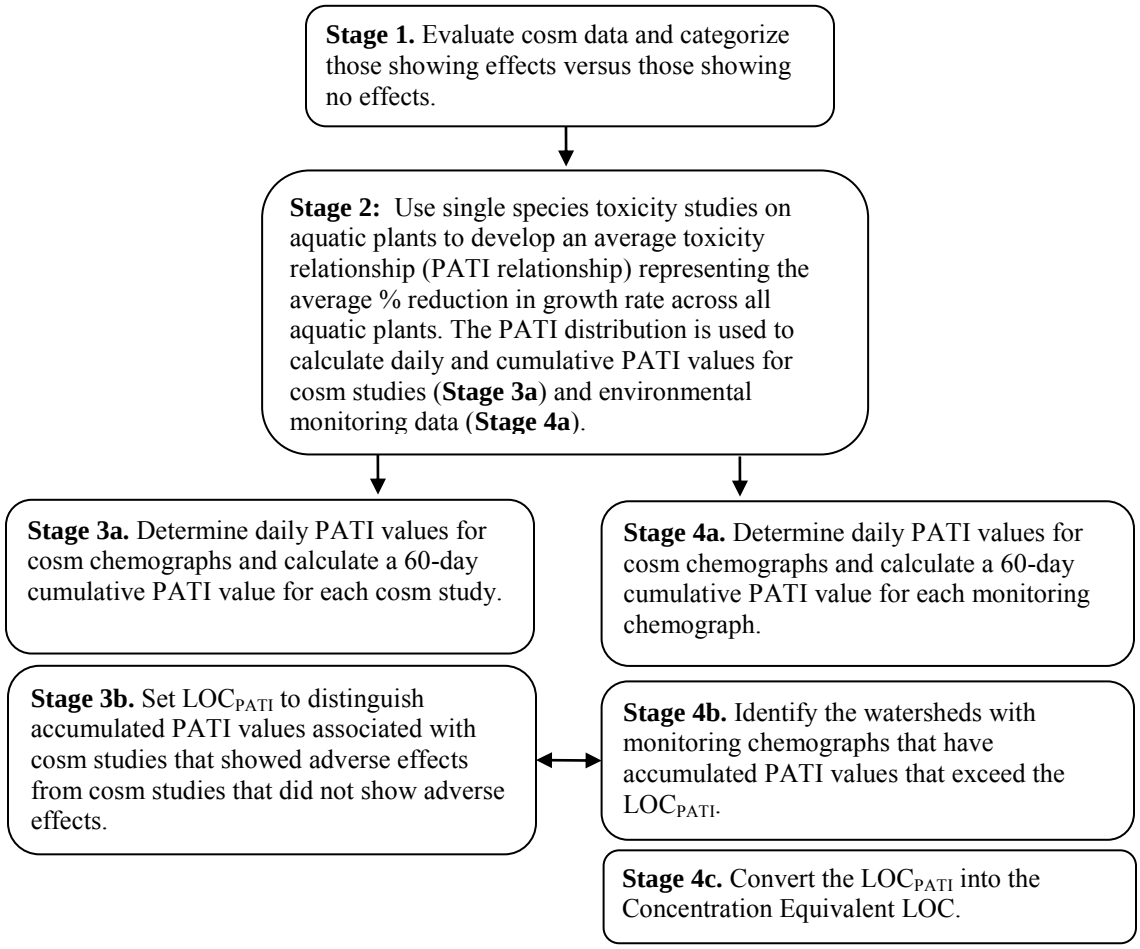


The primary goal is to be able to extrapolate the toxicity of different atrazine concentrations and length of exposure times from cosm studies to the concentrations and length of exposure occurring in the natural environment. It should be noted that the Comprehensive Aquatic Systems Model (CASM) was evaluated in the context of this goal; however, it was judged unsuitable for use on a national scale (**Appendix F**). Although CASM can have utility for certain site specific assessments, it was found to be too uncertain for the purpose here of a generic tool for the relative toxic impact of different exposure time series. Furthermore, risk characterizations using CASM provided no clear added value, differing negligibly from characterizations from much simpler methods, and involving uncertain and complex parameterization. As an alternative to CASM, EPA developed the Plant Assemblage Toxicity Index (PATI), which uses single-species aquatic plant toxicity data to estimate the relative severity of any atrazine concentration on an aquatic plant community. Additional tools such as species sensitivity distributions or the calculation of the 5th percentile hazard criterion (USEPA 2012b), result in similar results of the methodology using PATI but do not account for the durations of exposure needed to assess risk to aquatic plant communities.

The LOC methodology is a four stage process (**Figure 15**) that uses single-species plant toxicity data and cosm studies to discern what atrazine exposure patterns and concentrations can cause adverse effects on aquatic plant communities. With this methodology an LOC is

developed which, together with monitoring data, can be used to identify watersheds where atrazine levels may result in adverse effects to the aquatic plant community structure and function.

Figure 15. The four-stage process to set an LOC for atrazine

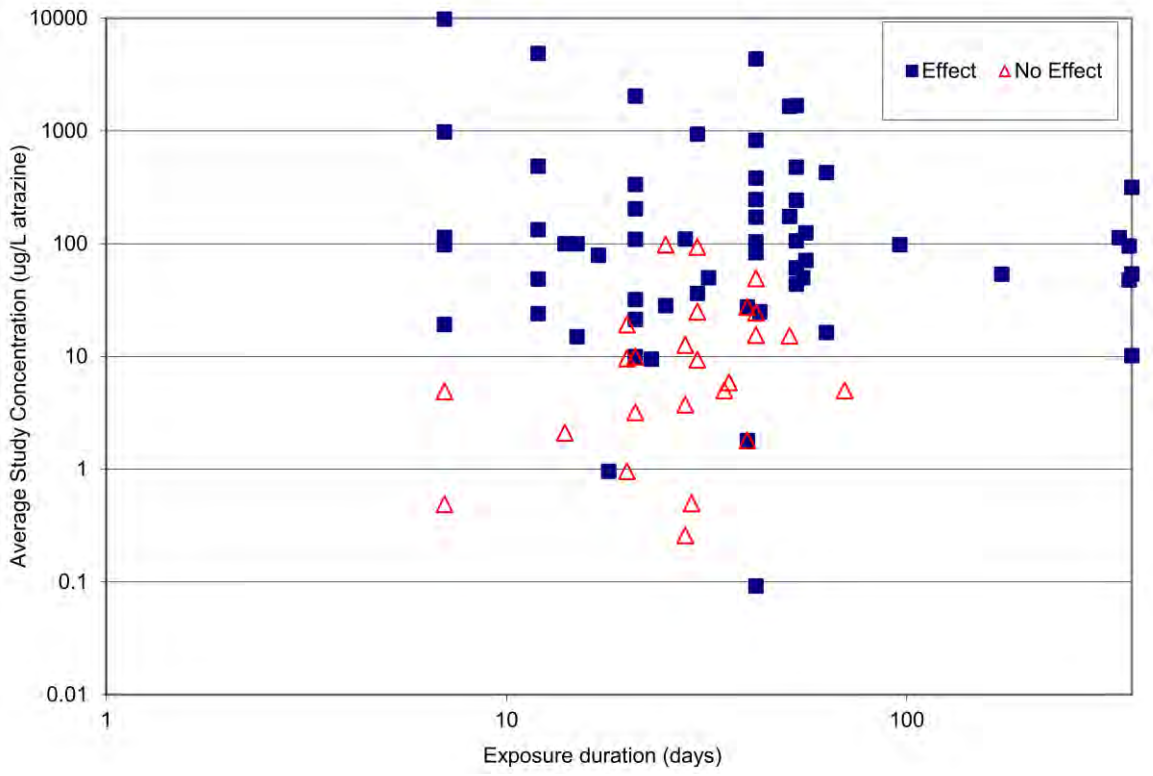


Stage 1: Summarize Toxic Effect to Communities Based on Microcosm and Mesocosm Studies.

For atrazine, an extensive set of cosm studies have documented effects of atrazine on plant community structure and productivity (**Appendix D).** These tests can be used to decide what 60-day cumulative value of PATI should be considered a LOC. In all, EPA is using 87 atrazine exposure values from 46 published articles on effects of atrazine on cosm systems (**Figure 16**). These 46 studies were selected from the larger pool of candidate studies because they met the established pre-screening and data quality criteria (**Appendix D**). The EPA reviewed each of the cosm studies that met the quality criteria in order to determine if atrazine-related effects were observed and at what atrazine concentration. Examples of atrazine-related effects observed in

the cosm studies included reductions in aquatic plant biomass, concentration of chlorophyll *A*, rate of photosynthesis (^{14}C uptake and oxygen production), and shifts in aquatic plant community structure (*e.g.*, species composition and diversity) relative to a control.

Figure 16. Effects of atrazine on experimental ecosystems as a function of exposure duration and average concentration. Squares denote adverse effects, open triangles no effects.



Stage 2: Summarize Toxic Effect Across An Aquatic Plant AssemblageBased on Single Species Toxicity Tests.

As noted above, a primary requirement for this methodology is to estimate the relative effects of different exposure time series on aquatic plant communities, in order to relate effects in different cosm exposures to each other and to extrapolate these effects to exposures in natural systems. PATI estimates such relative effects based on an aggregate of the toxicity relationships determined for individual aquatic plant species. This assemblage of test species is used as a surrogate for aquatic plant communities (only with regard to the relative effects of

different exposure time series). PATI is described and evaluated at length in **Appendix E** and is only summarized here (additional options and updates to PATI are provided in **Appendix M**).

PATI represents an expansion of the Species Sensitivity Distribution (SSD) concept commonly used in aquatic risk assessments. SSDs summarize available toxicity tests as a statistical distribution of toxicity endpoints (e.g., EC_{50}s – median effect concentrations) across different taxa (**Table 19**). PATI expands on this concept by, considering the entire toxicity relationship for plant taxa rather than the single level of effect embodied in EC_{50}s and by determining the average effect across all taxa rather than focusing on a single taxon at a specific percentile in the SSD. PATI thus provides a more complete description of the reduction in productivity of an assemblage of plants and of the driving force for atrazine effects on aquatic plant communities.

The toxicity relationship for each taxon is the relationship between the reduction in growth rate and the concentration of atrazine. For example in the middle panel of **Figure 17**, curve #1 shows that as atrazine concentration increases, the percent of growth rate reduction also increases. With higher concentrations there is a reduction in the growth of the taxon (*i.e.*, there is a toxic response). This curve represents the toxicity relationship for a single plant taxon. PATI assembles the toxicity relationships from many different taxa of plants and calculates the average toxicity relationship. This represents the average reduction in growth rate across all taxa and concentrations and is called the **PATI relationship** (**Figure 17**, lower panel). At 50 µg/L, the average effect (growth rate reduction) over all genera is 19%, providing the PATI value in the bottom panel (arrow). Thus, rather than just providing the percentage of taxa that have an EC50 below some concentration (e.g., 50 µg/L corresponds roughly to the 16th percentile on the SSD), PATI describes the percent reduction in plant production for the entire assemblage (weighting each taxon equally) (**Appendix E**).

Table 19. Compiled data regarding atrazine toxicity to aquatic plants. All data pertain to the specific growth rate (SGR) of the plant. Compilation includes the EC_{50} for the SGR, a steepness measure, and the SGR of the control (SGR_c) under test conditions. Italicized EC_{50}s denote values whose estimation required information on SGR_c and/or steepness from other studies.

	Piecewise Linear Model		Sigmoidal Threshold Model		Logistic Model		Appendix D Report Values (Logistic)			
Genus	**SGR EC_{50}**	**Steep**	**SGR EC_{50}**	**Steep**	**SGR EC_{50}**	**Steep**	**SGR EC_{50}**	**Steep**	**SGR_C**	**Reference**
Ankistrodesmus	112.7	1.017	105.2	1.485	104.3	1.412	104	1.41	0.33	Burrell et al. 1985
	138		127		124		119			Larsen et al. 1986
Chlamydomonas	421.7	0.516	386.8	0.677	378.3	0.652	378	0.65		Kallqvist and Romstad 1994
	147		142		139		*141*		1.06	Schafer et al. 1993
	78		71		70		*67*			Larsen et al. 1986

Table 19. Compiled data regarding atrazine toxicity to aquatic plants. All data pertain to the specific growth rate (SGR) of the plant. Compilation includes the EC_{50} for the SGR, a steepness measure, and the SGR of the control (SGR_c) under test conditions. Italicized EC_{50}s denote values whose estimation required information on SGR_c and/or steepness from other studies.

	Piecewise Linear Model		Sigmoidal Threshold Model		Logistic Model		Appendix D Report Values (Logistic)			
Genus	**SGR EC_{50}**	**Steep**	**SGR EC_{50}**	**Steep**	**SGR EC_{50}**	**Steep**	**SGR EC_{50}**	**Steep**	**SGR_C**	**Reference**
	45		45		45		45			Hersh and Crumpton 1989
Chlorella	26.3	0.905	25.5	1.066	25.3	1.038	26	1.07	1.4-2.4	Faust et al. 1993
	37		37		37		37			Hersh and Crumpton 1989
	99.8	0.391	92.8	0.505	91.2	0.474	91	0.47	0.26	Burrell et al. 1985
	645		592		580		*557*			Larsen et al. 1986
	480		480		480		480			Stratton 1984
Scenedesmus	101		92		90		*87*			Larsen et al. 1986
	300		300		300		300			Stratton 1984
	39.1	0.662	39.2	0.729	38.9	0.716	*39*	0.73		Zagorc-Koncan 1996
Selenastrum	164	0.491	164	0.752	164	0.792	164	0.79	1.80	Mayer et al. 1998
									1.93	Radetski et al. 1995
	54.7	1.171	50.8	1.623	49.6	1.658	50	1.66	1.25	Caux et al. 1996
	100		100		100		100	1.50		Versteeg 1990
	134.5	0.533	130.2	0.656	130.7	0.617	131	0.62	1.75	Hoberg 1991A
	70		70		70		70			Turbak et al. 1986
	191.0	0.735	173.2	1.080	171.7	1.038	163	1.22	1.65	Roberts et al. 1990
	128.1	0.858	124.5	1.114	124.6	1.065	125	1.07	1.01	Gala and Giesy 1990
	110	0.656	110	0.906	110	0.900	110	0.90		Kallqvist and Romstad 1994
	202.1	0.565	205.0	0.791	200.6	0.787	201	0.79		Kallqvist and Romstad 1994
	231.4	0.691	237.9	1.026	236.4	1.012	236	1.01		van der Heever and Grobbelaar 1996

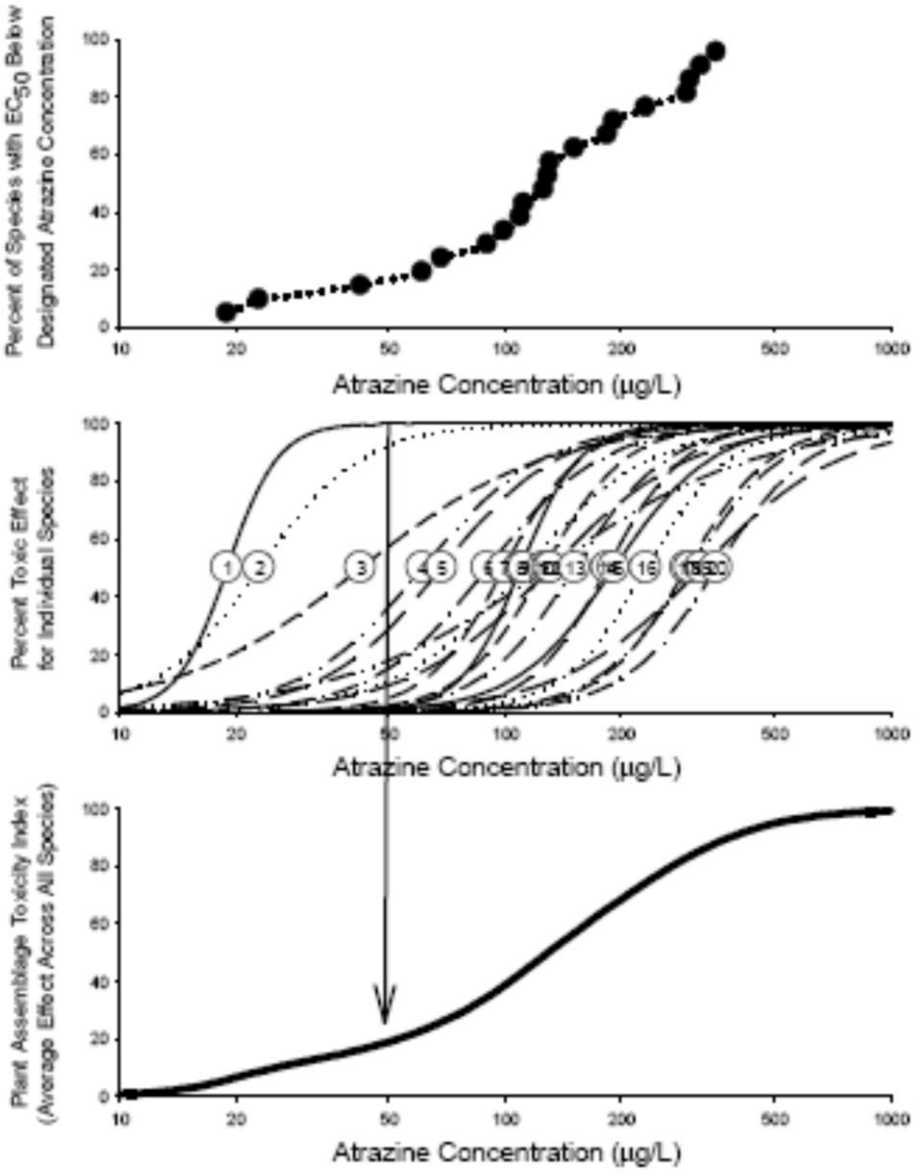
Table 19. Compiled data regarding atrazine toxicity to aquatic plants. All data pertain to the specific growth rate (SGR) of the plant. Compilation includes the EC_{50} for the SGR, a steepness measure, and the SGR of the control (SGR_c) under test conditions. Italicized EC_{50}s denote values whose estimation required information on SGR_c and/or steepness from other studies.

