White Paper in Support of the

Meeting of the FIFRA Scientific Advisory Panel

on the

Examination of Mesocosm and Microcosm Studies for Evaluating the Effects of Atrazine on Aquatic Plant Communities

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Preamble

This document presents the U.S. Environmental Protection Agency's (EPA's) reevaluation of 11 atrazine microcosm and mesocosm studies[1] identified by the 2012 Federal Insecticide, Fungicide and Rodenticide (FIFRA) Scientific Advisory Panel (SAP) as warranting further review. These studies are part of EPA's Ecological Risk Assessment of atrazine and are specifically used in assessing the effects to aquatic plant communities.

The document consists of nine chapters. **Chapter 1** is background material, which includes a brief overview of atrazine (use, usage, environmental fate, and ecological effects), an overview of EPA's assessment of aquatic plant communities, the history as it relates to the use of microcosm and mesocosm studies to assess aquatic plant communities, and then the objective of this White Paper. The **Charge Questions** to the SAP can be found at the end of **Chapter 1** in the Objective section.

Following the background, **Chapters 2** through **8** present the reevaluation of the 11 cosm studies identified by the 2012 SAP as warranting further review. These chapters include a summary of the design and execution of the experiment associated with these studies, EPA's use of these studies in the 2016 Refined Ecological Risk Assessment, the major concerns and criticisms associated with these studies over the years from previous SAPs and public comments and finally, EPA's 2023 reevaluation and conclusions regarding these studies.

Chapter 9 summarizes EPA's conclusions from the 2023 reevaluation of the 11 cosm studies.

Supporting reference materials can be found near the end of the document in the appendices, including details about relevant documents referenced throughout the White Paper (see **Appendix A** and **Appendix B**).

[1] Complex experiments used to examine communities under semi-controlled conditions that simulate natural environments.

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CHAPTER 1. BACKGROUND

1.1 Purpose

To protect aquatic plant communities from the effects of the herbicide atrazine, the U.S. Environmental Protection Agency (hereafter 'EPA') developed an aquatic plant community-based concentration-equivalent level of concern (hereafter 'CE-LOC'). The CE-LOC is determined using a combination of single-species aquatic plant toxicity studies and microcosm/mesocosm (hereafter 'cosm') studies. The cosm studies included in the CE-LOC calculation can be defined as complex experiments used to examine aquatic plant communities under semi-controlled conditions that simulate natural environments. Endpoints for these cosm studies were defined as single determinations of the response of one or more components of the aquatic plant community (*e.g.,* phytoplankton, periphyton, macrophytes) for a defined individual atrazine test concentration as it relates to the controls in the study.

From 2002 to 2016, EPA considered over 70 cosm studies obtained from the open literature or that were submitted to EPA by the registrant Syngenta Crop Protection. The 2012 Federal Insecticide, Fungicide, and Rodenticide (FIFRA) Scientific Advisory Panel (SAP) (hereafter 'SAP') identified 11 of those cosm studies used by EPA (Table 1 of USEPA, 2012b; also APPENDIX B. TABLE 1 FROM THE 2012 SAP MEETING MINUTES**APPENDIX B**. TABLE 1 FROM THE 2012 SAP MEETING MINUTES here) as warranting further review because of concerns about study design or performance flaws, as well as EPA's interpretation of the results. In 2022, EPA received additional public comments about the 11 cosm studies, along with renewed requests to convene an SAP meeting regarding the studies (USEPA, 2022b; 2022c).

Considering this input, the purpose of this 2023 White Paper is to present EPA's 2023 reevaluation of these 11 cosm studies and associated publications, specifically the inclusion/exclusion decision, and if appropriate, the results associated with the specific endpoint(s) from each study. This 2023 reevaluation includes reconsideration of the design and execution of the experiment associated with each study, over two decades of comments, and the results associated with each endpoint. In the end, EPA's reevaluation aims to address the concerns surrounding these 11 cosm studies that were raised by past SAPs and substantive public

comments related to the concerns raised by the panel, including those received from Syngenta Crop Protection[2], the Triazine Network[3], and the Center for Biological Diversity (CBD).

This White Paper provides an overview of atrazine, its history as it relates to the cosm studies, chapters focused on the 11 studies (and associated publications when necessary), and supporting reference materials. The **Charge Questions** to the 2023 SAP can be found at the end of this chapter.

1.2 Overview of the Use, Usage, Fate, and Effects of Atrazine

Atrazine, a chlorotriazine photosystem II inhibitor[4] first registered in 1958 in the U.S., is an herbicide used to control annual broadleaf and grass weeds. It is primarily used on corn, sorghum, and sugarcane crops, but is registered for a wide variety of uses including wheat, macadamia nuts, guava, soybeans, fallow crop lands, and non-agricultural use sites (*e.g.*, turfgrass). Between the years 2013 through 2017, an annual average of 72 million pounds of atrazine were applied to treat an average of 75 million acres of agricultural crops, with the majority being to corn in terms of both pounds applied (87%) and acres treated (88%) (USEPA, 2021).

Based on laboratory studies, atrazine is mobile in soil (FAO, 2000) and persistent in the environment (Goring *et al*., 1975) (average K_{oc} of 75 mL/g-oc and half-lives from 49 to 608 days in soil and water). The main routes of dissipation are microbial degradation under aerobic conditions, runoff, and leaching. Atrazine can break down into multiple degradation products including deethylatrazine (DEA), deisopropylatrazine (DIA), 2-hydroxy-6-ethylamino-4-amino-s-triazine (DIHA), 2-hydroxy-4-isopropylamino-6-amino-s-triazine (DHEA), diadealkylatrazine (DACT) and hydroxyatrazine (HA). Because of its persistence and mobility, atrazine has the

[2] Syngenta Crop Protection comments include those from Jeffrey Giddings with Compliance Services International (CSI), a Syngenta contractor. The comments from Syngenta Crop Protection and Jeffrey Giddings are collectively referred to as "Syngenta" in this White Paper.
[3] The Triazine Network comments include those from Dwayne Moore with Intrinsik Environmental Services, a Triazine Network contractor. The comments from the Triazine Network and Dwayne Moore are collectively referred to as "Triazine Network" in this White Paper.
[4] In plants, triazine herbicides such as atrazine inhibit photosynthesis by reversibly binding with a protein complex of the Photosystem II in chloroplasts, thereby inhibiting the transfer of electrons and the subsequent formation and release of oxygen (Schulz *et al.,* 1990).

propensity to move into surface and groundwater, which is confirmed by the widespread detections of atrazine in surface and groundwater samples (USEPA, 2021).

Based on the mechanism of action (*i.e.*, disruption of photosynthesis), atrazine is expected to be toxic to most flowering and non-flowering vascular plants, as well as multicellular and unicellular algae, which all rely on photosynthesis. To investigate this, there are many single-species laboratory aquatic plant toxicity tests available, which represent all major lineages. The most sensitive aquatic non-vascular plants tested with atrazine were the chlorophycean "green" algae, *Stigeoclonium tenue*, and the cyanobacterium "blue-green algae" *Oscillatoria lutea*, which showed a 67% and 93% reduction in chlorophyll production at 1 µg active ingredient (a.i.)/L, respectively. For vascular aquatic plants, the most sensitive species was *Elodea canadensis* with a 50% reduction in root dry-weight at 4.6 µg a.i./L (USEPA, 2016).

1.2.1 The Use of Cosm Studies to Assess Aquatic Plant Communities

EPA conducted a screening-level risk assessment that evaluated the potential risk of atrazine exposure to aquatic plants using single-species aquatic plant toxicity data (for the most recent, see USEPA, 2016). In addition to single-species data, EPA evaluated toxicity to aquatic plant communities using community-level data given the large number of available cosm studies and the stated risk management concern in reregistration for aquatic plant communities. Aquatic plant communities are vital to both aquatic and terrestrial food webs and, in turn, ecosystem integrity (Error! Reference source not found.**).** The complex interactions among members of the community can be investigated using cosm studies. For example, in a cosm study, researchers can assess numerous species, multiple trophic levels, direct and indirect[5] effects, community structure and function, and chemical fate and effects simultaneously, and often in more "natural" conditions (*e.g.*, temperature fluctuations, precipitation) than laboratory-based experiments provide. While extremely useful tools, these studies are both time and resource intensive, which not only limits the number of studies available in the open literature, but also the breadth of the experimental design (*e.g.*, replication, treatments, duration). This, coupled with the complexity of

[5] Indirect effects are effects that occur through interactions with other affected biotic (*e.g.*, predator, prey) or abiotic (*e.g.*, nutrients, dissolved oxygen) factors.

the communities[6] and the semi-controlled conditions, can render the results variable and challenging to interpret.

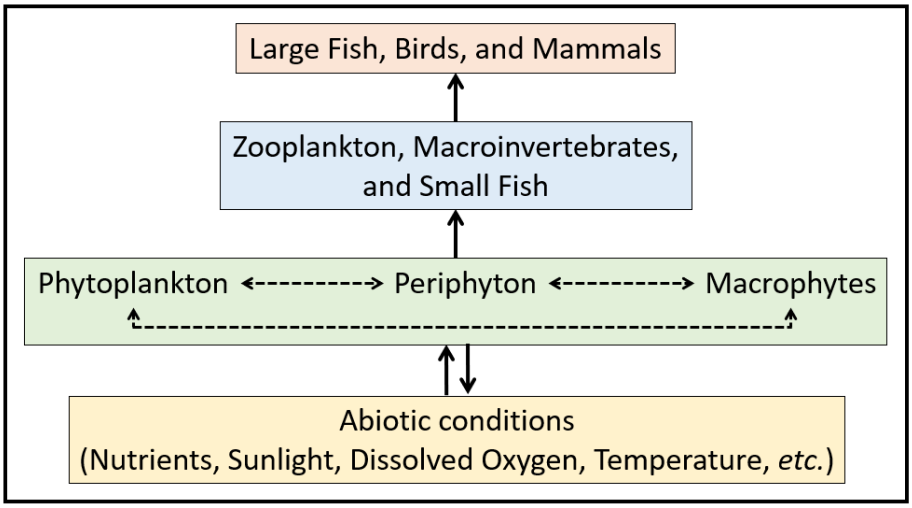

Figure 1.1. A simplified depiction of the interaction of abiotic conditions and trophic levels in aquatic ecosystems

In comparison to the required laboratory test guideline studies[7], cosm studies are often not submitted to EPA for consideration in risk assessments and when they are available for a chemical, there are usually only a few available. However, since the early 2000's, EPA has been able to incorporate cosm studies into the atrazine risk assessment because there are a large number of atrazine cosm studies available in the open literature, as well as submitted by Syngenta. These studies collectively provide insight into the effects of atrazine at the aquatic plant community level. In contrast to the specific standards established for laboratory test guideline studies on individual species, greater flexibility can be given to atrazine cosm studies in terms of study complexity, variability in design, and minor deficiencies. This flexibility is appropriate because the atrazine cosm database is not intended to represent toxicity to a specific organism or community under specific conditions. Rather, the cosm database is intended to capture as much environmental variability as possible given the intent to protect aquatic plant

[6] A multi-species grouping of test organisms may represent a "community" in the sense of the natural collection of organisms in an environment or an "assemblage" in the sense of a subset of a community or an artificially constructed assemblage of organisms. In this White Paper, EPA refers to cosm study systems with multi-species group of test organisms as "communities".

[7] Ecological data requirements are listed in the Code of Federal Regulations: https://www.ecfr.gov/current/title-40/chapter-I/subchapter-E/part-158/subpart-G/section-158.660 and aquatic plant (Group D) guidelines can be found here: https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines

communities at a national level. Therefore, atrazine cosm studies were only excluded if they did not meet basic scientific validity criteria (*e.g.*, study did not include controls) or had considerable uncertainty about atrazine being the causal mechanism of observed effects. Based on EPA's review of the large quantity of atrazine-related cosm studies, EPA found that aquatic plant communities may be more resilient to atrazine exposure than predicted by the effects and responses observed in single-species laboratory acute toxicity studies (USEPA, 2003). Potential impacts on the aquatic ecosystem include, but are not limited to, reduced biological diversity, reductions in food items for animals, reductions in fish spawning and nursery habitat, increased erosion potential, and reductions in overall water quality. Therefore, EPA's consideration of cosm studies helps provide a more realistic estimation of the potential adverse effects of atrazine on aquatic plant communities and thus helps protect vital food webs, ecosystem function, and ecosystem services (**Figure 1.1**).

1.2.2 The History of the Use of Cosm Studies in the Ecological Assessment of Atrazine

EPA first introduced the concept of using cosm studies for setting an aquatic plant community-based level of concern (LOC)[8] for atrazine in the 2003 Interim Reregistration Eligibility Decision (IRED) for Atrazine (USEPA, 2003). This marked the beginning of a 20-year discussion on cosm studies, exposure estimates, and threshold setting (**Figure 1.2**).