	Piecewise Linear Model		Sigmoidal Threshold Model		Logistic Model		Appendix D Report Values (Logistic)			
Genus	**SGR EC_{50}**	**Steep**	**SGR EC_{50}**	**Steep**	**SGR EC_{50}**	**Steep**	**SGR EC_{50}**	**Steep**	**SGR_C**	**Reference**
	185.3	0.407	215.3	0.599	222.4	0.610	223	0.61		van der Heever and Grobbelaar 1997
	104.4	1.302	101.7	1.694	101.1	1.610	101	1.61	0.97	Parrish 1978
	90		83		81		*78*			Larsen et al. 1986
Stigeoclonium	367		336		330		*317*			Larsen et al. 1986
Ulothrix	184		169		166		*159*			Larsen et al. 1986
Cryptomonas	574.9	0.827	516.3	1.106	493.5	1.151	494	1.15		Kallqvist and Romstad 1994
Cyclotella	479.9	1.079	471.2	1.290	461.8	1.215	462	1.22		Kallqvist and Romstad 1994
	102.9	0.615	102.1	0.721	100.2	0.674	100	0.67		Millie and Hersh 1987
	117.7	0.607	117.1	0.696	114.4	0.646	114	0.65		Millie and Hersh 1987
	236.2	0.922	224.9	1.031	225.2	0.996	225	1.00		Millie and Hersh 1987
Navicula	217.8	0.919	216.6	1.104	216.7	1.080	217	1.08	1.03	Hughes et al. 1988
Anabaena	70		70		70		70			Stratton 1984
	280		280		280		280			Stratton 1984
	470		470		470		470			Stratton 1984
	767.2	0.497	718.5	0.621	705.7	0.588	706	0.59	0.76	Hughes et al. 1988
	317		294		292		*286*			Larsen et al. 1986
Microcystis	168.4	1.043	167.0	1.350	164.2	1.252	164	1.25	0.55	Parrish 1978
	557.2	0.569	609.3	0.806	602.8	0.768	605	0.77		Kallqvist and Romstad 1994
Synechococcus	154.9	0.504	136.6	0.606	136.0	0.593	136	0.59		Kallqvist and Romstad 1994
Ceratophyllum	25.2	0.979	23.6	0.718	24.4	0.813	24	0.81	0.035	Fairchild et al. 1998
Elodea	104.6	0.230	73.9	0.244	64.7	0.226	65	0.38		Forney and Davis 1981

Table 19. Compiled data regarding atrazine toxicity to aquatic plants. All data pertain to the specific growth rate (SGR) of the plant. Compilation includes the EC_{50} for the SGR, a steepness measure, and the SGR of the control (SGR_c) under test conditions. Italicized EC_{50}s denote values whose estimation required information on SGR_c and/or steepness from other studies.

	Piecewise Linear Model		Sigmoidal Threshold Model		Logistic Model		Appendix D Report Values (Logistic)			
Genus	**SGR EC_{50}**	**Steep**	**SGR EC_{50}**	**Steep**	**SGR EC_{50}**	**Steep**	**SGR EC_{50}**	**Steep**	**SGR_C**	**Reference**
	<38		<38		<38		<38		0.011	Fairchild et al. 1998
	141.6	0.348	199.6	0.532	203.6	0.519	204	0.52	0.088	Hoberg 2007
Hydrilla	151.0	0.440	118.4	0.604	111.0	0.630	118	0.99		Hinman 1989
Lemna	207.1	1.161	201.9	1.261	201.9	1.235	202	1.24	0.279F	Hoberg 1991B
	102.4	1.045	95.4	1.410	93.2	1.327	93	1.33	0.252W	Hoberg 1993B
	61.3	1.076	65.2	1.621	66.2	1.704	49	1.71	0.226W	Hoberg 1993C
	128.9	0.425	124.7	0.462	115.4	0.420	115	0.42	0.205F	Fairchild et al. 1998
	225.8	0.886	222.7	1.190	224.2	1.142	224	1.14	0.225F	Hughes et al 1988
	102		97		95		95			Kirby and Sheehan 1994
	91.0	1.017	90.2	1.221	89.9	1.183	90	1.18	0.396W	Desjardin 2003
Myriophyllum	<150		<150		<150		<150		0.01?	Fairchild et al. 1998
Najas sp.	14.5	1.359	14.5	1.750	14.5	1.670	15	1.67	0.066	Fairchild et al. 1998
Potamogeton	53.7	0.549	61.6	0.728	62.8	0.695	63	0.69		Forney and Davis 1981
Vallisneria	141.5	0.355	153.9	0.436	140.7	0.401	141	0.40		Forney and Davis 1981

Figure 17. Comparison of toxicity relationships for 20 plant genera (middle panel), the SSD of EC50s for these genera (top panel), and the plant assemblage toxicity index (bottom panel, PATI = the average of the curves in the middle panel) (from Erickson 2012).



In the LOC methodology, the PATI relationship is used to specify the average reduction in plant growth rate for each day (daily PATI value) in both the cosm studies and the chemographs available from environmental monitoring data. Because of the rapid recovery of growth rates after atrazine exposures (*e.g.*, Abou-Waly *et al.*, 1991, Desjardin *et al.* 2003), daily PATI values need not consider residual toxicity from exposures on previous days, but rather only the toxicity for the current day's exposure.

The cumulative effects of an exposure through time (*i.e.,* the total toxic severity of an exposure time series) will take into account the total effect on the community. The EPA addresses this total effect by summing the daily PATI values to produce a "**cumulative PATI value.**" Such a

summation cannot be indefinite, but rather is limited to an "**assessment period,**" and this limit must reflect judgments about cumulative effects and the duration of the available cosm data.

Because atrazine exposure outside the assessment period is considered inconsequential by PATI, the assessment period needs to be long enough to encompass (a) exposures of significance to establishing LOC_{PATI} from the cosms (**Figure 16**) and (b) effects expected from seasonal field exposures (*e.g.*, **Figure 14**). However, it should not be any longer than necessary, in order to avoid uncertain inferences regarding (a) cumulative effects of low concentrations and (b) widely separated exposures that are independent regarding ecological effects.

The 60-day assessment period was chosen because it would include all or almost all periods of significant exposure in the AEEMP monitoring data, and would also encompasses the duration of all but a few of the cosm studies. A few additional considerations regarding this period relative to the treatments in the cosm studies should be noted (**Appendix E**):

- It is slightly shorter than the longest cosm study treatment with no effect. If the assessment period is significantly shorter than treatments with no effect, this will under-represent how substantial exposures could be without causing effects and thus be too restrictive.
- For those treatments with effects, a shorter period will also be too restrictive by assuming that less exposure is needed to elicit effects than actually is involved (*e.g.*, an effect observed over a 60-day exposure would be assumed to require less exposure than actually was required). This consideration does not pertain to the few cosms with extremely long durations, because they simply verify significant effects for high PATI values. For the LOC, the important treatments with effects are those whose exposures near to those without effects.
- That 60-day exposure is longer than many cosm treatments with effects is not an issue, provided the effects from these shorter exposures will still be considered unacceptable from the perspective of this longer assessment period. For example, if a 30-day exposure showing effects had been monitored for another 30 days without exposure, the effects during the first 30 days would be considered unacceptable despite any recovery that occurred during the second 30 days).

One drawback to assessment periods longer than 63 days is that there is limited data from cosm studies that extend beyond this duration. The Agency determined the 60-day assessment period was most representative of the available data because most cosm studies were in the 7-63 day duration range, and the LOC values derived for the 60-day assessment period should be protective of the shorter time periods**.**

Stage 3: Calculate a Level of Concern for Aquatic Plant Communities Based on the PATI Relationship and the COSM Studies.

To establish an LOC for aquatic plant communities based on PATI, the first task is to calculate a cumulative PATI value for each cosm study. Daily PATI values for a cosm exposure are first calculated by applying the PATI relationship (e.g., **Figure 17**) to each day's concentration. The cumulative PATI value for the cosm exposure is then based on the 60-day period that has the greatest cumulative PATI value. For example if a cosm study has an atrazine concentration of 50 µg/L and that concentration is held constant, based on the PATI relationship (**Figure 17**) the daily PATI value is 19%, and the 60 day cumulative PATI is 1140%-days. After this cumulative value has been calculated for all of the cosm studies the values are then combined with the effects/no effects classifications determined in Stage 1.

The relationship of the cosm studies cumulative PATI values to their effects/no effects classification(s) (see **Figure 16** and **Figure 18**) (**Appendix D**) is used to specify the LOC_{PATI} (the LOC in cumulative PATI values). **Figure 15** provides a binary plot of cosm treatment effects/no effects determinations versus their calculated 60-d cumulative PATI values. The LOC_{PATI} is set as the cumulative PATI value that corresponds to a 50 percent probability of an effect based on a logistic binary regression conducted to determine the probability relationship **(Appendix E).** In other words, at a PATI score of 130, there is a 50:50 chance of having adverse effects. EPA risk management determined a 50 percent cutoff due to a variety of factors including variability in sensitivities of the cosm studies, magnitude and duration of effects observed in the cosm studies, statistical uncertainty in the calculation of the LOC_{PATI} and ecological relevance of observed effects in the cosm studies. This LOC_{PATI} is expressed in PATI values and needs to be converted to a concentration-based LOC to be more easily used by the regulatory agencies. This conversion is discussed in Stage 4 below.

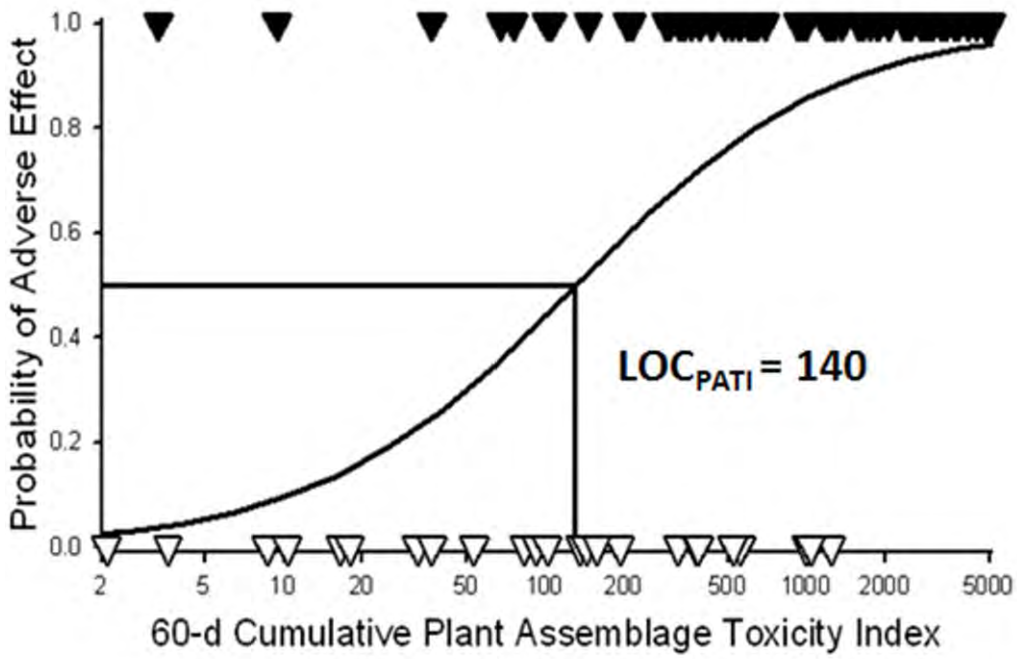


Figure 18. Cosm studies plotted as effect (closed triangle)/no-effect (open triangles) versus PATI fitted to a logistic relationship for the probability of an effect versus PATI, this probability being 50% when PATI equals 140.

Stage 4: Determine if Watersheds Exceed the Concentration Equivalent Level of Concern Based on the PATI Distribution and the Environmental Monitoring Data.

The first task is to calculate a cumulative PATI value for each environmental monitoring site. A daily PATI value is calculated for each day of the study, by taking the concentration for each day and finding the corresponding PATI value from the PATI relationship (see **Figure 17** for an example). After each day has been calculated the 60-day period that has the greatest cumulative PATI value is recorded. For example if an environmental monitoring site had an atrazine chemograph as shown in the top panel of **Figure 19**, based on the PATI relationship (**Figure 17**) the daily PATI value would be variable depending on the daily concentration, and the maximum 60-day cumulative PATI would be 150. The monitoring sites with cumulative PATI values greater than the LOC_{PATI} would be predicted to cause adverse effects to the ecological communities in those lakes, streams or rivers.

To assess whether the LOC_{PATI} is exceeded in natural systems, the next step is to quantify the difference between each of the environmental monitoring sites and the LOC_{PATI}. The cumulative PATI value from each monitoring site chemograph (*e.g*. 150) is divided by the LOC_{PATI} (**Figure 18**). This number is called the Effects Exceedance Factor (EEF), and is similar to risk quotient

methods in most EPA risk assessments as it identifies high- or low-risk situations (for examples visit: EPA Risk Characterization).

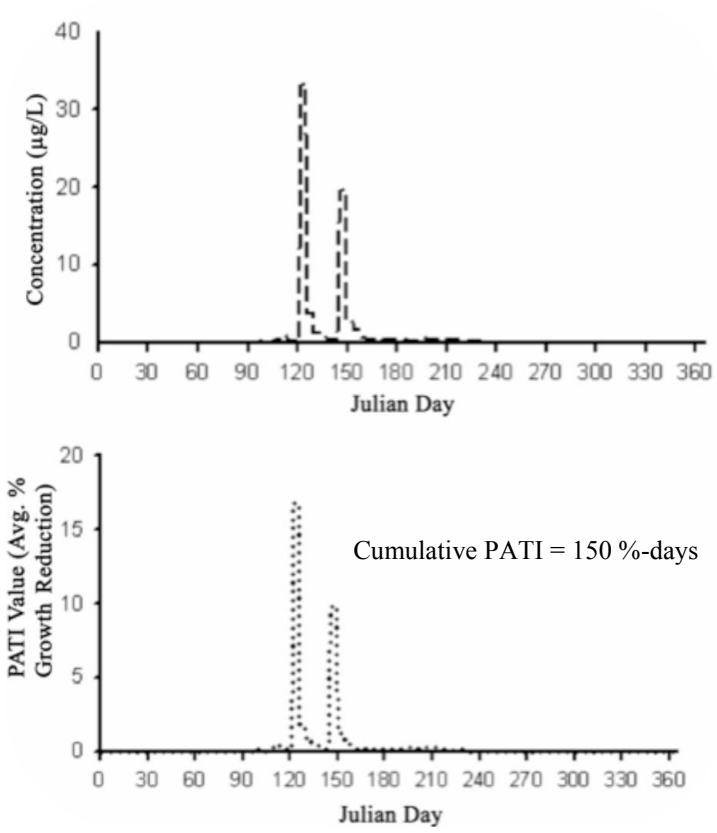


Figure 19. Typical atrazine exposure chemograph from monitoring data (top panel). The calculated daily PATI values and cumulative PATI value for a 60-day window for the example chemograph in the top panel.

To more easily use this information the LOC_{PATI} is converted into a concentration-based LOC called the Concentration Equivalent Level of Concern (CE-LOC). The CE-LOC is determined as the concentration at which the monitoring site cumulative PATI value is equal to the LOC_{PATI}, or in other words, the EEF equals 1. In calculating this CE-LOC, EPA will use Syngenta's monitoring data (AEEMP 2004-2011; http://www.epa.gov/opp00001/reregistration/atrazine/atrazine_update.htm). In **Figure 20**, the points represent AEEMP environmental monitoring sites (2004-2011), plotted by their average concentration for the chosen 60-day exposure window, and by their EEF. Additional calculations were carried out to determine the variability in CE-LOC values based on different analytical/statistical methods (these analyses are discussed further in **Sections 14.3 and 14.4).** A discussion of how the CE-LOC will be implemented is presented in **Chapter V.**

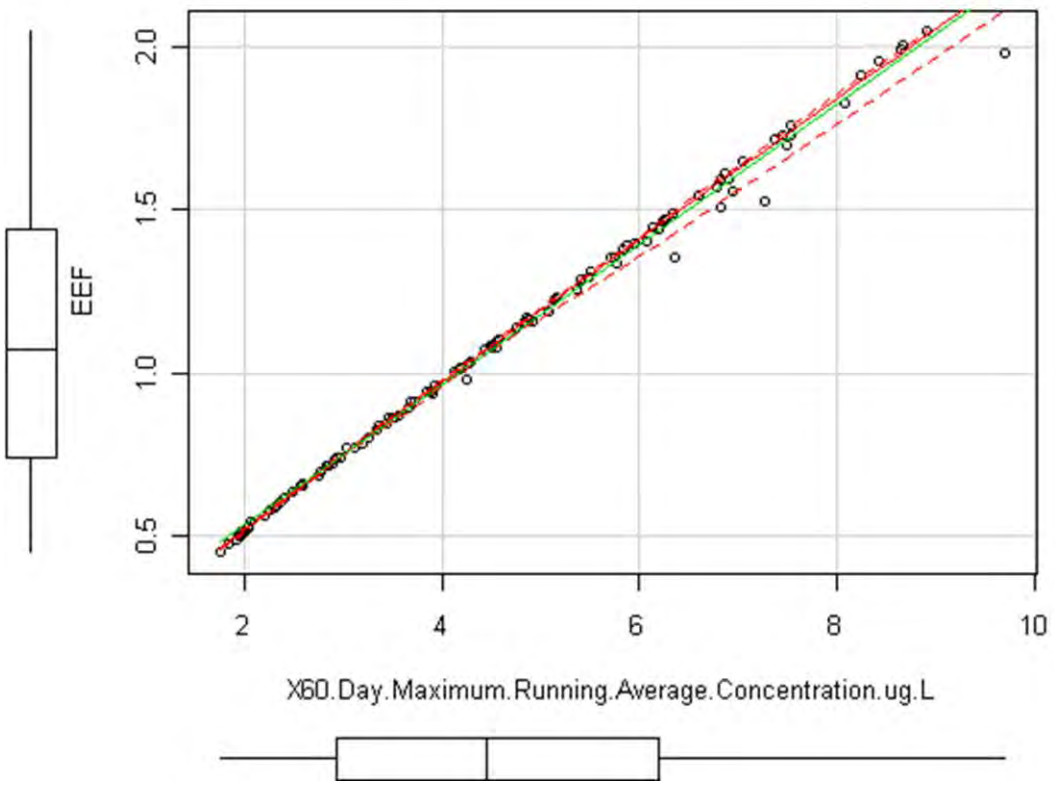


Figure 20. Plot of maximum 60-day average concentrations against the Effects Exceedance Factor (EEF) for the AEEMP Site Year Data (2004-2011). See **Appendix G** for further details on the regression results. Box plots along each axis describe the distribution of each variable.