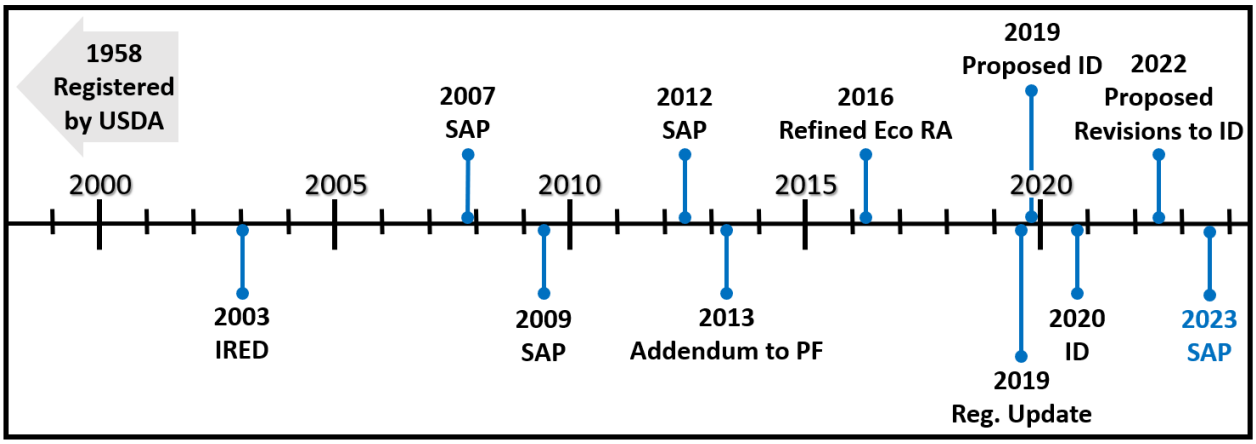

Figure 1.2. Timeline of relevant events. Events represent various regulatory and external peer review activities related to the assessment of the effects of atrazine to aquatic plant communities including the interim Reregistration Eligibility Decision (IRED), the Problem Formulation (PF) in support of the Registration Review of atrazine, the Refined Ecological Risk Assessment (Eco RA), and the proposed Interim Decision (ID).

[8] The LOC became known as the aquatic plant community-based concentration-equivalent LOC (CE-LOC) in 2009.

Around the time of the 2003 IRED, a screening and scoring approach for the cosm studies was developed from a workgroup consisting of EPA scientists and the broader scientific community, including registrant scientists (Gonzalez-Valero *et al.*, 2003). The scoring approach followed a method similar to Brock *et al.* (2000; often referred to as "Brock Scores"). In addition, an initial list of cosm publications was identified by the workgroup[9]. Using this list, if a publication was included, an identification number (hereafter 'endpoint') was assigned to each concentration tested within the experiment to represent the whole community. These endpoints aided in tracking and eventually represented the datapoints used in LOC calculations. The endpoints were then assigned a single number from "Brock Score 1" (no effect) to "Brock Score 5" (significant effect without return to control levels for more than 56 days) based on the response of one or more aquatic plant taxa from that concentration to represent the overall effect to the aquatic plant community (*i.e.*, one score per endpoint based on the response of phytoplankton, periphyton and/or macrophytes).

Ultimately, the 2003 IRED included 77 endpoints from 35 references in the cosm database[10] used to calculate the LOC, including several studies evaluated in this White Paper [*i.e.*, Lampert *et al.*,1989, Detenbeck *et al.*, 1996, the Kosinski publications, and those associated with the University of Kansas experiments].

Following the 2003 IRED, EPA went to the SAP several times. In December 2007, an SAP was convened to address the potential for community-level effects to aquatic plants in Midwestern streams, but the SAP ultimately did not comment on the cosm studies (USEPA, 2007; 2008). In May 2009, EPA again convened an SAP meeting that was focused on aquatic plant communities (USEPA, 2009a). In terms of the cosm studies, the SAP concluded that the description of the screening and scoring approach (Brock Score method) for the cosm studies was inadequate and needed to be refined. Additionally, the SAP, for the first time, commented on individual cosm studies, including Lampert *et al.* (1989), the Kosinski publications, and those associated with the University of Kansas experiments (USEPA, 2009b), which are discussed further in this White Paper.

[9] It is important to note that some cosm experiments are discussed in multiple publications, so some endpoints may have multiple references associated with them.

[10] The "cosm database" contains studies that passed the screening criteria used at the time and were then scored using the scoring approach employed at the time. Endpoints in the cosm database at that time went on to be used in the calculation of the level of concern (LOC; referred to as the "CE-LOC" from 2009 to present).

Following the 2009 SAP, EPA developed a new set of screening criteria derived from peer reviewed sources from 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). These screening criteria were intended to identify studies with confounding study design and performance elements to allow greater confidence in the study results. The criteria included a prescreen, which required that: (1) treatments were exposed to only atrazine (*i.e.*, not mixtures); (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. If a study passed the prescreen criteria, it was further screened using additional criteria that assessed basic elements such as the use of controls and the use of at least two replicates per treatment group (the full screening criteria can be found in USEPA, 2011, Appendix D of USEPA, 2012a, or Appendix G.1 of USEPA, 2016).

If a study passed the screening criteria, it went on to be scored before being used to calculate the CE-LOC. Because of the way EPA had implemented the Brock Scores in its previous scoring approach (*i.e.*, either an effect on the aquatic plant community or no effect; hence, a de facto binary approach), EPA decided to move away from the five tier Brock Score method and switched to a simpler binary approach where Brock Scores of 1 (no effect) and 2 (slight or transient effect) were scored as "no effect," while Brock Scores of 3 (clear effect with recovery[11]) or higher were scored as "effect." The revised scoring criteria followed the same basic principles discussed in Brock *et al.* (2000) and de Jong *et al.* (2008). In short, each endpoint was evaluated by a panel of EPA scientists where they determined if an effect had occurred at a specific test concentration based on a statistically significant difference from the control or best professional judgement in the absence of a statistical analysis, taking into consideration characteristics such as magnitude, duration, replication, variability, and recovery. This scoring decision for an endpoint is referred to as the "effect/no-effect conclusion" in this White Paper. Using the updated screening criteria and scoring approach, EPA assessed an additional 38 cosm studies recommended by the 2009 SAP, including the Seguin *et al.*

[11] 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.

publications discussed later, which made for a total of 73 studies assessed. In total, 46 studies passed the new screening criteria (15 new studies) and were included in the cosm database used to calculate the CE-LOC, and 87 endpoints from those studies were scored as having an effect or no effect on the aquatic plant community (USEPA, 2011).

In June 2012, the SAP reconvened to discuss the preliminary Problem Formulation written in support of the registration review of atrazine, including specific questions relevant to the CE-LOC (USEPA, 2012a; 2012b). The SAP recommended changes to the cosm database, including restricting the cosm endpoints to more environmentally-relevant atrazine exposure concentrations and durations. The SAP also proposed that several studies be excluded from the cosm database and identified concerns with the effects/no-effects conclusions for several endpoints, all due to study design issues, performance flaws, and EPA's interpretation of results. In total, the SAP had concerns with only 11 specific studies, which can be separated into seven groups (see **Appendix B** and the study chapters for the full comments from the SAP):

1) Lampert *et al.* (1989)
2) University of Kansas – 1979 experiment [deNoyelles *et al.* (1982, 1989); Kettle *et al.* (1987)]
3) University of Kansas – 1981-1983 experiment [Carney (1983); deNoyelles *et al.* (1989); Dewey (1986)]
4) Detenbeck *et al.* (1996)
5) Kosinski (1984)
6) Seguin – 2001 publications [Seguin *et al.* (2001a), Seguin *et al.* (2001b)]
7) Seguin – 2002 publication [Seguin *et al.* (2002)]

The 2012 SAP stated that the other cosm studies beyond these 11 did not need to be reassessed because there was agreement among the panel on including the studies and EPA's effect/no-effect conclusions for the endpoints within the studies.