14. History of the Aquatic Plant Community LOC Methodology and the Effects on the LOC from Implementation of Suggestions by Scientific Advisory Panels.

14.1. A Synopsis of the Changes Incorporated into the Current Aquatic Plant Community LOC Methodology.

The EPA made the following revisions to the LOC approach based on recommendations made by the SAPs in 2007 and 2009:

- Modifications to CASM after the 2007 review

- Critical evaluation of CASM and consideration of alternatives to CASM,
- Re-evaluation the suitability of the original cosm endpoints based on peer-reviewed acceptance criteria (66 of the original 77 endpoints remained).
- Re-classification of the endpoints from the 1-5 Brock score scale to an effect/no effect determination (5 of the original endpoints were re-classified from a Brock score of 2 [treated as "no effect" in the analysis] to "effect"
- Addition of one endpoint from one of the original cosm studies that was not previously included)
- Incorporation of 20 additional cosm endpoints from new studies recommended by the SAP
- Change in the LOC method from balancing the absolute numbers of Type I/II errors to a logistic regression approach
- Replacement of the assumed constant nominal atrazine concentration over the duration of the cosm study with time-variable atrazine concentrations

14.2. Modifications to the Method for Determining the LOC for Aquatic Plant Communities (Based on the Suggestions from the 2009 SAP).

New COSM Studies and Old COSM Reclassifications

The original cosm data set was comprised of 35 studies and 77 endpoints for CASM development. The Agency evaluated 38 additional studies recommended by the 2009 SAP, and re-evaluated the 35 studies using a rigorous set of acceptance criteria (**Appendix D**). The new cosm study dataset now includes 46 studies and 87 endpoints (plotted in **Figure 16**). In this current cosm data set, the cosm effects were changed from the 5-tier Brock score to an effect-no effect classification. Of the original cosm studies, 5 endpoints that were previously classified as the equivalent of "no effect" (Brock Score of 2) were reclassified as "effect" under the revised analysis.

- Endpoint #51 (Brockway *et al.*, 1984): the effects were based on a 25% reduction in phytoplankton oxygen production occurring the first day of a twelve-day study at 50 µg/L. Recovery was not observed during the study period.

- Endpoint #52 (deNoyelles *et al.*, 1982, 1989): the effects were based on a 50% decline in ^{14}C-uptake and 50% decline in phytoplankton biomass. All effects were statistically significant. Recovery of both ^{14}C-uptake and biomass was not specified at this level, but assumed to be ≥3 weeks, given the lower magnitude of effect and recovery at the higher concentrations.

- Endpoint #58 (Lampert *et al.*, 1989): the effects were based on a 50% decrease in chlorophyll-a and oxygen saturation for the phytoplankton community at 1 µg/L in the

18-day study. Reductions in oxygen may have been related to daphnid mortality; however, chlorophyll-a reductions were considered to be treatment related. Recovery was not observed.

- Endpoint #59 (Pratt *et al.*, 1988): the effects were based on 35% decrease in dissolved oxygen, slight reductions in magnesium and calcium levels for this 21-day study at 32 µg/L. All effects were statistically significant. Recovery was not reported in the study.

- Endpoint #60 (Pratt *et al.*, 1988): the effects were based on a 35% decrease in dissolved oxygen, a 60% reduction in chlorophyll-a, and slight reductions in magnesium and calcium levels for this 21-day study at 110 µg/L. All effects, except chlorophyll-a reduction, were statistically significant. Recovery was not reported in the study.

Changes to the calculation of the LOC_{PATI}

The second suggestion from the 2009 SAP was to modify the way that cosm endpoints were used to estimate the LOC_{PATI}. Instead of determining the LOC_{PATI} as the PATI value at which a balance of absolute numbers of effect endpoints fall below and no effect endpoints fall above the value, which is problematic where the numbers of effect and no effect endpoints are unbalanced, the Agency now uses a probability of adverse effects (**Appendix E**). The relationship of the probability of effect in the cosms to the PATI value determined for each cosm exposure is determined using binary logistic regression. The LOC_{PATI} is the point at which there is a 50% probability of an effect (**Figure 18**).

Final calculation of the Concentration-Equivalent LOC

The Agency uses the AEEMP monitoring sites (EPA-HQ-OPP-2003-0367-0206) and the LOC_{PATI} to derive a single concentration-duration endpoint, the CE-LOC. The CE-LOC can be derived using a variety of methods and assessment periods.

In investigatory studies of the effect of assessment period on the CE-LOC, the LOC_{PATI} was calculated for 7, 14, 30, 60 and 90-day assessment periods **(Table 20)** using the 2011 Cumulative PATI model and the full cosm dataset (**Appendix D**). The CE-LOC was calculated by conducting two linear regressions of the EEFs for each duration versus the maximum running average for each duration, one using all the data and one using only those points with 0.5<EEF<2.0. The CE-LOC was estimated as the concentration on the regression line corresponding to EEF=1.0 **(Table 20**). At shorter assessment periods, 7 to 60 days, the linear regression was a poor fit of the data (*i.e.*, missing the center of the distribution at EEF=1). The linear regression on only the 0.5<EEF<2.0 portion of the data set, resulted in a good fit of the data and was used to establish the CE-LOC.

TABLE 20. Effect of averaging period and method of derivation on the percent of AEEMP site/years exceeding the CE-LOC.					
Averaging Period	**7-Day**	**14-Day**	**30-Day**	**60-Day**	**90-Day**
Cumulative PATI Value (%-days)	65.5	107.4	132.7	140.0	141.8
CE-LOC (µg/L) Linear Regression (Entire Data Set)	19.0	15.6	8.6	4.2	2.8
CE-LOC (µg/L) Linear Regression (0.5-2.0 EEF Range)	18.0	14.9	8.3	4.2	2.7

14.3. Analyses of Driving Factors Affecting the CE-LOC

This section evaluates the relative impacts of recommendations made by the 2007 and 2009 Scientific Advisory Panels (USEPA 2007b and USEPA 2009a) on the CE-LOC **(Table 21).** Based on the 2003 method, for the 60-day duration, the preliminary trigger was 17.5 µg/L. The direct comparison between the preliminary trigger and CE-LOC endpoints derived from PATI is problematic because a different set of cosm data, LOC approach, expanded set of field chemographs, and atrazine concentration profile, have been used. In addition, the CASM model used in that preliminary derivation has changed since 2003 based on SAP recommendations.

Table 21. Comparison of effect of LOC methods, cosm exposure characterization, and cosm datasets on resulting 60-day PATI model-derived LOCs and concentration-equivalent LOCs					
LOC Method	**Cosm Data**	**Cosm Exposure**	**Changes**	**LOC_{PATI}**	**CE-LOC**
Old	Original 77	Constant Nominal	2003 Preliminary	NA	17.5[a]
LOC BASED ON 2007 SAP RECOMMENDATIONS					
Old	Original 77	Constant Nominal	Estimated change in LOC switching from preliminary version of CASM to updated version of CASM	NA	11.7[b, c]
Old	Original 77	Time-Variable	Changed representation of cosm atrazine concentrations from assumed nominal to actual concentrations over time	NA	7.2[b, c]
Old	Original 77	Constant Nominal	Switched from CASM to PATI to derive the LOC after 2007 SAP recommendations for modifying CASM, additional sensitivity analyses	4.97	9.6
Old	Original 77	Time-Variable	Changed representation of cosm atrazine concentrations from assumed nominal to actual concentrations over time	4.24	8.1
LOC BASED ON 2009 SAP RECOMMENDATIONS					
New	Original 77	Time-Variable	Changed LOC from balancing absolute numbers of Type I/II errors to logistic regression	4.15	7.9

Table 21. Comparison of effect of LOC methods, cosm exposure characterization, and cosm datasets on resulting 60-day PATI model-derived LOCs and concentration-equivalent LOCs					
LOC Method	**Cosm Data**	**Cosm Exposure**	**Changes**	**LOC_{PATI}**	**CE-LOC**
New	Original screened re-evaluated	Time-Variable	Screened 77 original studies with new acceptance criteria: - Dropped 7 effects endpoints (6, 11, 12, 16, 20, 21, 43) - Dropped 4 no effects (55, 56, 57, 74) Re-evaluated the 66 remaining original cosm endpoints that passed the new acceptance criteria (broken down in steps)		
			(a) Changed endpoint durations to match observed effect	4.55	8.7
			(b) Changed 5 studies from no effect (original Brock score of 2) to effect (51, 52, 58, 59, 60)	3.01	5.5
			(c) Added a second endpoint from the Lambert study (58b)	2.89	5.3
New	New Revised Cosm Set	Time-Variable	Added 20 endpoints from new cosm studies	2.33	4.2
LOC BASED ON MODIFICATIONS TO THE MODEL					
New	New Revised Cosm Set	Time-Variable	Changed from an average PATI value to Cumulative PATI.	140.0	4.2
New	Modified New Rev. Set	Time-Variable	Changed 5 of original cosm endpoints from effect back to original no effect determination)	235.0	7.4

[a]The 2003 concentration is not a CE-LOC, but a trigger concentration.
[b]For calculation of the CE-LOC when implementing the CASM model in the process, both the concentration and EEF were Log_{10} transformed. All subsequent regressions were conduced using linear regression of untransformed data.
[c]CASM was implemented with a logistic toxicity relationship for the intitial single species toxicity data to be consistent with the current version of PATI. The version of CASM presented to the 2007 SAP used a sigmoidal-threshold toxicity relationship.

Effect of 2007 CASM Changes

One consequence of the 2007 SAP was the recognition by all parties that the initial CASM model was unrealistic and needed modification. Changes were made that provided a more realistic depiction of a midwestern stream and this version was used for evaluations leading up to the 2009 SAP. To establish the impact of these changes on the difference between the CE-LOC and the preliminary screening value, the modified version of CASM used for the 2009 SAP

was applied with the same cosm data and LOC method as the 2003 evaluations. These CASM-based LOCs were then applied to the same AEEMP data used for current CE-LOC derivations, in order to derive what the EEFs and CE-LOC would be for the modified version of CASM. This resulted in a CE-LOC of 11.7 µg/L, compared to the screening value of 17.5 µg/L. In other words, correcting only the deficiencies of the 2003/2007 CASM version and applying it to a more extensive and realistic set of field data than used in 2003 caused the CE-LOC to be 29% lower than the 2003 trigger.

Change from assumed constant nominal to time-variable atrazine concentrations over the duration of the cosm study.

The original analysis of cosm studies (for the 2003 preliminary trigger concentrations and the 2007 SAP) assumed atrazine concentrations remained constant throughout the duration of the study. However, for a majority of the cosm data, atrazine concentrations declined throughout the study period. As part of the revisions leading up to the 2009 SAP, chemographs were developed for each cosm treatment (these chemographs were also reviewed by Syngenta's consultants). Using the modified CASM with these new time-variable chemographs, while still implementing the 2003 CE-LOC methodology, results in a CE-LOC of 7.2 µg/L (**Table 21**). A significant drop is to be expected because constant concentrations indicate a higher concentration was needed to cause effects in the cosms than was actually present. Again, by addressing only the changes recommended by the 2003 SAP, there is more than a two-fold difference between the preliminary trigger and the CE-LOC. This is before considering the switch to PATI, the change in the CE-LOC method, and changes in cosm data. The 2003 and 2007 SAP evaluations presented in **Table 21** were intended to be preliminary illustrations of methodology rather than providing assessment concentrations and were recognized at the time to require additional changes, so that this difference between the trigger and the CE-LOC is to be expected.

Switch from CASM to PATI

Based on the recommendations from the 2007 SAP, as an alternative to CASM, EPA developed PATI. PATI was developed after the change to the time-variable chemographs, however to compare to the earlier CE-LOCs, PATI was modified to use the constant concentration chemographs used in earlier versions of CASM. The resulting CE-LOC for comparison to the constant nominal concentration for CASM, explained in the earlier step, is 9.6 µg/L for PATI. This reflects the change from CASM to PATI. The resulting CE-LOC for the time variable concentrations is 8.1 µg/L, the same as the time variable CASM based value.

Change the LOC determination approach from balancing Type I/II errors to logistic regression.

The old LOC approach balanced the absolute number of effect endpoints that fell below the LOC with the number of no-effect endpoints above the LOC. However, because there are fewer no-effect endpoints in the cosm dataset, this allows for a higher percentage of no-effect endpoints above the LOC than effect endpoints below the LOC. The 2009 SAP expressed concern about the approach and recommended exploring alternative approaches.

The new LOC approach is based on the relative probability of an adverse effect, linking the PATI index value with the 50^{th} percent probability of an adverse effect. The change from the old (original) LOC approach to the logistic regression approach using the original cosm dataset (77 cosm endpoints) resulted in the CE-LOC dropping from 8.1 to 7.9 µg/L.

Re-evaluation of the original 77 cosm endpoints

The 2009 SAP made several recommendations regarding the original set of cosm studies EPA used for the LOC determination, ranging from the Brock scoring effects determination to the suitability of some studies for use (based on a review Syngenta submitted to the docket for the 2009 SAP). The re-evaluation included several steps, which have been broken out as separate increments in **Table 21:**

(a) The studies were screened against acceptance criteria based on number of controls, exposure, experimental design, statistical methods, and data interpretation (Appendix D). The re-evaluation resulted in 11 of the original 77 endpoints being dropped, which included 7 effects endpoints and 4 no effects endpoints. During the re-evaluation process, some endpoint durations from the original evaluation were revised to match the effects endpoints. Twenty-five durations were adjusted, with 15 effects durations increased and 10 effects durations decreased. This resulted in a net increase in the CE-LOC to 8.7 µg/L

(b) The next sequential change was a change in the effects endpoint classification from the 1-5 Brock score to a binary effect (1) / no effect (0) score. This resulted in a change from no effect (Brock score 2) to effect for 5 of the original cosm endpoints (Appendix D): #51 (Brockway *et al.*, 1984), #52 (deNoyelles *et al.*, 1982, 1989), #58 (Lampert *et al*., 1989), #59 and #60 (Pratt *et al*., 1988). The effective classification of the other endpoints remained unchanged. This resulted in the greatest change in the CE-LOC from 8.7 µg/L to 5.5 µg/L

(d) The re-review also resulted in adding an effects endpoint from the Lampert et al. (1989) study, identified at #58b, which showed an effect at a concentration of 0.1 µg/L, and resulted in a slight reduction in the CE-LOC from 5.5 to 5.3 µg/L (Appendix D).

Next to the revision in the CASM model recommended by the 2003 SAP, the re-evaluation of the cosm endpoints and, in particular, the re-classification of 5 of the studies from an original no-effect to effect (Appendix D), resulted in the greatest reduction in the CE-LOC.

Incorporating additional endpoints from new cosm studies recommended by the 2009 SAP.

The 2009 SAP provided EPA with a list of additional cosm studies that were not included in the original set of cosm studies. The Agency's review of these studies, using the study acceptance criteria, added 15 new studies with a total of 20 new endpoints (13 effect endpoints, 7 no effect endpoints; Appendix D). The addition of the new cosm endpoints to the existing revised endpoints resulted in a change in the CE-LOC from 5.3 to 4.2 µg/L.

Changed from an average PATI value to Cumulative PATI.

The initial development of PATI as presented at the 2009 SAP used the average PATI value over the assessment period. The change to cumulative PATI better reflects the intent to describe cumulative effects. Because the effects index is intended to describe total toxic impact, the approach to address time is simply to sum the daily PATI values to provide a cumulative PATI. The summation units of this cumulative PATI are analogous to the ppb-days or, more familiarly, with degree-days used to describe the total heating or cooling impact of seasonal weather. A fundamental aspect of such a summation is that a certain reduction in growth over 1 d is treated as being of equal importance as half that reduction persisting for 2 d, a quarter of that reduction persisting for 4 d, etc. This summation cannot be continued indefinitely, but rather is limited here to a 60-day period. The change from an average PATI to the cumulative PATI does not change the EEFs or CE-LOC, because they are mathematically equivalent.

14.4. Uncertainty in the Calculation of the LOC_{PATI} and CE-LOC

There are several calculations in the derivation of the CE-LOC that incorporate uncertainty into the final numbers. These include the the PATI relationship, the COSM classifications, the estimation of the 50^{th} percentile of the effects/no effects distribution (binary logistic regression), and the final conversion to the CE-LOC. A discussion in of the the uncertainty based on the original toxicity data, as well as the assumptions made concerning using the average PATI relationship rather than the most or least sensitive taxonomic group for the PATI relationship is provided in **Appendix E**.

The logistic PATI relationship may lead to heavy tails emphasizing greater effect at the tails than predicted from the toxicity data. To test the effects of this on the final CE-LOC two additional

PATI relationships, piecewise-linear, and threshold sigmoidal, were calculated and the results were compared to the CE-LOC described above. The results indicated that the logistic toxicity relationship was more protective than either the sigmoidal-threshold (5.1 µg/L) or linear toxicity relatinonships (5.6 µg/L).

The LOC is presented as a range in concentrations due to the uncertainty involved in the classification of a few COSM endpoints. The reclassification of 5 of the cosm endpoints from "no effect" to "effect" had the largest impact on the CE-LOC, resulting in an approximate 40% reduction in the revised baseline 60-day CE-LOC. If the classification of 5 of those endpoints that were previously considered to be "no effect" were changed back to "no effect", the CE-LOC would be 7.4 µg/L. Based on the results of these analyses EPA has determined the CE-LOC range to be between **4-7 µg/L.** This means that those fresh water and estuarine/marine monitoring sites with a **60-day running average at or above 4 µg/L** have atrazine concentrations that are above the lower bound of the CE-LOC, and that permanent or irreversible change in aquatic plant community structure, function, and/or productivity would be expected.

The greatest uncertainty is surrounding the calculation of the 50^{th} percentile (LOC_{PATI}) of the binary logistic regression of effects/no-effects data (*e.g.,* **Figure 18**). To investigate this, the 95^{th} confidence intervals (CI) around the LOC_{PATI} for both the 4 µg/L and 7 µg/L results were calculated by multiplying the standard error, provided as output from the PATI software, from each of the analyses by 1.96 and finding the upper and lower CI by adding or subtracting from the respective LOC_{PATI}. The upper and lower confidence intervals were divided by the LOC_{PATI} to obtain an EEF for each of the upper and lower confidence intervals around the LOC_{PATI}. The concentrations representing the upper and lower confidence intervals were then calculated using the linear regression equation for calculating the CE-LOC. This results in a 2-10 µg/L range of uncertainty around the 4 µg/L, and 3-15 µg/L for the 7 µg/L CE-LOC.