In the 2013 addendum to the Problem Formulation (USEPA, 2013), EPA addressed the 2012 SAP's recommendation to restrict the endpoints to more environmentally-relevant atrazine exposure concentrations and durations. The SAP did not provided values for these limits, so EPA

used monitoring data to inform appropriate limits on duration and concentration. For the duration restriction, the Atrazine Ecological Exposure Monitoring Program (AEEMP) relies upon a 240-day monitoring data survey window to capture the typical seasonal fluctuations of atrazine exposures in the corn producing regions of the Midwest. Therefore, EPA decided to limited endpoints in the cosm database to those with exposure durations less than 240 days. In total, EPA identified six endpoints potentially impacted by the new 240-day duration restriction and up for consideration for removal (see **Chapter 4** for more details). For the concentration restriction, EPA identified a peak non-spill related concentration of atrazine in the natural environment as 237.5 µg/L (later updated to 375 µg/L in USEPA, 2016). EPA decided to use 500 µg/L (approximately two times the measured peak value) as an upper-bound concentration to limit endpoints in the cosm database for the analyses. This upper bound was intended to be higher than the highest monitoring data sample because uncertainty in the monitoring data peak values may result in underestimates of peak exposures. As a result of this concentration limit, 11 endpoints were removed from the cosm database, including two relevant to this White Paper (USEPA, 2013; see **Chapter 6** for more details).

In response to the 2012 SAP's concerns about the 11 specific studies, EPA reevaluated the studies and presented the reevaluation in the 2016 Refined Ecological Risk Assessment for Atrazine (USEPA, 2016). In conducting the 2016 reevaluation, EPA considered comments from the 2012 SAP and the public. The reevaluation did not result in a change in EPA's understanding or interpretation of the 11 studies. Thus, all 11 studies remained in the cosm database, and the effect/no-effect conclusions were not changed. Ultimately, for the 2016 Refined Ecological Risk Assessment, the cosm database contained 86 endpoints from 47 references after adjustments were made for the new duration (240 days) and concentration (500 µg/L) limits. These endpoints were used to calculate a CE-LOC of 3.4 µg/L as a 60-day average concentration[12].

After the publication of the 2016 Refined Ecological Risk Assessment, EPA issued the 2019 Regulatory Update, 2019 Proposed Interim Decision, and the 2020 Interim Decision (USEPA, 2019a; 2019b; 2020, respectively). For these documents, an alternative analysis was completed that involved removing or changing the 11 specific cosm studies in the cosm database. Although

[12] 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.

EPAs interpretation of these studies did not change, EPA conducted this alternative analysis to demonstrate how the CE-LOC would change if modifications were made to the cosm database. In 2022, the Proposed Revisions to the Atrazine Interim Registration Review Decision document was published and stated that EPA would be using the CE-LOC of 3.4 µg/L from the 2016 Refined Ecological Risk Assessment for regulatory decision making and mitigating the effects of atrazine in the aquatic environment (USEPA, 2022b; 2022c). Following the 2022 publication, EPA received additional public comments about the 11 cosm studies, along with renewed requests to convene an SAP meeting regarding the studies.

1.3 Objective

In response to the 2022 comments and requests, EPA has again reevaluated the 11 cosm studies identified by the 2012 SAP (**Appendix B)**, and associated publications when necessary, for their inclusion, and if appropriate, the effect/no-effect conclusions for specific endpoints from the studies. Prior to the reevaluation, the 11 studies and associated publications were divided into seven distinct groups based on the unique experiments they discussed. These seven study groups are discussed in **Chapters 2** through **8** of this 2023 White Paper. The objective of these seven chapters is to present EPA's new 2023 reevaluation, which includes a review of the methods, results, conclusions, EPA's use, and previous concerns from these studies to address the issues raised by prior SAPs and public comments.

The objective of the upcoming 2023 SAP meeting is to solely discuss EPA's 2023 reevaluation and conclusions of these seven study groups that comprise the 11 cosm studies with the following charge to the panel:

The Charge Questions for the 2023 SAP meeting:

Question #1: Please comment on EPA's decision to exclude the Lampert study group (**Chapter 2**).

Question #2: Please comment on EPA's decision to include the 1979 University of Kansas study group (**Chapter 3**) and update the effect/no-effect conclusions for the two included endpoints (see **Table 1.1**).

Question #3: Please comment on EPA's decision to include the 1981-1983 University of Kansas study group (**Chapter 4**) but exclude four endpoints and update the effect/no-effect conclusions for the two remaining endpoints (see **Table 1.1**).

Question #4: Please comment on EPA's decision to exclude the Detenbeck study (**Chapter 5**).

Question #5: Please comment on EPA's decision to include the Kosinski study group (**Chapter 6**), add two additional endpoints, and update the effect/no-effect conclusions for the four included endpoints (see **Table 1.1**).

Question #6: Please comment on EPA's decision to include the 2001 Seguin study group (**Chapter 7**) and update the effect/no-effect conclusions for the two included endpoints (see **Table 1.1**).

Question #7: Please comment on EPA's decision to include the 2002 Seguin study (**Chapter 8**) and make no changes to the effect/no-effect conclusion for the single included endpoint (see **Table 1.1**).

To aid in the discussion of the **Charge Questions**, **Table 1.1** below summarizes the 2016 Refined Ecological Risk Assessment effect/no-effect conclusions for the endpoints associated with the 11 cosm studies (and associated publications) and then summarizes the 2023 proposed changes to those studies and endpoints. This is all discussed in **Chapters 2** through **8**. Overall, EPA is proposing to exclude some studies and endpoints, while adding and adjusting others.

The SAP's feedback on EPA's 2023 reevaluation and revised conclusions regarding these studies will inform how EPA ultimately uses these 11 cosm studies.

Table 1.1 . Comparison of the 11 cosm studies in 2016 and 2023. The cosm endpoint numbers, concentrations, and calls (*i.e.*, "Exclude" or if "Include", effect/no-effect conclusion for the endpoints) from the 2016 Refined Ecological Risk Assessment (*i.e.*, "EPA 2016") and the new 2023 reevaluation presented in this White Paper (*i.e.*, "EPA 2023") for seven groups that comprise the 11 studies in question and other associated publications.