CHAPTER V. METHOD FOR DETERMINING THE VULNERABLE WATERSHEDS

15. Identifying Watersheds without Available Monitoring Data.

15.1. Identifying Watershed Criteria for Sites that Exceed the Aquatic Community LOC for Atrazine

The Atrazine Ecological Exposure Monitoring Program (AEEMP) was a part of the data requirement imposed as a condition of reregistration for atrazine. Two objectives of the AEEMP were to (1) estimate the extent of watersheds in corn and sorghum areas with water bodies that exceed the atrazine CE-LOC for aquatic community effects, and (2) use watershed attributes to identify other watersheds where these higher atrazine exposure areas are likely to occur (USEPA 2003c). This section recaps the EPA's 2009 analysis of watershed parameters characteristic of those watersheds that are most vulnerable to prolonged, elevated atrazine concentrations and describes future directions in watershed assessments for atrazine (USEPA 2009a). It is important to note that the CE-LOC has changed since 2009, so the following analyses would need to be repeated with the current CE-LOC.

15.2. Factors Contributing to Watershed Vulnerability

The primary objective of the EPA's 2007 watershed analysis was to identify those characteristics that distinguish watersheds with waters that frequently exceed the aquatic community CE-LOC for atrazine and to use those characteristics to identify other, similarly vulnerable watersheds. The AEEMP watershed properties were used to identified the presence of soils with shallow, drainage-restrictive layers in areas with atrazine use as a characteristic that distinguished monitoring sites that exceeded the aquatic community CE-LOC in multiple years (*e.g.,* MO-01, MO-02, and NE-04) from those sites that did not exceeding the CE-LOC during the sampling period (USEPA, 2007b, and 2009a). In follow-up analyses, EPA evaluated a number of atrazine use, weather, soil, and hydrology properties reflective of runoff-prone conditions using both simple statistical comparisons and discriminate analyses. USEPA (2009a) provides a full list of watershed parameters that were considered and analyses conducted.

For the 2009 SAP, US EPA used multivariate analyses to evaluate the importance of a variety of parameters to account for the variability in atrazine exposures among the AEEMP monitoring sites. Basic cluster analyses indicated that MO-01, MO-02, and NE-04 (3 sites that exceeded the CE-LOC in multiple years), as well as NE-05 and NE-07 (sites that exceeded the CE-LOC once) tended to group together based on soil factors. Discriminant analyses confirmed the importance of the percentage of soils with (1) a depth within 50 to 60 cm to a drainage-restrictive layer (defined by saturated hydraulic conductivity, K_{sat}, values of less than 1 to 1.25 µm/s), (2) a high-to-very-high runoff potential, and/or (3) hydrologic group D soils in

distinguishing sites that exceed the CE-LOC in multiple years. All three of these soil parameters reflect runoff-prone conditions in the watershed.

Multivariate discriminant analysis showed the importance of weighting the percentage of watersheds with high runoff-prone soils, based on one of the three parameters described above, with the atrazine use intensity for the watershed (**Figure 21**). The first canonical model provided a sharp distinction between sites that exceed the CE-LOC in multiple years (in blue) and the remaining sites. The second canonical model provided some separation between the sites that exceeded the CE-LOC in 1 year (green) and the sites that did not exceed the CE-LOC during the monitoring study (red).

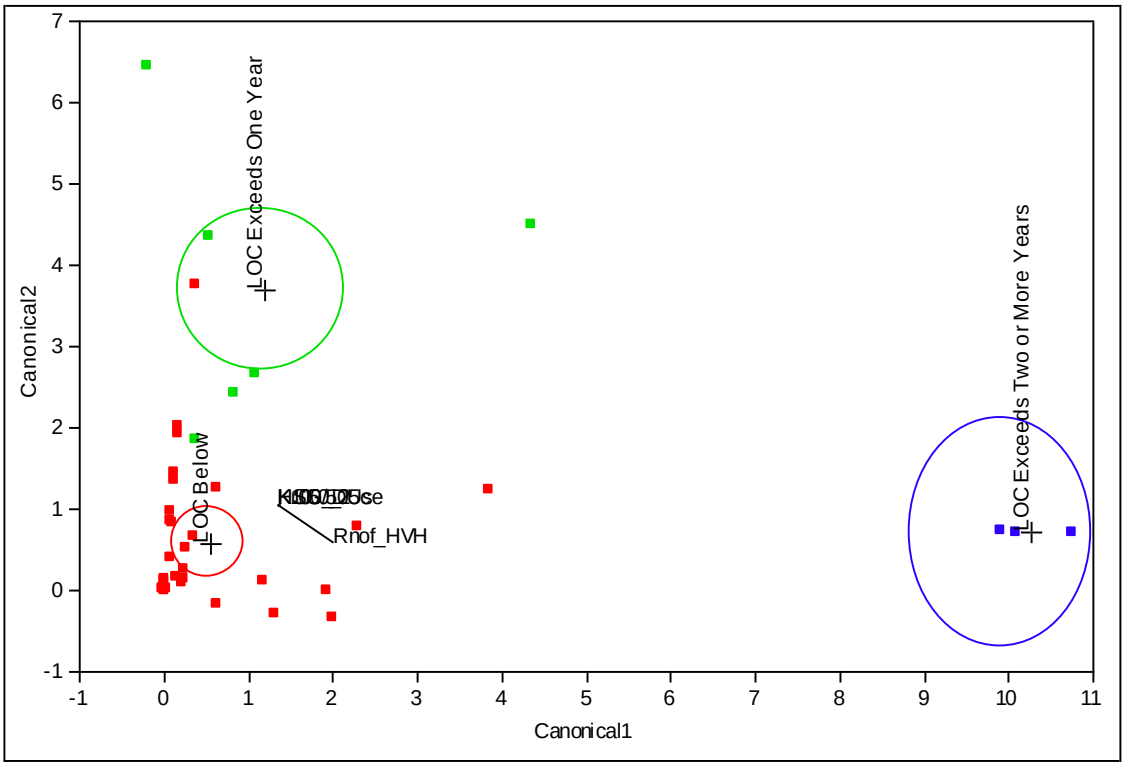


Figure 21. Analysis of discriminating factors in distinguishing between sites that exceeded the CE-LOC in multiple years (in blue), exceeded the CE-LOC once (green), and did not exceed the CE-LOC (red)

The US EPA further evaluated these watershed characteristics to identify those that, singly or in combination, best provide a clear distinction between the 3 AEEMP sites that exceed the CE-LOC in multiple years and the 31 sites that did not exceed the CE-LOC during the sampling period (USEPA, 2009a). The percentage of the upstream catchments containing soils with shallow drainage-restrictive layers (as defined above) provided a clear separation between the

3 sites that exceeded the CE-LOC in multiple years and the 31 sites that did not exceed the CE-LOC during the study period (**Figure 22**).

The red dashed lines in **Figure 22** group the 40 AEEMP sites into those that exceeded the CE-LOC in 2 or more years (to the left of the first red line), 1 year (between the two red lines), or 0 years (to the right of the second red line) during the study. The dark blue dotted line marks the lowest percentage of shallow, drainage restrictive soil layers among the watersheds that exceeded the CE-LOC in multiple years, while the yellow dotted line marks the highest percentage of shallow, drainage-restrictive soil layers among the watersheds that did not exceed the CE-LOC. Two of the six sites that exceeded the CE-LOC in 1 out of 3 years of the study (NE-05 and NE-07) were also above the upper threshold.

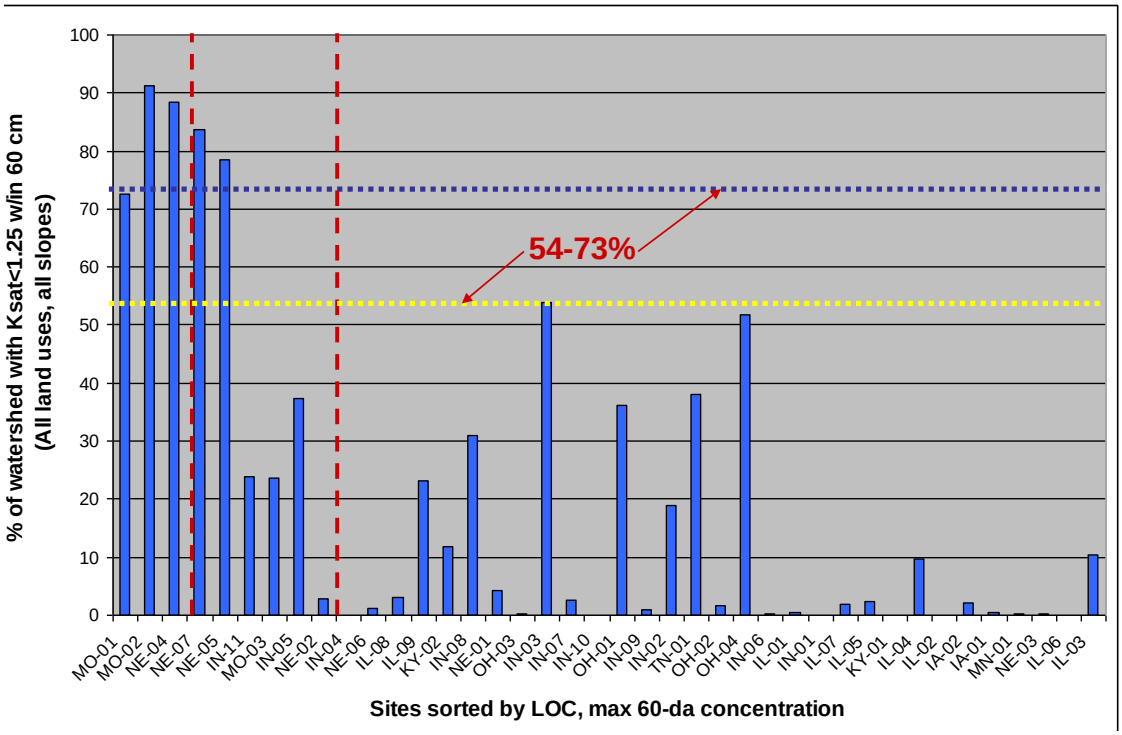


Figure 22. Percent of upstream catchments containing soils with shallow (<60 cm), low-Ksat (<1.25 um/s) layers for the 40 AEEMP monitoring sites. See text for description of the meaning of the red, blue and yellow lines.

Adjusting the fraction of the watershed with shallow, drainage restrictive layers by the atrazine use intensity improved the distinction between the 3 sites that exceeded the CE-LOC in multiple years and the remaining 37 sites (**Figure 23**).

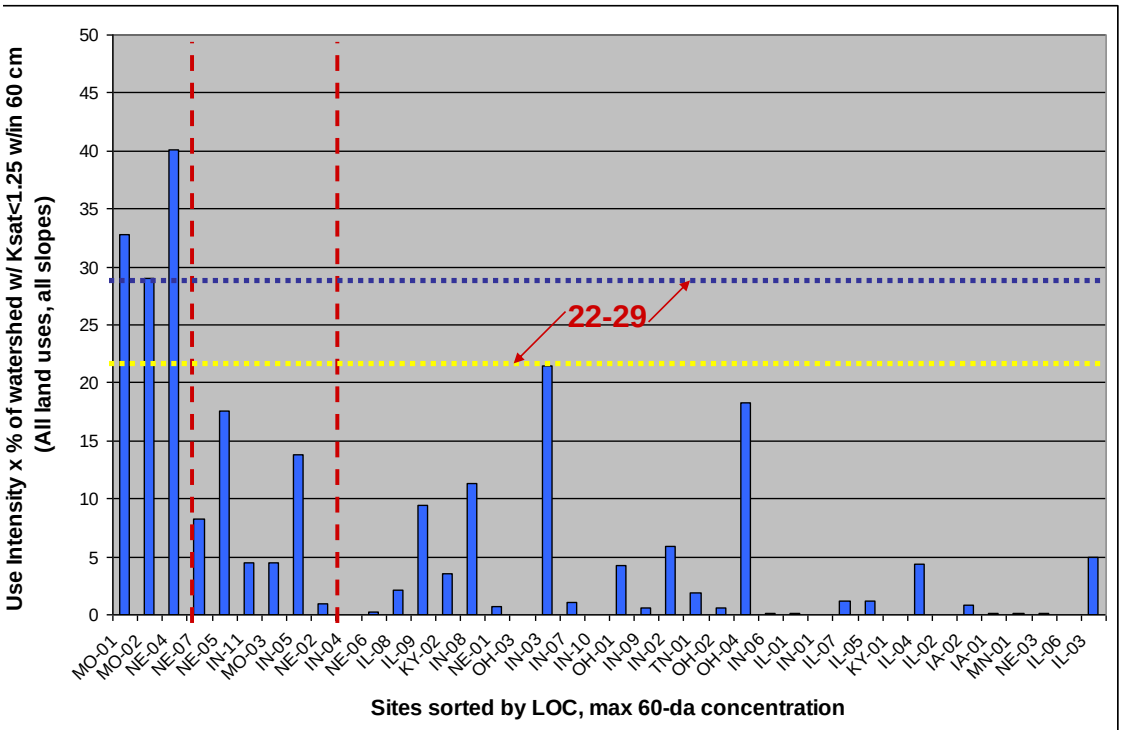


Figure 23: Atrazine use intensity adjusted percent of upstream catchment containing soils with shallow (<60 cm), low-Ksat (<1.25 um/s) layers for the 40 AEEMP monitoring sites. See the text for explaination of the red, blue and yellow lines.

Figure 24 shows the areal extent of 1:100,000 scale National Hydrography Dataset (NHD+) catchments in the Midwestern US that meet the criteria shown in **Figure 23**. The dark red in the map represent catchments that have use-intensity adjusted percentages of drainage-restrictive soils greater than the upper threshold value (blue dashed line in **Figure 23**); the lighter red in the map represent catchments that have values between the upper and lower (yellow dashed line) threshold value. The NHD+ catchments are smaller than the catchment areas represented by the 40 AEEMP monitoring sites, which ranged from 9 to 64.5 mi^2 (23 to 167 km^2) in area. Waters are more likely to exceed the aquatic community CE-LOC for atrazine in areas where contiguous NHD+ catchments meet the vulnerability criteria than in areas where only isolated catchments exceed the CE-LOC. Thus, **Figure 24** should be viewed as representing a maximum potential vulnerability area rather than the actual extent of vulnerable watersheds. As an illustration, **Figure 28** identifies headwater streams that exceed EPA's watershed vulnerability criteria (shown in blue and purple on the map) by beginning at the uppermost stream reach and continuing downstream until properties drop below the criteria. Thus, it excludes areas shown in **Figure 24** that occur farther downstream and, when added into the larger drainage area, would be below the vulnerability criteria.

While Syngenta also identified the presence of shallow, drainage-restrictive soil layers as a key attribute of watersheds that are most vulnerable to atrazine runoff, the available

documentation provided to the EPA focused primarily on the characteristics of MO-01 and MO-02 in defining vulnerable watershed criteria (Miller *et al.*, 2009; Prenger *et al.*, 2009). Syngenta further narrowed the characteristics by stipulating that the soil conditions must be tied to the presence of slopes ≥2% under cropland, and used the percentage of corn or sorghum as an indicator of atrazine use intensity (**Figure 25**).

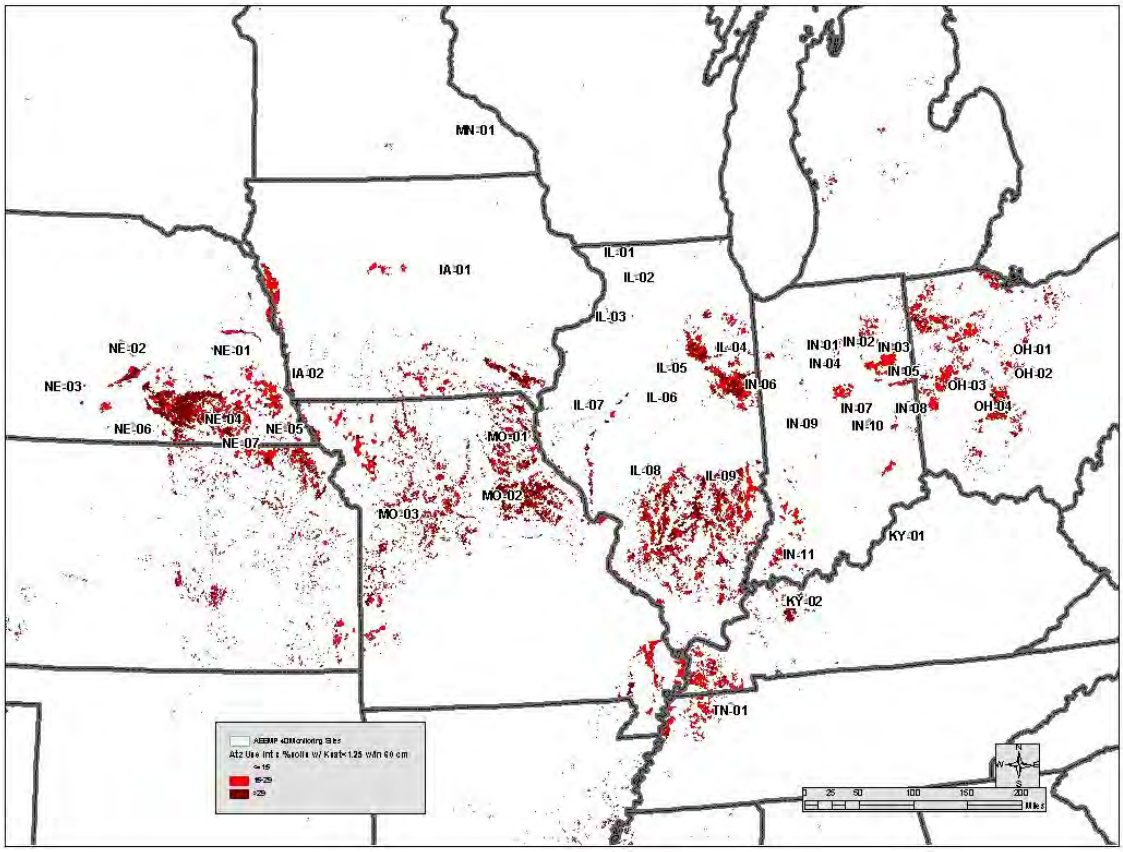


Figure 24: NHD+ catchments in the Midwestern US meeting US EPA's watershed vulnerability criteria for atrazine.

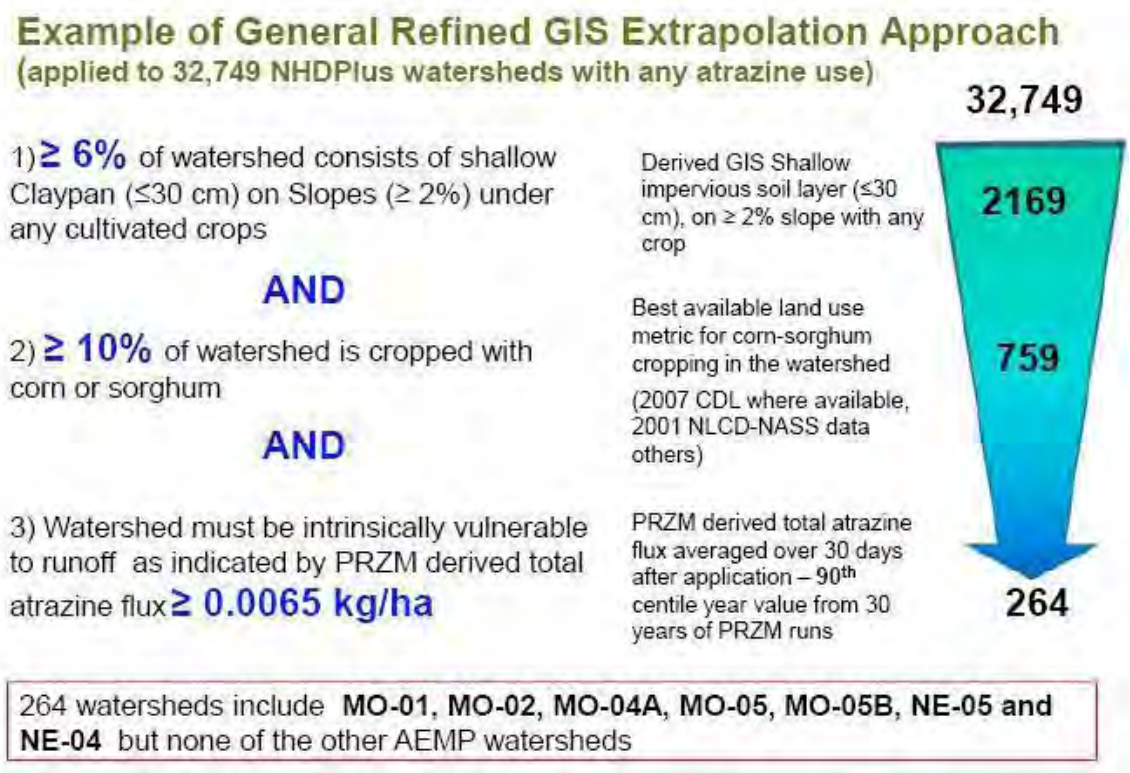


Figure 25: Registrant's criteria for selecting watersheds similar to the AEEMP sites that exceeded the CE-LOC in multiple years. Source: Syngenta.

Although Syngenta's approach did not specifically attempt to distinguish between the AEEMP sites that exceeded the CE-LOC in multiple years and the sites that did not exceed the CE-LOC, the soil-slope-crop criteria, weighted by the fraction of the watershed in corn or sorghum, did distinguish between the two groups (**Figure 26**). However, the additional Pesticide Root Zone Model (PRZM) calculated atrazine flux – 90th percentile of 30 years of 30-day average atrazine flux – did not distinguish between AEEMP sites that exceed the CE-LOC in multiple years and sites that did not exceed the CE-LOC (**Figure 27**). Based on the 90th percentile of the 30-day average concentrations for the AEEMP watersheds, 15 of 31 sites that did not exceed the CE-LOC have PRZM values greater than the lowest of the sites exceeding in multiple years (**Figure 27**).

Because PRZM does not specifically model the type of subsurface flow that may result from the shallow restrictive layers identified in the AEEMP sites and that exceeded the CE-LOC in multiple years, the US EPA would not expect the PRZM values to distinguish between the AEEMP sites.

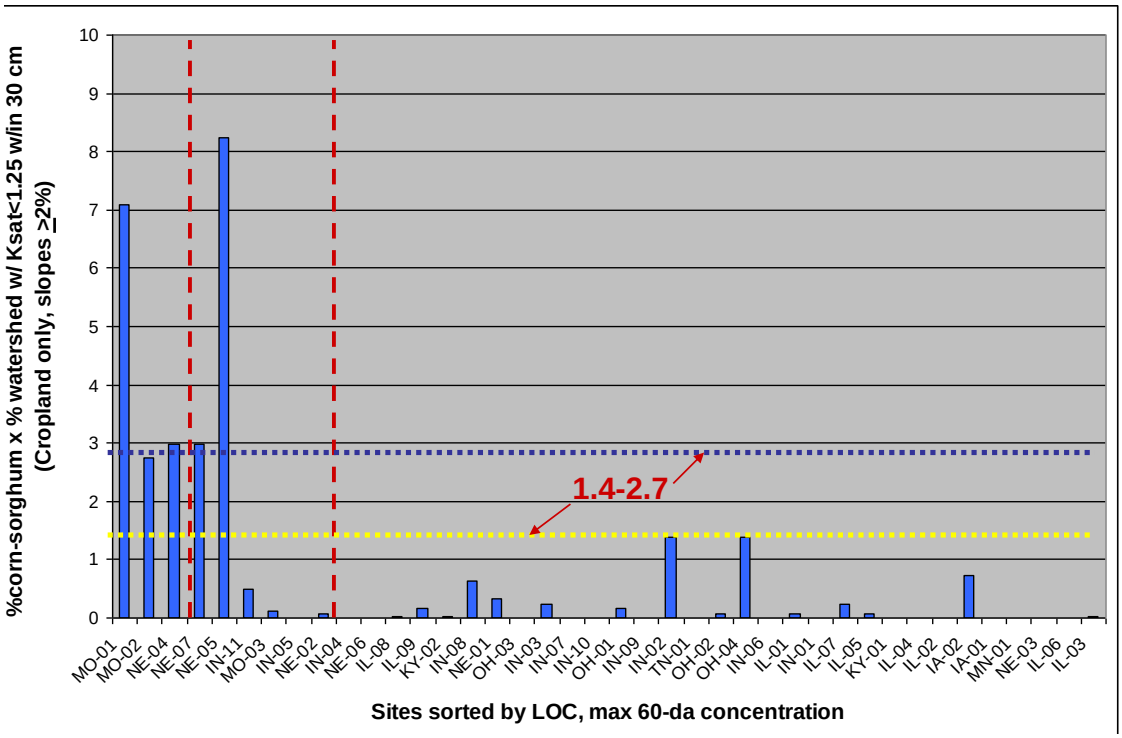


Figure 26: Percent of AEEMP watershed areas with soils that have a K_{sat}<1.25 um/s within 30 cm of the surface on slopes >2% under cropland x % corn or sorghum (as a fraction). See the text for the description of the red, blue and yellow lines.

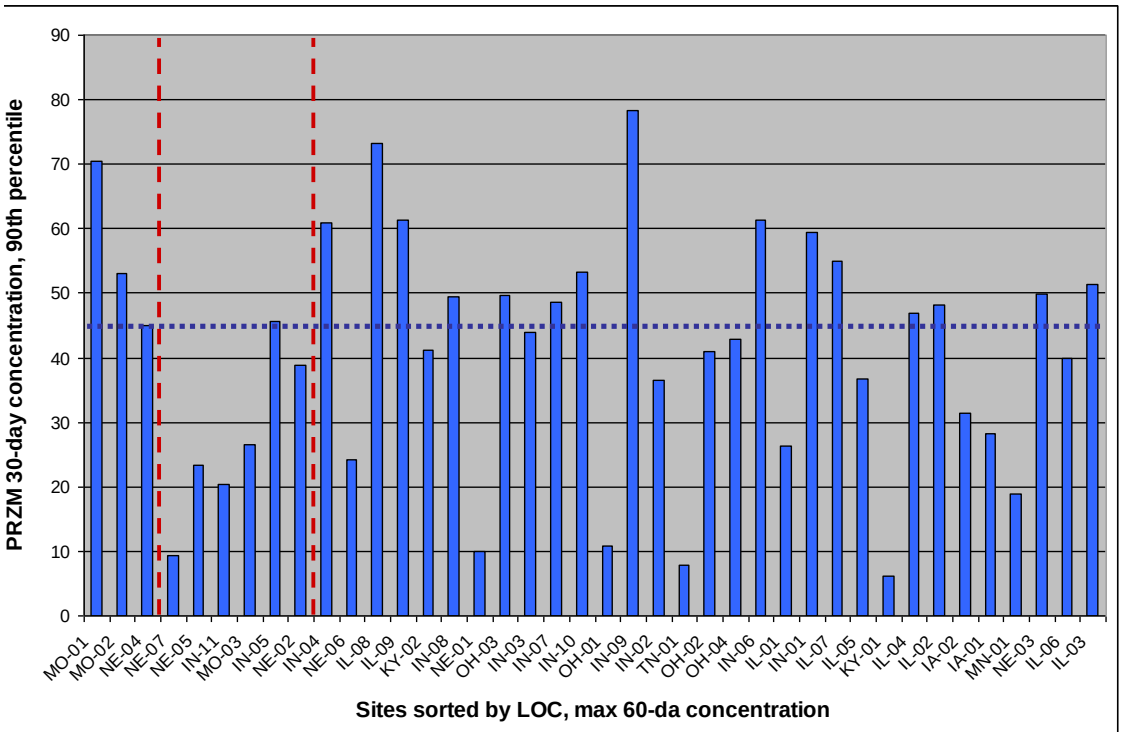


Figure 27: 90th percentile of 30-day average PRZM atrazine flux concentrations calculated for the 40 AEEMP sites. See the text for the description of the red and blue lines.

15.3. Evaluating the Watershed Vulnerability Criteria

While EPA and Syngenta identified the presence of soils with shallow, drainage restrictive layers, differences in the ultimate watershed criteria have resulted in two sets of watershed criteria with different threshold values (see **Table 22** below). The minimum threshold values listed in the table were identified by EPA based on a comparison of the sites that exceeded the CE-LOC in multiple years and the sites that did not exceed the CE-LOC during the study period.

Table 22 - Comparison of Watershed Criteria Used to Identify Sites that Exceed the CE-LOC in Multiple Years		
Watershed Parameters	**EPA Criteria**	**Syngenta Criteria** [3]
Drainage restriction / Ksat value	≤ 1 µm/s [1]	≤1.25 µm/s
Depth to drainage restrictive layer	50 / 60 cm [1]	30 cm
Land use	All land uses (entire watershed)	Cropland only
Slope	No slope cutoff	Slopes ≥ 2%
Minimum % of watershed area meeting the threshold values	54-73% [2]	5-6% [2]
Adjustments to % area threshold	x atrazine use intensity	x % of corn or sorghum in the watershed x 90th %ile of the 30-day average PRZM concentration [3]
Lower bound on threshold value	22 [2]	0.6 [2]
Upper bound on threshold value	29 [2]	1.3 [2]

[1] In the May 2009 SAP, EPA developed watershed criteria for drainage restrictive layers using 1 µm/s for the K_{sat} value and a depth of 50 cm. For this analysis, we used criteria of 1.25 µm/s and 60 cm because that coverage was already available for the entire atrazine use area.

[2] The lower end of the threshold range represents the highest value found for any of the 31 monitoring sites that did not exceed the CE-LOC in any of the monitoring years. The upper end of the range represents the lowest value found for the 3 sites that exceeded the CE-LOC in multiple years.

[3] This is EPA's reproduction of Syngenta's documented criteria approach documented. While Syngenta passed the watersheds through three separate filters, EPA worked through a combination of watershed parameters identified by Syngenta in order to apply an upper and a lower bound on the threshold criteria.

Consistent with the May 2009 FIFRA SAP recommendations (USEPA, 2009a) to evaluate both sets of criteria, this section details a methodology to evaluate the two approaches. Despite similarities in the basic vulnerability criteria, EPA's and Syngenta's watershed parameters often identify different areas as vulnerable (**Figure 28**). The map shows headwater watersheds similar in size to the AEEMP monitoring sites that either meet EPA's vulnerability criteria (shown in blue), Syngenta's criteria (in red), or both criteria (purple). While headwater watersheds meeting Syngenta's criteria occur primarily in the upper Midwest, from Ohio to Nebraska and Kansas, the EPA's criteria includes areas in southern Illinois (in a claypan area similar to that in northeastern Missouri where MO-01 and MO-02 occur) and in parts of Louisiana and Texas.

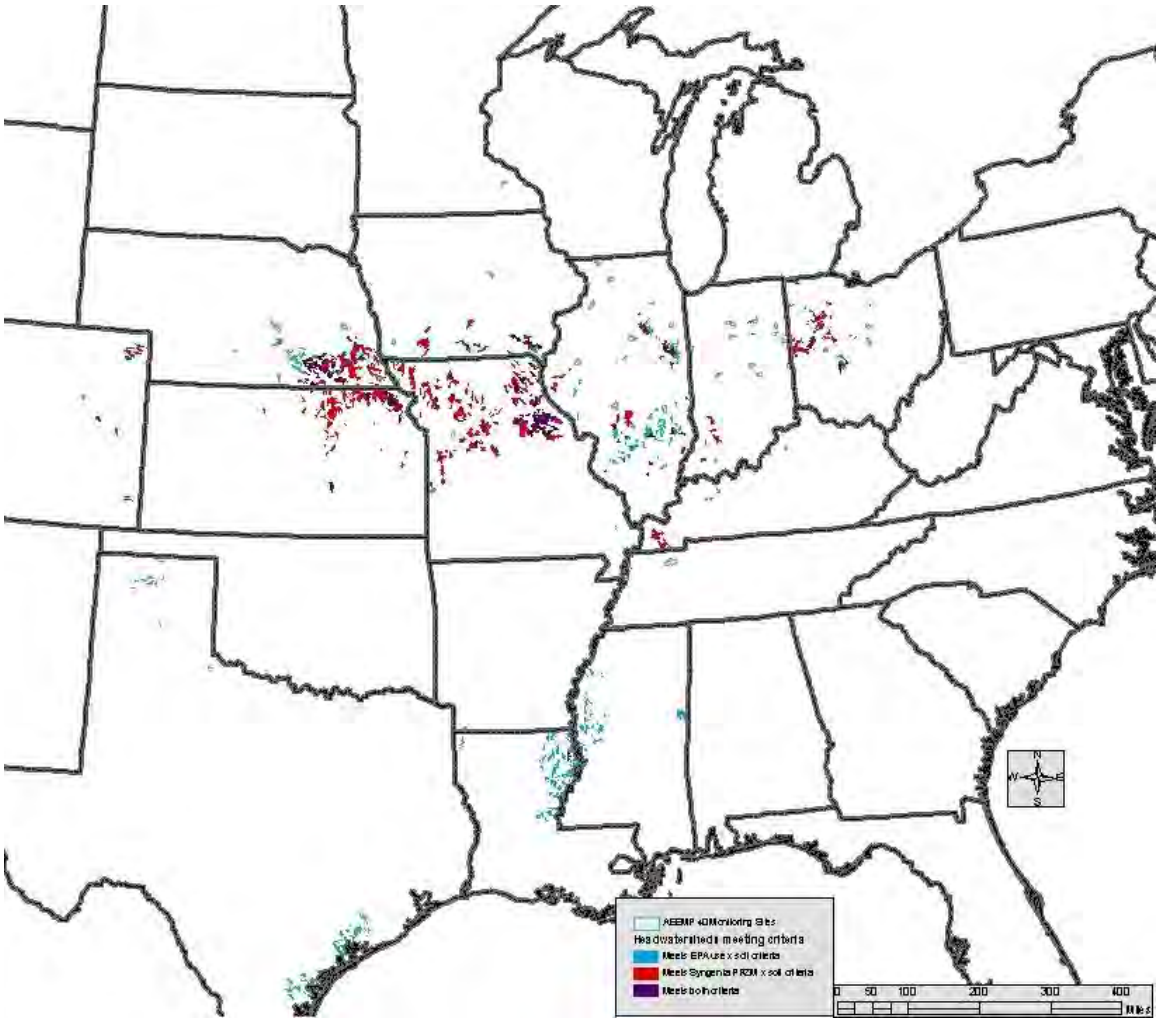


Figure 28: Headwater watersheds that meet US EPA's (in blue), Syngenta's (red), or both (purple) watershed vulnerability criteria for atrazine.

In order to better evaluate the differences between the two sets of criteria, the US EPA requested that Syngenta conduct an additional monitoring study that could be used to refine the vulnerability criteria. The EPA recommended that monitoring sites provide a statistical representation of headwater watersheds binned according to criteria (EPA or Syngenta) and range in threshold. The range is represented as between the lower and upper threshold, greater than the upper threshold, or a set less than the lower threshold could also be included).

The main focus for additional monitoring was to evaluate the two criteria approaches (EPA's watershed analysis vs Syngenta's watershed analysis) used for identifying watersheds that are likely to exceed the CE-LOC in multiple sampling years. A secondary focus was to ensure a geographical representation of the sites, particularly in areas where the availability of monitoring data is limited.

Ultimately, 25 sites were selected to represent either of the two criteria, including a set that met both. They selected sites to represent watersheds that either fell between the two threshold values or were greater than the upper threshold value. Monitoring began in the spring of 2010. The EPA will evaluate the results of the monitoring study once the third year of monitoring is completed and once Syngenta submits a full report, including the statistical sampling design, results of the monitoring, and a full set of watershed characteristics. The EPA anticipates receiving this report in time to evaluate it as a part of the registration review for atrazine.

Additionally, the US EPA is currently evaluating other monitoring data sets from state monitoring programs and from the USGS NAWQA program. These monitoring data will provide the EPA with additional sites that can be used to evaluate the watershed vulnerability criteria.

15.4. Further Evaluations of Watershed Criteria: The Cornbelt Watershed Regression for Pesticide (WARP) Model

The 2009 SAP recommended using an effects index or concentration metric, rather than categorical CE-LOC thresholds (*i.e.*, exceeded the CE-LOC in multiple years vs. did not exceed during the study) in order to take advantage of data from all 40 AEEMP sites (USEPA 2009a). The Panel encouraged the development of a "Cornbelt Watershed Regression for Pesticide (WARP) Model" and recommended considering additional data related to application (planting dates, timing of atrazine application), weather (rainfall intensity and duration), soils and hydrology (runoff propensity index, composite curve numbers, watershed geometry), and management (riparian buffers/setback areas, tillage, conservation practices, etc.). Some of the recommended parameters can be collected on a national scale while others may only be available, if at all, on a local scale. Readily available parameters available throughout the atrazine use area are potentially useful in providing a national or regional atrazine vulnerability assessment that can be used to identify areas of likely concern for atrazine. Less readily available parameters may be useful for watershed-specific evaluations that attempt to more narrowly pinpoint the causes and sources of atrazine residues in water.