Chapter: Group	References	EPA 2016			EPA 2023			
		Endpoint Number[F]	Nominal Conc. (µg/L)	Call	Endpoint Number[F]	Nominal Conc. (µg/L)	Call	Summary of Rationale
Chapter 2: Lampert	Fleckner (1988)[AB]; Lampert *et al*. (1989)	58b, 58	0.1, 1	All scored as "Effect"	-	-	Exclude	Uncertainty regarding potential solvent interaction.
Chapter 3: University of Kansas – 1979 Experiment	deNoyelles and Kettle (1980)[A]	2	20	All scored as "Effect"	52 3	20 500	No Effect Effect	#1 and #2 not associated with 1979 experiment. #52 rescored due to minimal differences in phytoplankton and no quantification or presentation of macrophyte results.
	deNoyelles *et al.* (1982)	52, 3	20, 500					
	deNoyelles *et al.* (1989)[C]	52, 3	20, 500					
	Kettle (1982)[AB]	NA	NA					
	Kettle *et al.* (1987)	2, 1	20, 500					
	Larsen *et al*. (1986)[ABC]	NA	NA					
Chapter 4: University of Kansas – 1981-1983 Experiment	Carney (1983)[D]	2, 4, 5, 1	20, 100, 200, 500	All scored as "Effect"	2 4	20 100	No Effect Effect	#1 and #5 removed due to duration (>240 days). #41 removed due to duplication. #42 removed due to one replicate. #2 rescored due to no phytoplankton results and no effect on macrophytes.
	Dewey (1986)[D]	2	20					
	deNoyelles and Kettle (1983)[AB]	NA	NA					
	deNoyelles *et al.* (1994)[A]	2, 1	20, 500					
	deNoyelles *et al.* (1989)[C]	2, 4, 41, 42, 5, 1	20, 100, 100, 200, 200, 500					
	Huggins (1990)[AB]	NA	NA					
	Huggins *et al*. (1994)[AB]	NA	NA					
	Larsen *et al*. (1986)[ABC]	NA	NA					

Chapter: Group	References	EPA 2016			EPA 2023			
		Endpoint Number[F]	Nominal Conc. (µg/L)	Call	Endpoint Number[F]	Nominal Conc. (µg/L)	Call	Summary of Rationale
Chapter 5: Detenbeck	Detenbeck *et al.* (1996)[D]	22, 23, 24, 25	15, 25, 50, 75	All scored as "Effect"	-	-	Exclude	Excluded based on study design, execution, and results reporting.
Chapter 6: Kosinski	Kosinski (1984); Kosinski and Merkle (1984)[AE]	28, 44	10, 100	All scored as "Effect"	28 44 28a 44a	10 100 10 100	Effect No Effect No Effect No Effect	#28a and #44a added to account for distinct experiments. #44, #28a, and #44a scored as "No Effect" because either no significant effect or slight/transient effect.
Chapter 7: Seguin – 2001 Publications	Seguin *et al.* (2001a)[D]	84, 83	2, 30	All scored as "Effect"	84[G]	2	No Effect	#86 and #85 removed due to combining[G]. #84 scored as "No Effect" because of potential confounding effects from succession
	Seguin *et al.* (2001b)[D]	86, 85	2, 30	All scored as "No Effect"	83[G]	30	Effect	
Chapter 8: Seguin – 2002 Publication	Seguin *et al.* (2002)[D]	87	30	All scored as "Effect"	87	30	Effect	No change

[A] Publications that were not part of the original 11 identified by the 2012 SAP but are associated with the 11 studies.
[B] Reference is not in Appendix G.2 of the 2016 Refined Ecological Risk Assessment (*i.e.*, the cosm database).
[C] deNoyelles *et al.*, 1989 and Larsen *et al.*, 1986 summarize both the 1979 and the 1981-1983 University of Kansas experiments. Therefore, they are present in both University of Kansas sections and are associated with all eight endpoints.
[D] These are the correct citations and will be used throughout – Not Carney and deNoyelles, 1986, Dewey *et al*. (1986), Detenback *et al*. (1996), or Sequin *et al.* (2001a, 2001b, 2002).
[E] 1000 and 10,000 µg/L treatments are not presented here because they were omitted in the Problem Formulation for the 2016 Refined Ecological Risk Assessment due to the new concentration limit set by EPA.
[F] Endpoint numbers were assigned to each concentration within the experiment(s) associated with that publication and were logged as a data point number.
[G] Seguin *et al.* (2001a) and Seguin *et al.* (2001b) represent the same study and were combined in the 2023 reevaluation. Therefore, endpoints #86 and 85 will be removed from the cosm database and endpoints #84 and 83 will now represent the two concentrations in this experiment.

CHAPTER 2. LAMPERT

2.1 Overview

Lampert *et al.* (1989) was one of the original studies included in the cosm database (since 2003) and was one of the 11 studies flagged by the 2012 SAP for reconsideration with the SAP recommending its exclusion (USEPA, 2012b). Previous criticisms of the Lampert *et al.* (1989) study cited specific information reported in Fleckner (1988), the German dissertation partially presented in Lampert *et al.* (1989). Although Fleckner (1988) violates the screening criteria of being written in English, EPA believes the dissertation provides additional detail and information that must be considered when evaluating the experiments reported in Lampert *et al.* (1989). Accordingly, EPA has translated and reviewed very limited portions of Fleckner (1988). These portions are pertinent to the understanding of the study as presented in the Lampert *et al.* (1989) publication and to better address the comments from the 2012 SAP:

> *This study should be excluded due to solvent bias as noted on p. 173 of Giddings et al. 2005; "decreased primary productivity" was actually increased in bacteria growth and respiration (See Appendix D, p. 5). The Panel recommended that this study should be dropped. The Panel was disappointed to see this study still included in the cosm dataset since the 2007 and 2009 SAPs indicated that it be dropped due [to] solvent bias.*

2.2 Experimental Design and Execution

EPA's present discussion and review of Lampert *et al.* (1989) only focuses on the reported outdoor mesocosm experiments (*i.e.*, additional experiments are described that do not examine aquatic algal communities), relies primarily on the methods as presented in Lampert *et al.* (1989) and where needed, provides supplementary information from Fleckner (1988).

It is unclear if reagent grade or a commercial product formulation of atrazine was used in the experiments. Lampert *et al.* (1989) is silent on this; however, Fleckner (1988) reports that the atrazine was at least 99% pure. This high level of purity suggests that reagent grade was used. The source of the atrazine used was inconsistently reported between Lampert *et al.* (1989) and Fleckner (1988): the former reported Ciba Geigy (Basel) as the source and the latter reported it

as Riedel-de Haën in Seelze, Germany[13]. One of the most controversial topics regarding the outdoor mesocosm experiment is the use of a solvent. Lampert *et al.* (1989) explicitly states that a solvent was not used; however, Fleckner (1988) indicates that ethanol (EtOH) was used to dissolve the atrazine. The authors state, "*All chemical and biological analyses were carried out according to routine protocols*"; however, the source of the protocols used is not stated (additional details are provided in Fleckner (1998); however, a complete translation was not considered necessary for this review). Particulate organic carbon (POC) was measured using an infrared carbon analyzer.