The EPA has begun analyses that use time-averaged atrazine concentrations and a variety of watershed parameters which vary spatially (soil and hydrology parameters) as well as temporally (atrazine use, land use, rainfall), and which use parameters and monitoring results from individual years rather than multi-year averages. Such refinements may better identify the relative contributions of atrazine use patterns and rainfall on atrazine concentrations detected in the streams.

The USGS recently developed and published a cornbelt WARP model (Stone and Gilliom 2011). The USGS evaluated how well a number of watershed characteristics (relating to pesticide use,

land use, agricultural management practices, soil properties, physical watershed characteristics, weather characteristics, and hydrologic properties) predicted various maximum time-average (14, 21, 30, 60, and 90 day durations) and 95th percentile atrazine concentrations in streams in the Corn Belt region. Stone and Gilliom (2011) used 37 of the AEEMP sites (2004-2007 sample years), 5 Heidelberg University NCWQR sites, and 2 NAWQA sites for model development, and 11 site/year combinations from the AEEMP and NCWQR and 10 sites from the Atrazine Monitoring Program for Community Water Systems (http://www.epa.gov/opp00001/reregistration/atrazine/atrazine_update.htm) for model evaluation.

The following watershed parameters provided the best fit for predicting atrazine concentrations in the cornbelt WARP model:

- Percentage of agricultural land with a soil-restrictive layer within the top 25 cm of the surface
- Total precipitation (in mm) during May and June
- Percentage of stream flow due to Hortonian overland flow
- Watershed area (km^2)
- Percentage of watershed that is artificially drained
- Atrazine use intensity (kg a.i. / watershed area in km^2)

These parameters accounted for 53 to 62 percent of the variability in the atrazine concentration measurements in the model-development sites (Stone and Gilliom, 2011). As the US EPA moves into the registration review phase for atrazine, it will further evaluate the utility of using the cornbelt WARP model as a means of identifying watersheds that are most vulnerable for exceeding the aquatic community CE-LOC.

The Cornbelt WARP model can be used to rank watersheds based on relative vulnerability based on estimated concentrations, similar to what EPA did to identify the original area of the most vulnerable watersheds to target for the AEEMP monitoring program (USEPA, 2009a). Because the model generates time-weighted concentrations (*e.g.*, maximum 60-day average concentration in a year), it may also be useful in identifying watersheds that have a greater potential for exceeding the aquatic plant community level of concern concentration. The EPA will be evaluating its utility in this regard during registration review.

16. Method for Comparing Monitoring Data to the Aquatic Plant Community CE-LOC

While reviewing this section, please consider the charge questions below.

SAP Questions:

- Please comment on the strengths and limitations of EPA's development and use of bias factors (Chapter V, Section 16.1) for addressing uncertainties in monitoring data.

Prediction of bias factors is dependent on the selection of an appropriate model. EPA illustrated (Chapter V, Section 16.1) both categorical and regression methods for prediction of bias factors based solely on the number of samples taken in the 2nd and 3rd quarters of the year (April 1st to September 30th).

- Please comment on EPA's prediction of bias factors from monitoring data using categorical or regression method approaches.

- Please comment on any additional methods for estimating bias factors that would be useful in this situation.

EPA illustrated (Chapter V, Section 16.1) both categorical and regression methods for estimation of bias factors as a function of the sampling frequency of monitoring data. Step-wise regression analysis indicates that watershed size and average flow rate in the 2nd and 3rd quarters of the year are not significant variables for prediction of bias factors. However, the number of samples in the 2nd and 3rd quarters of the year was found to be a significant variable, accounting for 46% of the variation in the bias factor.

- What other variables, if any, should be considered in the prediction of bias factors?

EPA examined (Chapter V, Section 16.1) the performance of various regression equations to assess the failure percentage for identification of monitoring site-years with true maximum 60-day average concentrations exceeding the CE-LOC for atrazine. This analysis showed that application of a bias factor, based on sample number during the 2nd and 3rd quarter of the year, substantially reduced the number of sites with underestimation of true maximum 60-day means.

- Given the EPA analysis, what other tests, if any, should be conducted to assess the performance of regression models for prediction of bias factors?

16.1. The Development of Bias Factors

Implementation of a PATI derived CE-LOC concentration requires addressing the uncertainty in capturing the true maximum 60 day mean concentration from monitoring programs of different designs. As discussed in the surface water monitoring section, sampling frequency among monitoring programs is variable. Sampling frequency, however, is an important consideration in the accurate estimation of pesticide concentrations in surface water. The 2011 FIFRA SAP recommended that the use of bias factors is an appropriate approach for addressing uncertainty in capturing true concentrations from monitoring data. This section provides an illustration of such an approach for the development of factors to address uncertainty in capturing the maximum 60 day mean from monitoring data. The bias factor serves as a protective multiplier of the actual concentration from monitoring data to account for uncertainty associated with sampling frequency. The general bias factor equation is as follows:

$$\hat{Y} = X * \text{BIAS FACTOR}$$

Where:

$\hat{Y}$ ***=Estimated True Maximum 60 day average atrazine conc.***
X= ***Maximum 60 day rolling mean atrazine conc. obtained from monitoring data***
Bias Factor=True maximum 60 day rolling mean atrazine conc./Estimated 5th percentile maximum 60 day rolling average atrazine conc.

The development of bias factors in this analysis is based on selected monitoring data from the AEEMP and NCWQR monitoring programs (**Appendix G**). Watershed characteristics and descriptive statistics of selected atrazine monitoring are shown in Table 26. The data were selected because they represent site-year chemographs with limited data infilling and long sampling periods (April 1st to September 17th). For this analysis, geographic and hydrologic properties were not used to discriminate the monitoring data. Missing data in each chemograph was infilled using a stair-step imputation between measured values. The measured atrazine concentration for each sampling day represents the highest measured value of the day, regardless of the monitoring method (grab *vs* autosample) or the number of samples taken within a given day.

Table 23. Watershed Characteristics for Selected AEMP and NCWQR Monitoring Sites					
				Maximum Peak	Maximum 60 day Average
Site	Year	Watershed Area (acres)	Average Flow (m^3/sec)	µg/L	
MO-05	2008	16192	0.3190	37.83	5.74
MO-05b	2008	116781	26.1526	36.83	7.04
MO-02	2009	18023	NR	155.20	16.83
MO-02	2008	18023	0.10074	56.60	7.57
MO-02	2007	18023	0.01920	16.18	3.46
MO-04a	2008	5382	0.8595	144.69	11.84
IN-11	2008	5780	1.3593	27.12	2.03
Sandusky	1995	800621	328.475	15.46	6.47

Bias factors were derived using a Monte Carlo sub-sampling process as presented to the 2011 FIFRA SAP (FIFRA SAP, 2011). A similar approach was used by Syngenta to develop bias factors from AEEMP and NCWQR data (Mosquin *et al*. 2011). Each constructed chemograph was randomly subsampled 10,000 times using subsampling intervals of 4 days, 7 days, 14 days, and 28 days. The sampling simulation was conducted using the Crystal Ball software programs (Crystal Ball® 2000 and Crystal Ball Predictor™, 1999) starting with a common seed (**Appendix G**). For each sampling realization, a random value from the custom distribution of values within the designated time interval was selected to represent a value at each sampling interval within the chemograph. These selected concentrations were then used to construct simulated daily chemographs of atrazine concentrations using a linear interpolation. From a distribution of the 10,000 simulated chemographs, the 5th percentile maximum 60 day rolling mean atrazine concentration was selected to derive the bias factor. Selection of the 5th percentile maximum 60 day rolling mean atrazine concentration would provide development of protective bias factors. Protective factors would ensure that when applied the estimated 60 day maximum mean concentration would be equal to or higher than the true 60 day maximum mean concentration. The bias factor was calculated by dividing the true maximum value from the original chemograph by the 5th percentile maximum 60 day rolling mean atrazine concentration from the Monte Carlo simulation (**Appendix G**).

Some uncertainties, limitations, and assumption in the development and application of the bias factor in this analysis are:

- Random sampling is simulated for the 2nd and 3rd quarters of the year. It is anticipated that systematic sampling in the field or sampling around rainfall events would alter bias factor estimation.

- Serial correlation of daily atrazine concentrations is not directly considered in the estimation of the bias factor. It is anticipated that serial correlation effects on bias factor estimation is dependent on sampling interval.
- The extent of data infilling for the development of an annual chemograph is expected to impact the reliability of the bias factor estimation. In this analysis, AEEMP monitoring data were selected for their low infilling amount as well as long duration of monitoring during the 2nd and 3rd quarters.
- Because the bias factors are based on monitoring data from the 2nd and 3rd quarters of the year, they are probably not applicable to monitoring sites with atrazine use in the 1st and 4th quarters.
- The bias factors in this analysis may not be representative of all atrazine use site-year combinations in the AEEMP and NCWQR monitoring data because they only represent 7 site-years.

The 60 day average monitoring bias factors for 4, 7, 14, and 28 day sampling intervals are shown in **Table 24** (**Appendix G**). The minimum bias factor is 1. There is an inverse relationship between the calculated bias factor and sampling frequency; the bias factor and it variability increases with a decrease in sampling frequency or an increase in sampling interval (**Figure 29**). Similar results were found in a preliminary analysis of AEEMP and NCWQR monitoring data for different geographic areas and watershed sizes (**Appendix G**). Among the various monitoring sites presented in Table 24, the average bias factor for maximum 60 day mean atrazine concentrations is -1.79 for the 4-day sampling interval, -2.56 for the 7-day sampling interval, -3.38 for the 14-day sampling interval, and -8.35 for the 28 day sampling interval. The bias factors are reported as negative numbers to represent the extent of underestimation from the true value.

Table 24. Atrazine Monitoring Bias Factor for Predicting the Maximum 60 day Average Concentration				
	Sampling Interval			
Site	4 day	7 day	14 day	28 day
MO-05b 2008	-1.47	-2.02	-3.89	-7.91
MO-05 2008	-1.89	-3.48	-3.36	-4.67
MO-02 2008	-1.94	-2.64	-1.94	-7.81
MO-02 2009	-1.90	-2.36	-3.10	-8.50
MO-04a 2008	-2.41	-4.27	-6.80	-22.34
IN-11 2008	-1.13	-1.92	-2.57	-5.08
SANDUSKY 1995	-1.17	-1.21	-2.00	-2.13
Descriptive Statistics				
Mean	**-1.79**	**-2.56**	**-3.38**	**-8.35**
Median	**-1.89**	**-2.46**	**-3.23**	**-7.86**
Maximum	**-2.41**	**-4.27**	**-6.80**	**-22.34**
Minimum	**-1.13**	**-1.21**	**-1.94**	**-2.13**

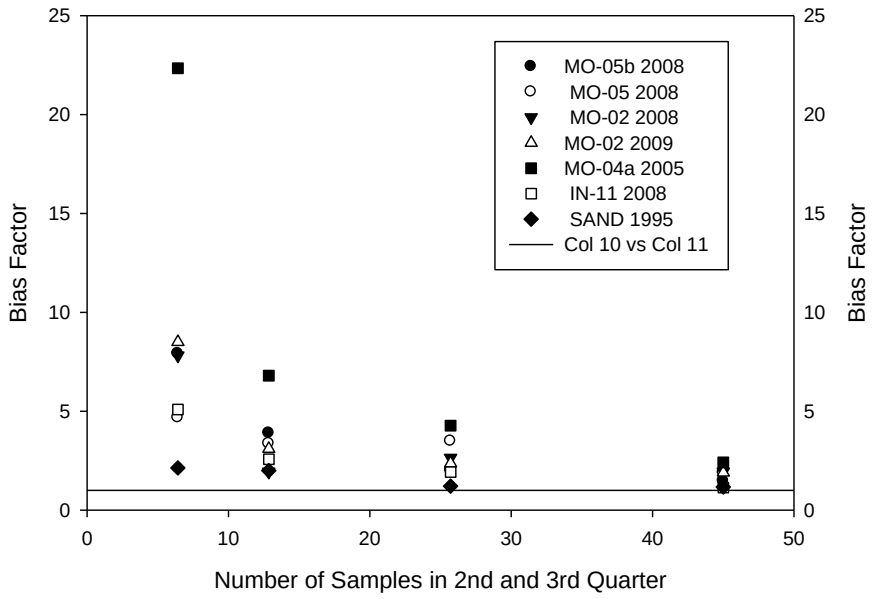


Figure 29. Relationship of Bias Factor and Sampling Frequency for Selected Watersheds from AEEMP and NCWQR Monitoring Programs.

Selection and use of bias factors may be accomplished by using either a categorical or regression-based approach. The sample number was determined by dividing 180 days (representing the 2nd and 3rd quarters) by the sampling interval. For this analysis, the various approaches are not expected to represent all atrazine monitoring sites due to limited geographic and hydrologic variability in the test data. Forward stepwise multiple regression, however, indicate that watershed size and average flow are not significant variables ($p < 0.45$) for prediction of bias factors. In contrast, sample number was a significant variable ($p<0.0029$) for predicting bias factors. Therefore, the development of bias factors is based on the relationship of sample number and bias factor.

A categorical approach would require the assignment of a unique bias factor for each sampling interval (**Figure 30**). Selection of the appropriate bias factor would assume the bias factor is fixed between the windows of sample intervals. For example, using an upper 95% confidence interval of the mean bias factors, a sampling frequency of 25 to 46 samples would be assigned a bias factor of 1.87(2); 12 to 26 samples would be assigned a bias factor of 2.94 (3); 7 to 13 samples would be assigned a bias factor of 4.01(4); and ≤ 6 samples would be assigned a bias factor of 10.83 (11). The resolution of bias factor assignment is dependent on the number of different sampling intervals used in deriving bias factors; fewer sampling intervals would invoke more uncertainty in the deviation and selection of the bias factor.

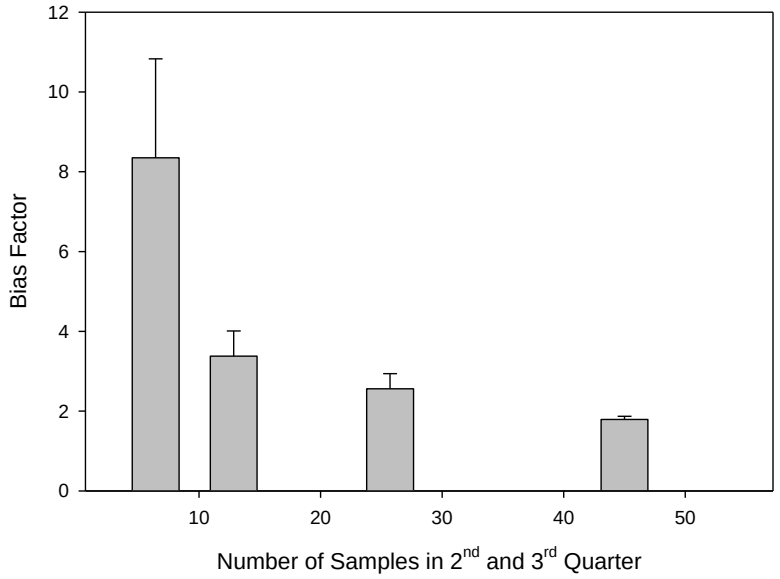


Figure 30. Categorical Designation of Upper 95% Confidence Limit of the Mean Bias Factor According to Sampling Frequency.

Another approach for assignment of bias factors is the use of regression equations to describe the relationship between sample number and bias factor (**Appendix K**). The development of a regression model would be an important tool for estimating the true 60 day maximum annual mean from monitoring data with different sampling frequencies. This approach allows prediction of bias factors for a large number of sampling intervals.

The first regression equation was derived using the SigmaPlot Regression Wizard (Version 11). This equation was selected to represent the relationship of bias factor and sample numbers in the 2nd and 3rd quarters. The best least-squares non-linear regression fit was a 4-parameter exponential decay model [y = 470.8251*exp (-0.0708*x) +-458.7698*exp(-0.0708*x); r^2=0.3304; p<0.02] where y is the bias factor and x is the number of samples (
Figure 31). The upper 95% confidence interval of the regression[y =1383.0681*exp (-1.067*x) +11.6378*exp(-0.0425*x); r^2=0.9993; p<0.0001)] was also fit and is presented on the graph the graph in red.

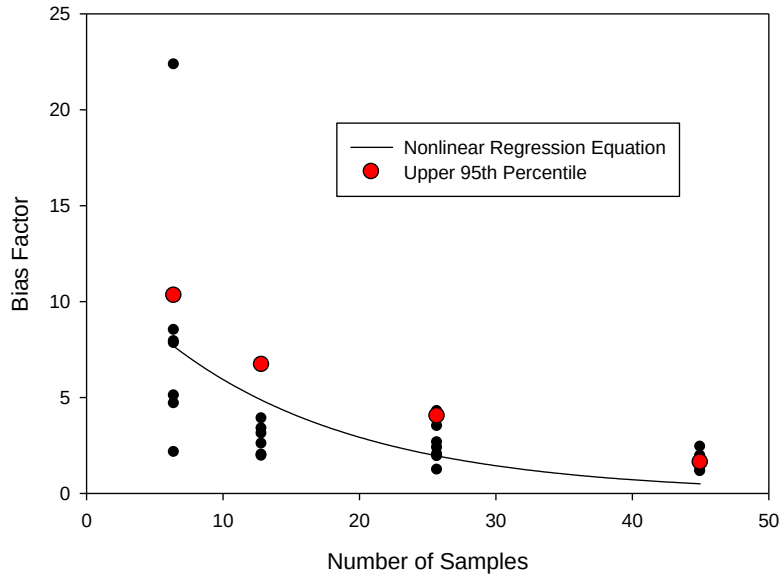


Figure 31. Nonlinear Regression between Number of Samples and 60 Day Average Bias Factors

A second approach was made by fitting the average bias factor against the number of samples in the 2nd and 3rd quarters. In this case, the best fitted equation was also a a 4-parameter exponential decay model [y= 170.5297*exp (-0.5580*x) + 4.1246*exp (-0.0186*x); r^2=1.00](**Figure 32**). Although the variability in bias factors is removed using this approach, it may provide a useful predictor of the mean bias factor as a function of the number of samples in the 2nd and 3rd quarters.