The cosm experiments were carried out in plastic bags mounted in an aluminum frame floating in Schöhsee, a moderately eutrophic 0.78 km^2 lake in Schleswig-Holstein, Germany. The lake has a small catchment area where no atrazine-treated crops are raised, and so the authors assumed no prior exposure to atrazine was expected to affect the community responses. The cosms were 1 m in diameter, were about 2.7 m long, and held 1.7 m^3 of water. Experiments were carried out in different seasons and with different atrazine concentrations (*i.e.*, nominal concentrations of 0.1, 1, 10, and 100 µg/L[14]). The 0.1 µg/L experiments were termed "warm", which indicated that the experiment was conducted in June/July and "cold, which indicated that the experiment was conducted in August/September. Each test concentration was tested with a natural community sourced from the lake at that time, such that the initial communities for each test concentration differed across experiments and test concentrations[15]. In each experiment, the cosms were filled with lake water that had been filtered through a 100 µm mesh to screen out large zooplankton, and then the bags were inoculated with equal amounts of zooplankton collected with a plankton net. Prior experiments had shown that all four cosms developed "*very similar*" communities when left untreated (prior experiments were not further described in Lampert *et al.*, 1989; EPA did not translate this portion of Fleckner, 1988). In each experiment

[13] From Fleckner (1988): "*Das 2,4-D zur Synthese wurde von der Fa. Merck in Dermstadt, das Atrazin (mindestens 99% rein) von der Fa. Riedelde Haen in Seelzen bezogen*." Translation: "*The 2,4-D for the synthesis was obtained from Merck in Dermstadt, the atrazine (at least 99% pure) from Riedelde Haen in Seelzen*."

[14] Lampert *et al*. (1989) does not specifically state that the reported concentrations are nominal; however, it is evident that they are nominal based on reported measured concentrations in the cosms as reported in Fleckner.

[15] From Fleckner (1988): " *Da die drei Versuche nacheinander bei vergleicbbaren Witterungsverhiltnissen (100 µg/1: 25.6.1981 bis 13.7.1981; 10 µg/1: 16.7.1981 bis 3.8.1981; 1 µg/1: 6.8.1981 bia 24.8.1981)…"* Translation: *"Since the three tests were carried out one after the other under comparable weather conditions (100 µg/l: June 25, 1981 to July 13, 1981; 10 µg/l: July 16, 1981 to August 3, 1981; 1 µg/l: August 6, 1981 to August 24, 1981)…*". Lampert *et al.* (1989) states that the 0.1 µg/l tests were carried out in June/July and September 1982 and again in August/September 1984 (results were not presented in Lampert *et al*., 1989).

(*i.e.*, tested concentration and not conducted concurrently), two cosms received atrazine and two served as controls. The location and placement of the enclosures was not reported in Lampert *et al*. (1989); however, Fleckner (1988) reports that all four cosms per experiment were attached to the same flotation frame (two controls and two atrazine treatments)[16] and that the experiment was carried out in a sheltered bay.[17] Each of the nominal experimental concentrations was tested with a different initial community and at a different time (*i.e.*, each experiment included only one test concentration and consisted of four cosms: two controls and two of the same nominal atrazine concentration). Experiments lasted "*typically*" "*no longer than three weeks to avoid extensive growth of periphyton on the walls of the bags*" (18 to 42 days for the atrazine experiments according to the reported data). Periphyton growth was not discussed further and may have added an additional source of variability among the replicates.[18] Samples for chemical and biological analysis, including zooplankton sampled by vertical 100-µm net haul, were taken "*at regular intervals*" (about every few days). Atrazine concentrations in the mesocosms were periodically measured during the experiment (about 3 to 4 day intervals) by gas chromatography[19].

2.3 EPA's Use as of 2016

In the 2016 Refined Ecological Risk Assessment (USEPA, 2016), EPA included two endpoints that were based on effects to phytoplankton in the outdoor enclosure studies reported in Lampert *et al*. (1998; **Table 2.1**). EPA identified effects at 0.1 µg/L (endpoint #58b) and 1 µg/L (endpoint #58). It is of note that the two test concentrations were tested at different times with different initial phytoplankton communities: the 1 µg/L concentration was tested once and the 0.1 µg/L

[16] As illustrated in a photograph of the floatation.
[17] From Flecker (1988): *"Die Experimente wurden in einer wind- und wellengeschutzten Bucht des Schohsees durchgefiihrt. Die Wassertiefen an den Untersuchungsstellen lagen zwischen 5 und 7 Metern."* Translation: *"The experiments were carried out in a bay of the Schohsee sheltered from wind and waves. The water depths at the investigation sites were between 5 and 7 meters."*
[18] Chlorophyll a measurements were reportedly of the phytoplankton. From Lampert *et al*. (1989): "*phytoplankton biomass did not differ between treatments and controls*" and from Fleckner: "*Als Maß fur die Phytoplanktonbiomasse wurde der Chlorophyll a- Gehalt der Fraktionen <250 µm und <30 µm (ungefähre Grenze der für das Zooplankton freßbaren Partikel) bestimmt*." Translation: *"The chlorophyll a content of the fractions <250 µm and <30 µm (approximate limit of the particles eatable by the zooplankton) was determined as a measure of the phytoplankton biomass."*
[19] Measured concentrations are not reported in Lampert *et al*. (1989); however, data of concentrations in the cosms are presented in Fleckner (1988).

concentration was tested three times, each at a different time and with a different initial phytoplankton community.[20]

Table 2.1. A summary of the cosm endpoints associated with this group that appeared in Appendix G.2 of the 2016 Refined Ecological Risk Assessment. These endpoints were used to evaluate the potential effects of atrazine on aquatic plant communities.

References	Endpoint Number	Duration (days)	Nominal Conc. (µg/L)	Plant Group	Results and Recovery	Effect/No-Effect Conclusion
Lampert *et al.* (1989)	58b	42	0.1	Phyto	Decrease in photosynthesis rate (1 of 2 replicates in coldwater experiment only, 60% decline) and oxygen saturation (approx. 40% reduction). No clear effect on chlorophyll *a* levels. Recovery: >42 d (oxygen), >25 d (photosynthetic rate)	Effect[A]
Lampert *et al.* (1989)	58	18	1	Phyto	50% decrease in chlorophyll *a* and oxygen saturation. Recovery: >18 d	Effect[B]

[A] Endpoints evaluated include chlorophyll *a* levels, oxygen levels, and photosynthetic rate. Recovery was observed for the photosynthetic rate in some enclosures.
[B] Reductions in oxygen may have been related to daphnid mortality. However, chlorophyll *a* reductions were considered to be treatment related.

As stated by EPA in 2016:

> *EPA has identified that significant effects to the community occurred after 7 days of exposure to atrazine at both 0.1 and 1 µg/L.*
>
> *The effects noted in the study for the 1 µg/L test concentration included:*
> - *percent oxygen saturation declines ~100 % on Day 7 to ~30-40% on Days 15 and 20.*
> - *50% decline in chlorophyll a between Day 7 and Day 20*
> - *particulate organic carbon increased between Day 7 and Day 20.*
> - *zooplankton density (i.e., daphnia, cyclops, bosimia, nauplii) all reduced in 1 µg/L test after 7 days.*

[20] From Fleckner (1988): " *Da die drei Versuche nacheinander bei vergleicbbaren Witterungsverhiltnissen (100 µg/1: 25.6.1981 bis 13.7.1981; 10 µg/1: 16.7.1981 bis 3.8.1981; 1 µg/1: 6.8.1981 bia 24.8.1981)…"* Translation: *"Since the three tests were carried out one after the other under comparable weather conditions (100 µg/l: June 25, 1981 to July 13, 1981; 10 µg/l: July 16, 1981 to August 3, 1981; 1 µg/l: August 6, 1981 to August 24, 1981)…*". Lampert *et al.*, 1989 states that the 0.1 µg/l tests were carried out in June/July and September 1982 and again in August/September 1984 (results were not presented in Lampert *et al*., 1989).

The effects noted in the study for the 0.1 µg/L test concentration included:

- *% oxygen saturation declines by >50% between Day 7 and Day 20 in warm water experiments [June/July]*
- *% oxygen saturation declines by >50% between Day 10 and Day 20 in cold water experiments [September]*
- *photosynthetic rate much lower than controls in the warm and cold water experiments after Day 1 until the period of time between Day 15 and Day 25 where recovery was noted.*
- *daphnid die-off occurred between Day 10 and Day 20.*

The results for two additional treatment levels (*i.e*., 10 µg/L and 100 µg/L) and a third experiment with 0.1 µg/L were also reported in Fleckner (1988). However, those endpoints have never been considered by EPA because the details of those results were only available in German and while mentioned in Lampert *et al*. (1989), the details were not reported.

EPA acknowledges that past reporting on endpoints has not been clear and inconsistencies have led to public comment on specific endpoint interpretation. However, given that the major criticism about this study has been that effects observed are not treatment related but are a response to the solvent used, EPA has decided to focus on the issues associated with the solvent and the confidence in the study.

2.4 Stakeholders' Major Concerns and Criticisms

The major and long-standing criticism about this study has been about the use of EtOH, lack of proper controls, associated difficulties with interpretation of the results, and potential impact on study validity, which were most recently pointed out by Syngenta (2022): "*The observed responses have been explained by multiple reviewers (Brock et al. 2000; Giddings et al. 2005; [US]EPA 2009b; Giddings 2012 [Syngenta, 2012a here]) as due to the solvent (ethanol, which was apparently not used in controls) rather than to atrazine*". The primary issue is that Lampert *et al*. (1989) expressly states that a solvent was not used, yet the dissertation (Fleckner, 1988) underlying this publication says that EtOH was used and there are questions about how much solvent may have been used. The implication has been that the use of EtOH could have caused or

contributed to the system going from autotrophic to heterotrophic due to the oxygen consumption used by microbes to degrade the EtOH and thereby confound interpretation of the results, particularly those such as changes in dissolved oxygen. Consequently, there has been concern about including this study in the cosm database because the observed effects were potentially not due to atrazine and therefore, not reliable (*e.g.*, USEPA, 2007; 2009b; 2012b).

2.5 EPA's 2023 Reevaluation

After consideration of all of the studies' limitations, the determinative concerns will be discussed here, including those brought up by past SAPs and public commenters.

EPA acknowledges and assumes that EtOH was likely used as a solvent in these cosm experiments as discussed in Fleckner (1988)[21]. The use of two control mesocosms are reported in both Lampert *et al*. (1989) and Fleckner (1988); however, both reports are silent on the composition of those controls. It is possible that both were negative (water only) controls, both solvent controls, or one of each. Given the silence in both publications, EPA assumes that they were likely water only controls. Although these issues are not part of the study acceptance criteria (USEPA, 2011), the lack of clarity on the use of EtOH and use of suitable controls is a major uncertainty that is considered as part of the study evaluation criteria. If it were clear that a solvent control had been run, then it would have been possible to evaluate the potential impact of EtOH (both negative and solvent controls) or account for potential impacts of EtOH (solvent control only).

EPA's previous evaluation of Fleckner (1988) identified that the publication does not report the amount of EtOH added to the mesocosm or how much atrazine was dissolved in the EtOH, but states that atrazine was dissolved in 5 mL of EtOH. The 2016 Refined Ecological Risk Assessment for Atrazine interpreted the amount of solution added as being a "*small-pipetted volume*" but also calculated a theoretical estimate of how much oxygen demand could occur had

[21] From Fleckner (1988) " *Zur Applikation in die Sacke mußten die Herbizide in ca. 5 mL Ethanol aufgelost und mit destilliertem Wasser auf 100 mL aufgefullt werden. Mit einer Pipette wurden dann gleichmaßig wahrend des Fullvorgangs jeweils kleine Volumina der Losungen in die diagonal zueinander stehenden Tanks eingebracht*." Translation: "*For application into the sacks [bags], the herbicides had to be dissolved in about 5 mL of ethanol and filled up to 100 mL with distilled water. With a pipette, small volumes of the solutions were continuously introduced into the diagonally adjacent tanks during the filling process*."

the entire stock solution containing atrazine and 5 mL of EtOH been added to a single mesocosm. EPA stated that:

> *The foremost concern with this study is the use of ethanol as a solvent. The data described by Lampert et al. (1989) were generated from a graduate research project (Fleckner 1981 [sic]). The authors describe using a concentrated stock of atrazine dissolved in 5 mL of EtOH and then diluted to 100 mL with deionized water. They report that they pipetted volumes to be added to each 1700 L cosm. EPA has calculated the total estimated oxygen demand for ethanol degradation based on a worst-case assumption by assuming that the entire volume of stock concentrate was added to an individual cosm (i.e., 5 mL of EtOH into one 1700 L cosm). The theoretical oxygen demand for this condition, in the absence of organic matter, is calculated to be 8000 mg of oxygen for the complete degradation of the 5 mL of EtOH. The reported temperature and percent oxygen saturation allows for the determination that there would be ~ 16,150 mg of oxygen in the solution of the cosm. So, roughly half of the oxygen would be used for EtOH degradation.*
>
> *The Fleckner study clearly states that the cosms were dosed with a small, pipetted volume of the stock solution, thus there would have been far less EtOH added to each cosm. The exact dosing volume and the concentration of the stock solution were not reported. The dissipation of EtOH in the cosms would have occurred much more quickly than the time frame of effects reported in the study. The half-life of EtOH in standing water is between 0.25 and 1 day through biodegradation and there is a high likelihood that EtOH would have vaporized from the solution, thus when the samples were taken from the cosms for chemical and biological testing, some of the EtOH would be lost with the 300 L head space air exchange*.

The previous translation of Fleckner (1988) by EPA concluded that a single small volume was the amount of stock solution added to the mesocosm, suggesting that the amount of EtOH added may have been negligible. However, the translation is now understood to mean that "volumes" were added, as in "*small volumes of the solutions were continuously introduced*." Although speculative given the qualitative nature of the description, this difference in translation suggests

that a larger volume of the solvent may have been added to each cosm, and the stock solution was added a little at a time while filling the cosms rather than all at once. This description is consistent with estimates of the amount of EtOH needed to dissolve atrazine (solubility in EtOH ca. 1 mg/mL[22]) and suggests larger amounts of EtOH were added to the tank than previously thought (*e.g.*, 1.7 mL EtOH per mesocosm for the 1 µg/L treatment group if the solution was made at the solubility limit[23]). Presumably no more than one 100 mL stock solution would have been added to a single mesocosm. However, there is uncertainty with respect to the higher concentrations tested (10 and 100 µg/L) given that only 5000 µg atrazine could be dissolved in 5 mL EtOH at the reported solubility limit.