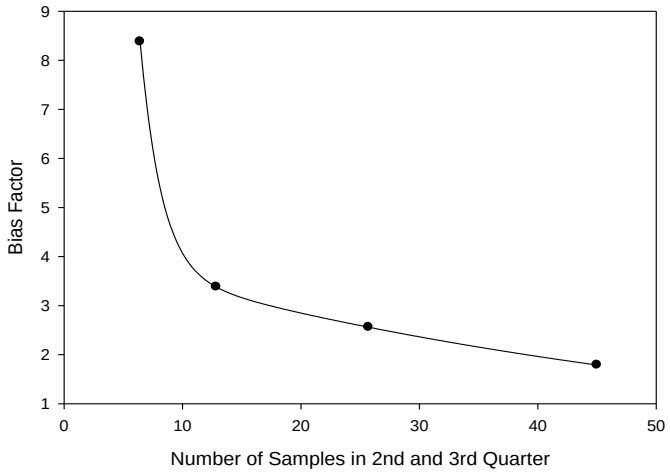


Figure 32. Nonlinear Regression of Number of Samples and the Mean 60 Day Average Bias Factor Across Watersheds (7 Site-Years)

A third approach was conducted using a 1st order linear model on log-transformed bias factor values. The resulting log- linear equation is log y= 0.78616 -0.013729, (r^2=0.4634, p<0.0001) (**Figure 33**). The upper 95% confidence interval and prediction intervals for the bias factors are also presented. The regression model for the 95% confidence interval is log y = 0.946326366-0.007773*X.

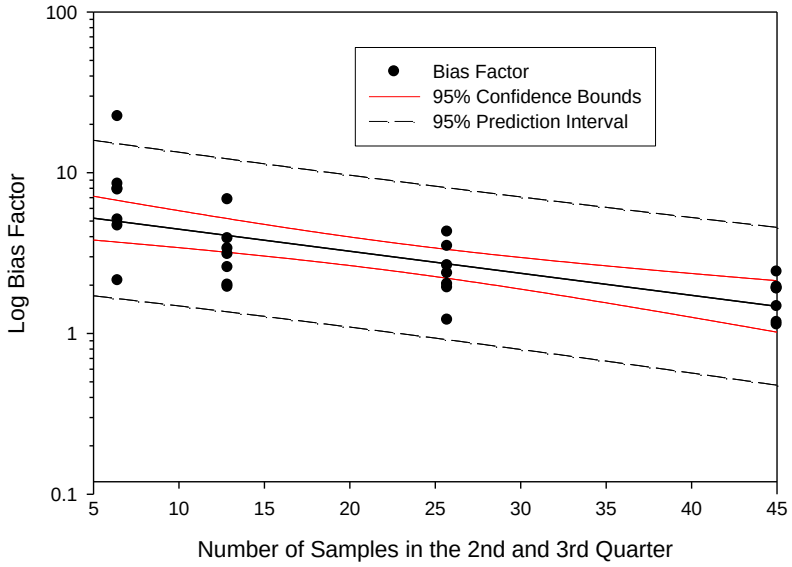


Figure 33. Relationship of Number of Samples and All Data 60 Day Average Bias Factor Across Watersheds

As expected, the highest variability in bias factors is associated with the 28-day sampling frequency. This is not unexpected because infrequent sampling leads to a greater likelihood that the peak atrazine concentration will not be measured. This is an important consideration since the estimation of the true average pesticide concentrations is dependent on capturing the peak concentration (USEPA**, 1998**). However, the magnitude of bias factors also appears to be dependent on the shape of the chemograph (**Figure 34**). From our data analysis, the MO-04a 2008 data had the highest estimated bias factors when compared to the other monitoring sites. Examination of the MO-04a 2008 chemograph shows it has fewer and sharper peaks when compared with the MO-05b 2008 and Sandusky 1995 chemographs. The sharpness and number of the chemograph peaks represents the measured temporal occurrence pattern. A similar observation was found in a preliminary analysis of other AEEMP and NCWQR monitoring data.

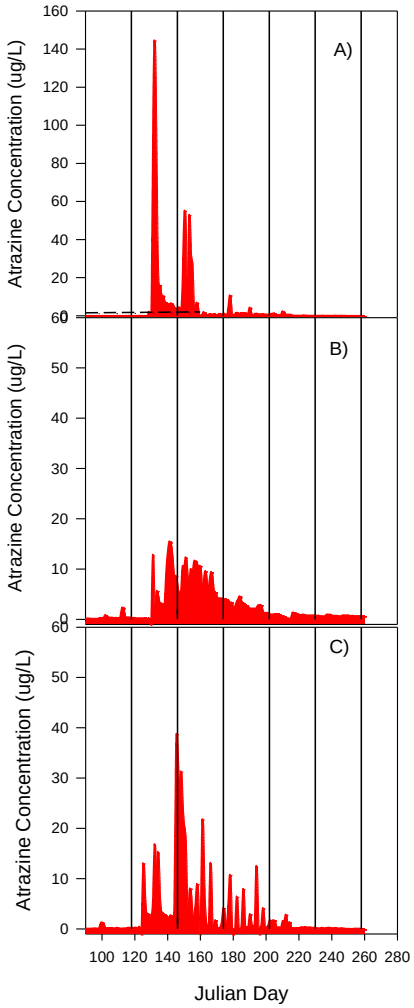


Figure 34. Difference in Chemograph Shapes and Height for Monitoring Site-Years Used for Development of Regression Equation: A.) MO-04a 2008; B) MO-05b 2008; and C) Sandusky 1995.

The predicted bias factors using the derived regression equations are shown in **Table 25**. As expected, each method provides some degree of over or underestimation of bias factor for a given sampling frequency. In general, the upper 95% confidence interval of the log-linear equation provided the most conservative estimates of bias factor at sampling frequencies less than 20 samples per 180 days. At sampling frequencies greater than 20 samples per 180 days, the upper 95% confidence interval of the non-linear equation provides more conservative estimates of bias factors. As discussed above, the greatest uncertainty in bias factor estimation is associated with the longest sampling intervals (28 days) or the fewest samples (6 samples in 180 days). Therefore, the applicability of bias factor estimation may have defined limits according to sample frequency. The lowest bias factor is constrained to 1. The highest bias factor in this analysis is 22.34 at the 28 day sampling interval. As discussed, presumably the magnitude of the bias factor appears to be related to the sharpness and number of the peaks.

Table 25. Estimated Bias Factors Using the Categorical and Regression-Based Estimation Methods.

Sampling Interval (Days)	Number of Samples Over 180 Days	Categorical Bias Factor	Average Non-Linear Bias Factor	Log Linear Bias Factor	Log Linear Bias Factor Upper 95% Confidence Interval	Non-Linear Bias Factor	Non-Linear Bias Factor Upper 95% Confidence Interval
4	45	1.87	1.79	1.55	3.95	1.00	1.72
7	26	2.94	2.56	2.79	5.58	1.95	3.90
10	18	2.94	2.96	3.53	6.40	3.37	5.42
14	13	4.01	3.38	4.13	7.02	4.85	6.74
20	9	4.01	4.61	4.65	7.52	6.37	8.03
28	6	10.83	8.38	5.02	7.88	7.65	10.30

To demonstrate the application of the bias factor approach using the regression equations, 190 available AEEMP site-year data sets were sampled using 4, 7, 10, 14, 20 and 28 day fixed sampling intervals. The various regression equations were used to estimate the factors (Table 12). Four chemographs were developed for each sampling interval by site-year combination. The chemographs were developed through systematic sampling at a fixed interval using the first 4 days of the monitoring interval as different starting dates. Each chemograph for the sampling interval-site-years was developed by stair-step imputation between sampled concentrations. The raw data and its summary for this performance analysis are provided in the **Appendix G**.

The purpose of the bias factor adjustment is to address uncertainty in estimating the true maximum 60-day mean according to sample frequency over 180 days. This analysis illustrates that estimation of the unadjusted maximum 60 day mean from 4, 7, 10, 14, 20, and 28 at uniform sampling intervals underestimates the true maximum 60 day mean at 82 to 94% of the sites (**Table 26**). As expected, the application of bias factors to adjust the maximum 60 day mean resulted in substantially fewer underestimations of true maximum 60 day mean. The regression equation with the fewest underestimations of true maximum 60 day mean is the upper 95% confidence interval of the log-linear equation.

Table 256. Percentage of the AEEMP site-years (N=190) that failed to meet or exceed the actual 60-day maximum running mean based on various sampling intervals.

Sampling Interval (Days)	60-Day Maximum Running Average, Unadjusted	Average Non-Linear Bias Factor	Log Linear Bias Factor	Log Linear Bias Factor Upper 95% Confidence Interval	Non-Linear Bias Factor	Non-Linear Bias Factor Upper 95% Confidence Interval
4	82	3	12	1	82	7
7	94	9	7	0	16	3
10	88	10	8	1	8	2
14	88	13	8	1	5	1
20	86	16	16	4	7	4
28	89	5	15	5	5	3

Bias factor estimation methods were evaluated to provide an illustration of various approaches for quantifying atrazine concentrations from monitoring programs with different sampling frequencies. In this case, the maximum 60 day mean concentration was used because it is the concentration CE-LOC derived from the PATI model. The bias factor adjusted maximum 60 day mean concentration from monitoring programs may be useful in identifying sites where additional monitoring may be required to assess potential ecological exposure from atrazine.

16.2. Translating Monitoring Data with the Bias Factor

The proposed method for determining whether a water body exceeds the Aquatic Plant Community LOC (CE-LOC), is as follows. The monitoring data for each year is collected and evaluated based on the following conditions:

1) A total of at least 4 samples across the year are required to construct a chemograph and calculate a 60-day maximum running mean.
    a. Summary statistics for the site are calculated.

b. A chemograph is constructed using a stair-stepping technique (linear interpolation preferred) with a minimum concentration of 0.16 µg/L (LOQ in the NAWQA Database) used as the concentration prior to the first measurement of the year,
c. The final annual measurement within a single data set is carried over 4 days, then the concentration is dropped to 0.16 µg/L for the duration of the year,
d. The maximum 60-day running mean is calculated from the chemograph.

2) The available monitoring data with at minimum 6 samples in the 2nd and 3rd quarters are adjusted with the bias factor approach.
   a. Log Linear Bias Factor upper 95% confidence interval on the mean is used as a multiplier to correct the maximum 60-day running mean to what would be expected from daily sampling at these sites.

3) Lastly the maximum 60-day running mean and the bias factor adjusted value are compared to the CE-LOC.

16.3. Results of Preliminary Review of Available National Monitoring Data.

Data Sources Evaluated as of 5/8/2012:

2003-2011 Finished Water; 2003-2011 Raw Water; AEEMP; CA; Heidelberg 1; Heidelberg 2; Heidelberg 3; Heidelberg 4; Heidelberg 5; Heidelberg 6; IA DNR 2003-2006; KS All Lakes and Wetland; KS Streams; KS WQ 2009-2011; MN MDA SW 2008-2011; MN MDA WQ 1993-2007; NAWQA; NE; PDP; WI 2008; WI 2009; WI 2010; WI 2011; USEPA Reservoir Data 1999-2000 (summary data provided in **Appendix G**)

- Total number of site-year combinations meeting criteria: 6917 site-years

Two general types of data were identified from the analysis of the available monitoring data, and were ranked based on the relative confidence in their results:

Bias Factor Applicable Data:

Sampling intervals 14 days or less (*i.e.,* 12 or more samples for the 2nd and 3rd quarters combined)

This temporal limitation to the 2nd and 3rd quarters was based on atrazine exposure typical in the AEEMP data. These data are considered of higher quality than are those with less frequent sampling. Bias factors for these datasets ranged from 3.36 to 7.13.

- Total number of sites meeting the criteria for bias factor use: 3385 site-years
- Total number of exceedances when bias factors are applied (4 µg/L): 2353 site-years (70%)
- Total number of exceedances when bias factors are applied (7 µg/L): 1886 site-years (56%)

Sampling intervals 28 days or less (*i.e.*, 6 or more samples for the 2nd and 3rd quarters combined)

This temporal limitation to the 2nd and 3rd quarters was based on atrazine exposure typical in the AEEMP data. These data are considered of less high quality than the previous set. Bias factors for these datasets ranged from 3.36 to 7.94.

- Total number of sites meeting the criteria for bias factor use: 5124 site-years
- Total number of exceedances when bias factors are applied (4 µg/L): 3125 site-years (61%)
- Total number of exceedances when bias factors are applied (7 µg/L): 2488 site-years (49%)

Unadjusted Data:

All available data were also reviewed based on the 60-day maximum running mean concentration. This includes data with sampling intervals greater than 14 days during the 2nd and 3rd quarters (i.e., fewer than 12 samples for the 2nd and 3rd quarters combined)

These data are considered for review but there is some uncertainty in the exceedances based on the infrequency of sampling. Because the bias factors were developed based on the AEEMP and Heidelberg data, their emphasis being on the 2nd and 3rd quarters, the applicability of the bias factor to data collected in other quarters is uncertain.

- Total number of sites assessed: 6917 site-years
- Total number of exceedances based on 60-Day maximum running mean (4 µg/L): 779 (11%)

- Total number of exceedances based on 60-Day maximum running mean (7 µg/L): 471 (7%)

16.4. Scope of National Aquatic Plant Communities Potentially Threatened by Atrazine Exposure.

The extent of the atrazine levels exceeding the Aquatic Plant Community CE-LOC (4 ppb) is reviewed in this section. According to the available monitoring data from Federal, State, Local and Registrant sources, the following states have exceedances of the CE-LOC (site specific details are reported in **APPENDIX G)**:

Based on 60-Day Running Means:

4 µg/L (21 States): AL, AR, FL, IA, IL, IN, KS, KY, LA, MD, MI, MO, MS, ND, NE, NJ, OH, TN, TX, VA, and WI

7 µg/L (15 States): AL, AR, IA, IL, IN, KS, LA, MO, MS, NE, OH, TN, TX, VA, and WI

Based on Bias Factor Adjusted Values, 28-day interval:

4 µg/L (30 States): AL, AR, CA, CO, FL, IA, IL, IN, KS, KY, LA, MD, MI, MN, MO, MS, NC, NE, NJ, NY, OH, OK, OR, PA, SD, TN, TX, VA, WA, and WI

7 µg/L (27 States): AL, AR, CA, FL, IA, IL, IN, KS, KY, LA, MD, MI, MN, MO, MS, NC, NE, NJ, NY, OH, OR, PA, SD, TN, TX, VA, and WI

An important limitation of the bias factor application used here is that those site-years with fewer than 6 samples gathered throughout the 2nd and 3rd quarters are not adjusted with the bias factor. Exceedances based on data collected in the 1st and 4th quarters provide additional insight into atrazine exposures outside of the primary application period for corn and sorghum crop areas and may reflect exceedances due to the other uses mentioned in Section 3.2 above or differences in use patterns due to the latitudinal gradient of seasonality. There were 14 states (110 site-years) that had too few samples within the 2nd and 3rd quarters for bias factor adjustments but had 60-day running averages above the 4 µg/L CE-LOC, and 11 states (48 site-years) above the 7 µg/L CE-LOC.

The monitoring data can also be used to test the performance of the vulnerable areas analysis (Section 15.3) that was based on the analysis of the AEEMP watershed characteristics. In this review of the monitoring data, the data were parsed into two categories “Prior to 2006” and

"2006-2011". These categories were selected to also review the potential effects of the reduction in the maximum allowable annual pounds/acre, enforcement starting in 2006.

The AEEMP monitoring sites in relation to the pounds of atrazine use per 1000 acres, the vulnerable areas analysis results, and the designated 303d atrazine impaired waterways are shown in **Figure 35**. What is reflected in the map regarding the vulnerability analysis (gold areas) is that exceedances are generally associated with the predicted areas. In addition, a number of states have identified atrazine related impairment to waterways. These impaired waterways reflect that atrazine exposures outside of the vulnerable areas analysis, and not associated with AEEMP monitoring sites, are also of concern.

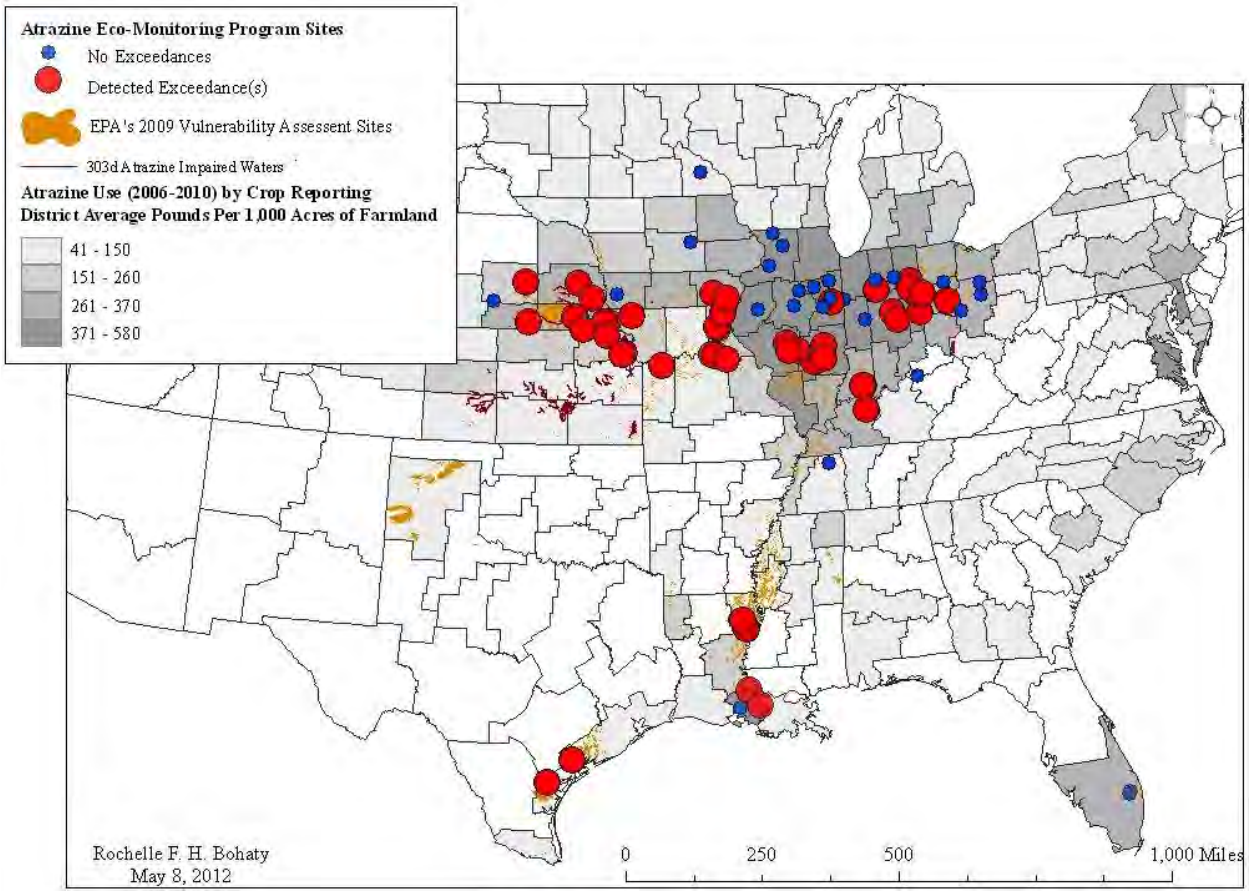


Figure 35. AEEMP monitoring program sites in relation to 303d atrazine impaired waterways (dark red areas), and the vulnerable areas (gold areas) identified through the methods discussed in **Section 15**. (The following 303d listings have not been mapped: ILC08_C33; ILC09_C09; ILC19_C19; ILCD01_CD01; ILCH01_CH02; ILJQ03_RJF; ILJQ03_RJG; ILN11_N11; ILN12_N12; ILNC07_NC07; ILNE05_NE05; ILOEB01_ROV; ILOIL01_ROT; ILOJ08_ROK; ILOZC01_SOC; ILROA_ROA; IL_SDH; IL_SDZO; IL_SOL)

To further investigate the atrazine exposure outside of the AMP and AEEMP monitoring program, the EPA reviewed the available monitoring data in a spatial context. The next several figures show NAWQA monitoring sites that have exceeded the CE-LOC. The NAWQA data set was selected for an example here because they contain the GIS localities for all of the monitoring sites. The other available monitoring programs may also have GIS localities for each of the monitoring sites, however this information was not provided to the EPA at this time.

The NAWQA sites that exceed the 4 µg/L and 7 µg/L CE-LOC based on the 60-day maximum running mean are shown in **Figure 36 and Figure 37** respectively. The distribution of exceeding sites compared to the vulnerability analysis seems to identify vulnerable areas; however a number of sites fall outside of the identified vulnerable areas. These sites make sense in the context of the pounds of atrazine used per 1000 acres. The NAWQA data results in 36 sites exceeding the 4 µg/L CE-LOC and 5 sites exceeding 7 µg/L between 2006 and 2011, in comparison to 123 and 41 sites exceeding from 1992-2005, possibly reflecting the reduced runoff based on the rate reductions implemented in 2006.

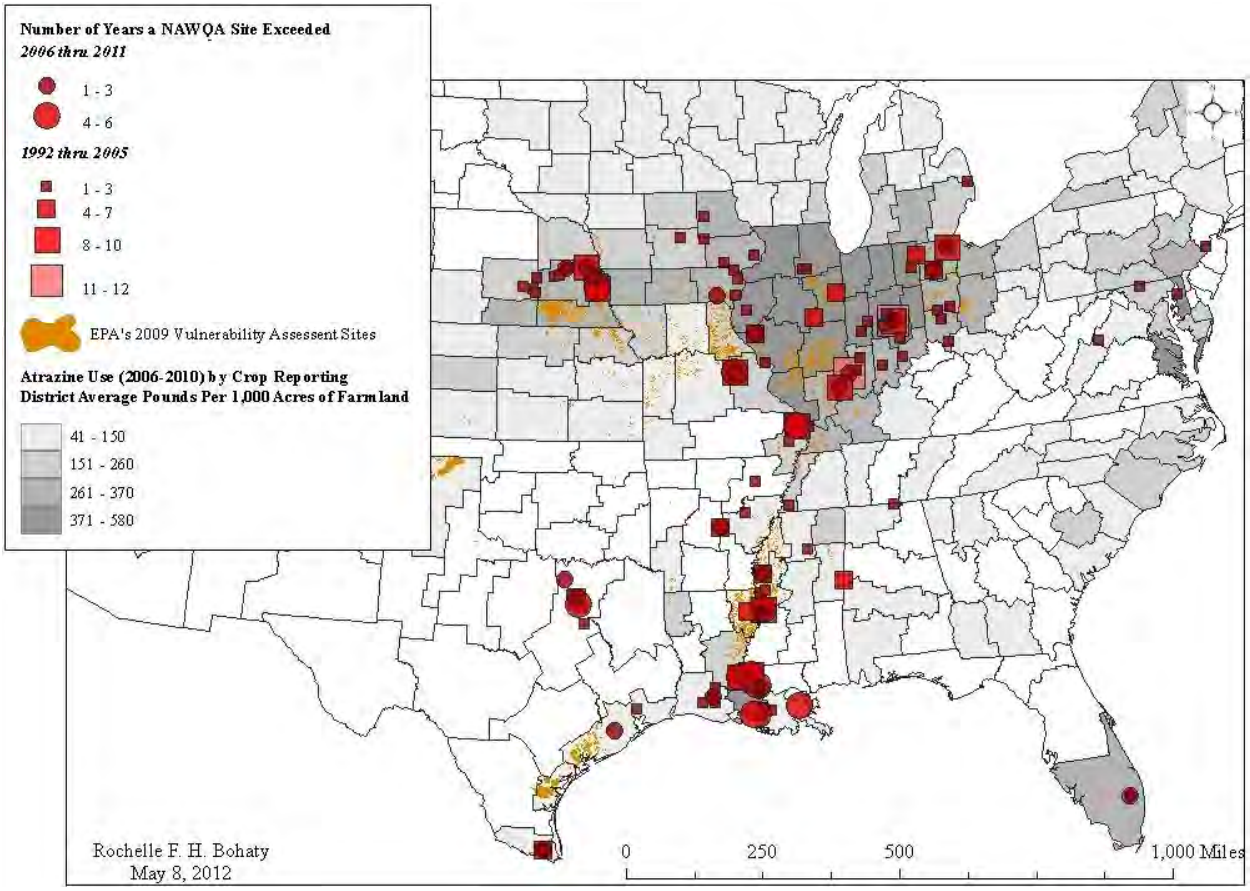


Figure 36. NAWQA monitoring sites that exceed the 4 µg/L CE-LOC based on the 60-day running average.

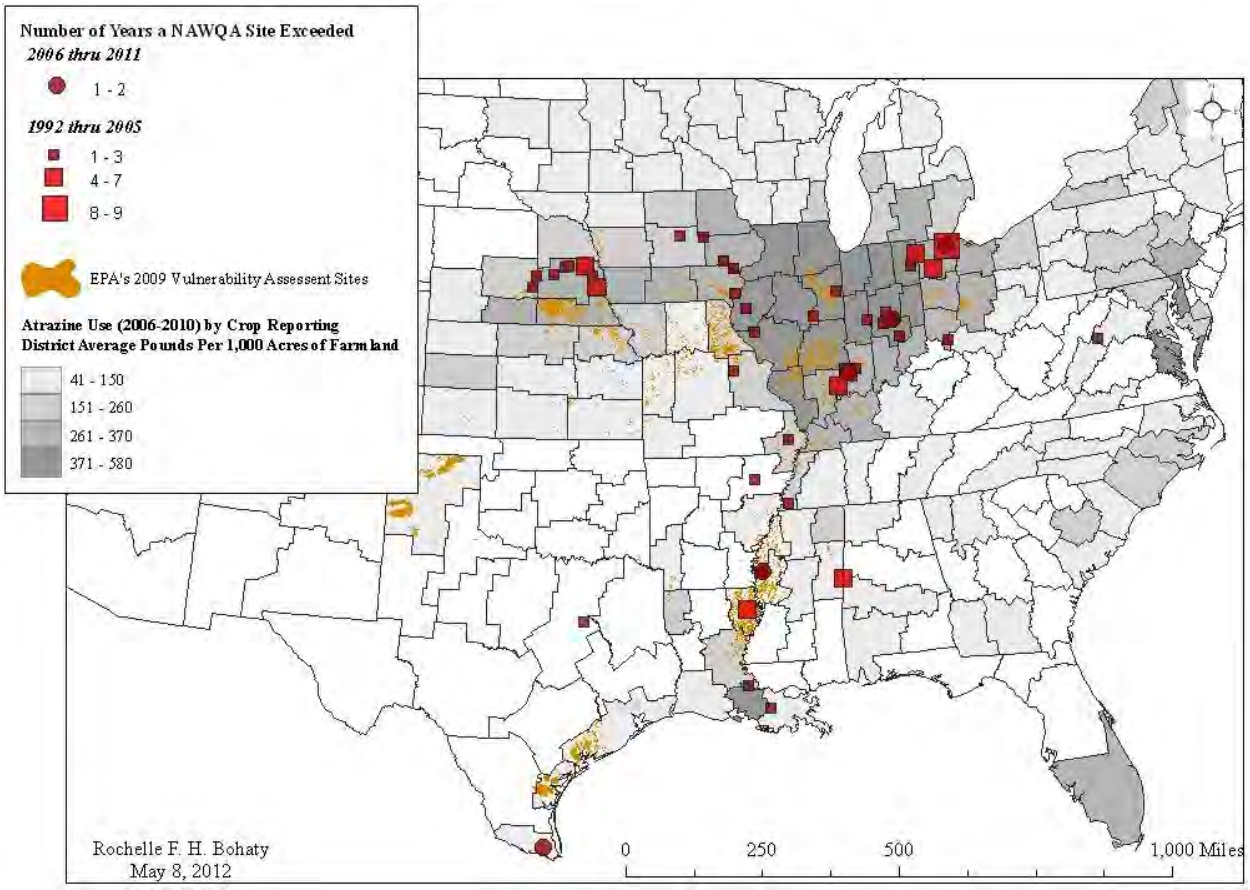


Figure 37. NAWQA monitoring sites that exceed the 7 µg/L CE-LOC based on the 60-day running average.

The NAWQA 4 µg/L and 7 µg/L CE-LOC exceedances based on the bias factor adjusted 60-day maximum running mean and a minimum of 6 samples across the 2nd and 3rd quarters are shown in **Figure 38** and **Figure 39**. Based on this distribution of exceeding sites, the most predictive feature is the amount of atrazine used per 1000 acres. This reduced set of the NAWQA data results in 47 sites exceeding the 4 µg/L CE-LOC and 38 sites exceeding 7 µg/L between 2006 and 2011, in comparison to 123 and 108 sites exceeding from 1992-2005, possibly reflecting the reduced runoff based on the rate reductions implemented in 2006.

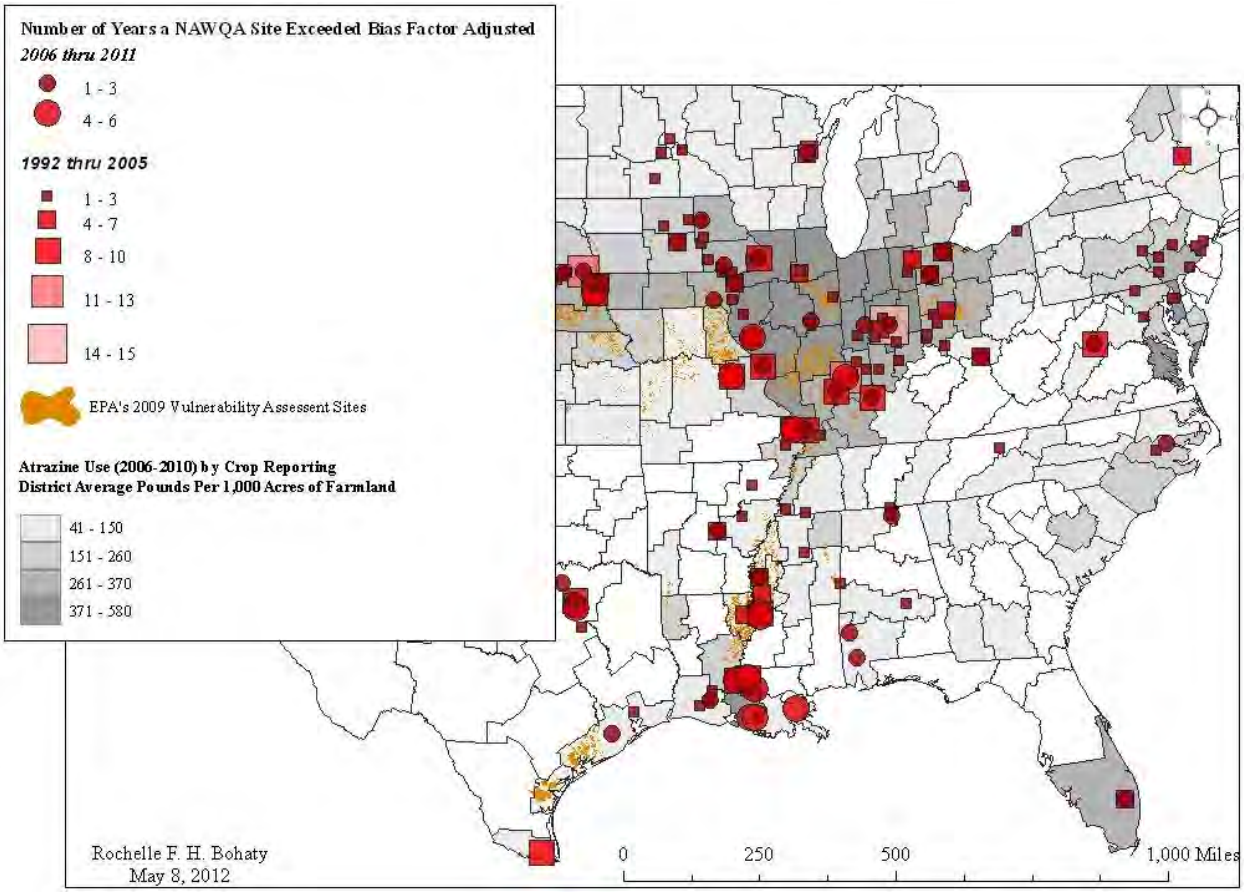


Figure 38. NAWQA monitoring sites that exceed the 4 µg/L CE-LOC based on the bias factor adjusted 60-day running average. Only those site-years with 6 or more samples in the 2nd and 3rd quarters were mapped.

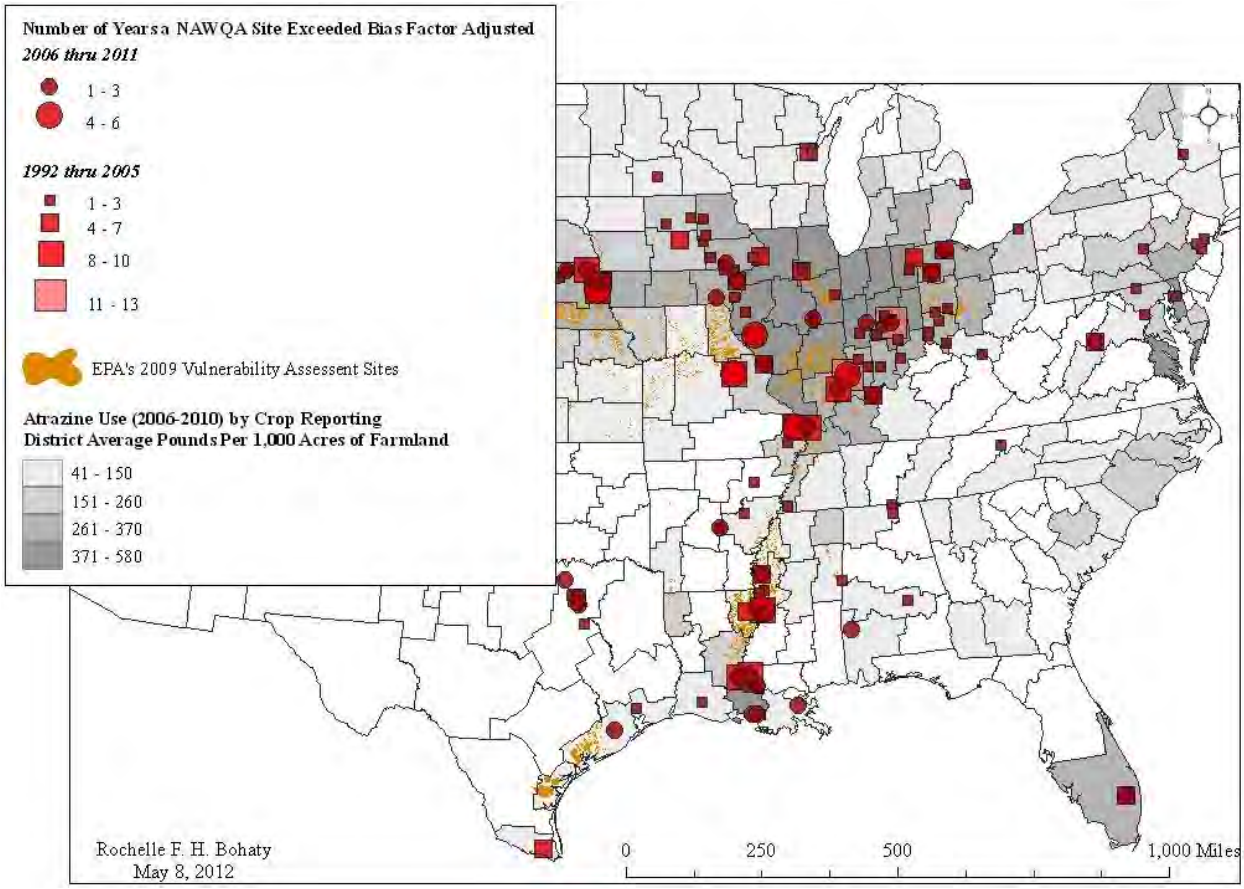


Figure 39. NAWQA monitoring sites that exceed the 7 µg/L CE-LOC based on the bias factor adjusted 60-day running average. Only those site-years with 6 or more samples in the 2nd and 3rd quarters were mapped.

The implementation of the Aquatic Plant Community CE-LOC results in a picture of the national scope of atrazine exceedances and reflects that the 2009 vulnerability analysis identified watershed characteristics that were predictive but not entirely representative of the exceedances seen in the available monitoring data. These results indicate that those sites that have exceeded the CE-LOC would require additional monitoring to obtain data for, at minimum, a 14-day interval for the likely periods of atrazine runoff. These times of year will vary on a nationwide scale due to what crop is being treated and the seasonality of use (*e.g.,* due to temperature).

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