Although there is uncertainty about the amount of EtOH that may have been added to each mesocosm, the cosm study acceptance criterion (0.5 mL solvent/L; USEPA, 2011) for maximum solvent concentration would have been met assuming that no more than 850 mL EtOH was added to the 1700 L mesocosm. The concentration of EtOH based on the Fleckner (1988) reported information would be well below that even assuming 5 mL EtOH had been added to the 1700 L mesocosm or if multiples of the stock solution had been added to achieve the higher concentrations of atrazine tested.

As indicated earlier, detecting if the solvent had an effect is unclear because it is unknown whether a solvent control was run. Assuming that the controls did not include the solvent, the magnitude of the drops in dissolved oxygen levels (**Figure 2.1**) are consistent with EPA's estimated dissolved oxygen consumption associated with EtOH degradation (see above).

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[22] Solubility of atrazine attained from https://cdn.caymanchem.com/cdn/insert/13375.pdf

[23] Hypothetical EtOH/cosm calculation

Amount of atrazine (µg) a cosm needed:

1 µg atrazine/L treatment * 1700 L cosm = 1700 µg atrazine per cosm.

Stock solution:

1000 µg atrazine/mL EtOH (max based on reported solubility) * 5 mL EtOH (see footnote 20) = 5000 µg atrazine/5 mL EtOH

Diluted to 100 mL with distilled water (see footnote 20) = 5000 µg atrazine/100 mL or 50 µg/mL

Dosing of the cosm:

1700 µg atrazine per cosm / (50 µg atrazine/mL stock solution) = 34 mL of stock solution added to each cosm for the 1 µg/L treatment

Amount of EtOH added:

34 mL of stock solution * (5 mL EtOH / 100 mL stock solution) = 1.7 mL EtOH/cosm

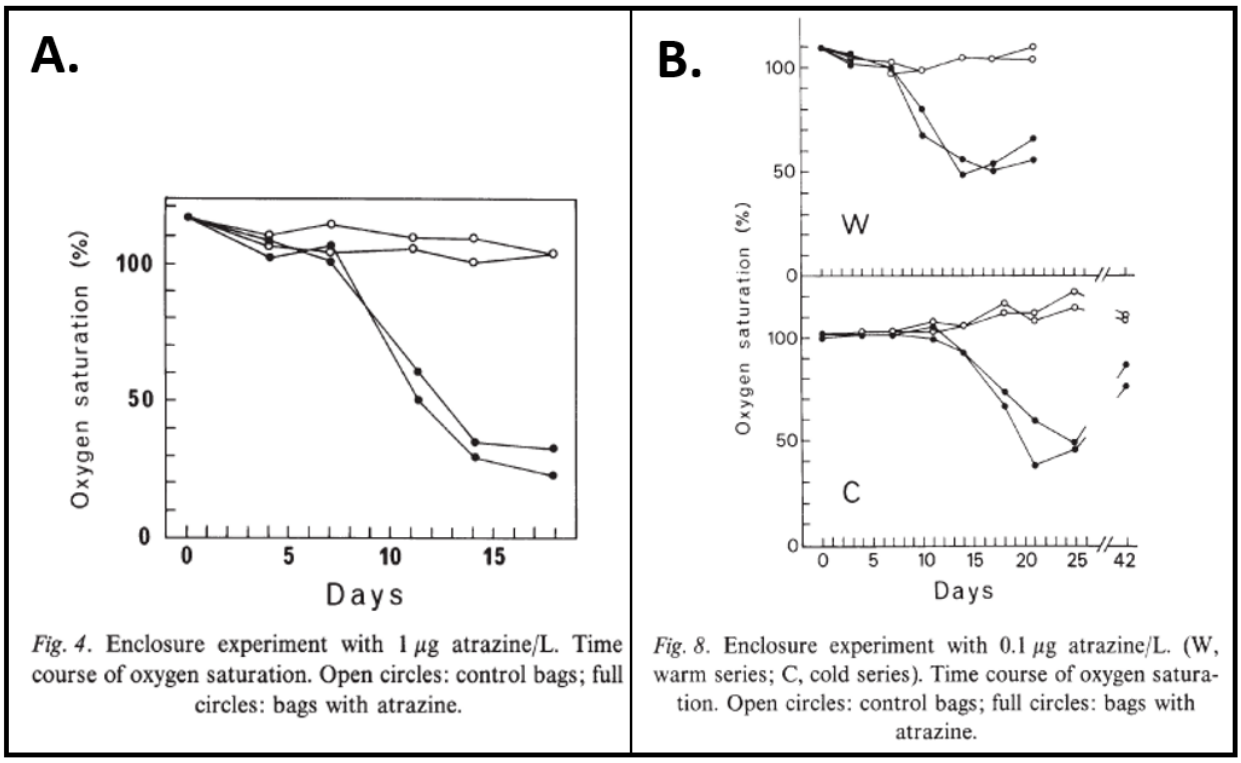


Fig. 4. Enclosure experiment with 1 μg atrazine/L. Time course of oxygen saturation. Open circles: control bags; full circles: bags with atrazine.

Fig. 8. Enclosure experiment with 0.1 μg atrazine/L. (W, warm series; C, cold series). Time course of oxygen saturation. Open circles: control bags; full circles: bags with atrazine.

Figure 2.1. Excerpts of Figure 4 (A) and Figure 8 (B) from Lampert *et al.* (1989)

The decline of dissolved oxygen, as seen in Lampert *et al*. (1989; **Figure 2.1**), was one of the variables EPA has associated with a potential effect of atrazine on the phytoplankton community within the cosms. EPA has also associated the decreased dissolved oxygen with the reduction in zooplankton survival. Zooplankton showed a sharp decline in abundance (**Figure 2.2**) around the same time as the sharp decline in dissolved oxygen (**Figure 2.1**). Given the potential for microbial activity related to zooplankton decomposition and EtOH breakdown, a plausible explanation for the reduction of dissolved oxygen may have been a change from an autotrophic community to a heterotrophic community. Although it is unclear what factor(s) contributed to the cascade of observed effects, the sharp drop in dissolved oxygen levels could be associated with microbial decomposition of the zooplankton as well as break down of EtOH (discussed above). In addition, the study authors indicate that the type of enclosure used may have had limited gas exchange with the surrounding water; thereby potentially enhancing the decrease in dissolved oxygen. In other words, there is considerable uncertainty if these dissolved oxygen drops were due to direct atrazine effects on the algal community given the other potential contributing factors.

Furthermore, Fleckner (1989) reports a similar set of cosm experiments conducted with 2,4-D dissolved in EtOH and those results also suggest the potential confounding influence of EtOH on the interpretation of the experimental results (discussed below).

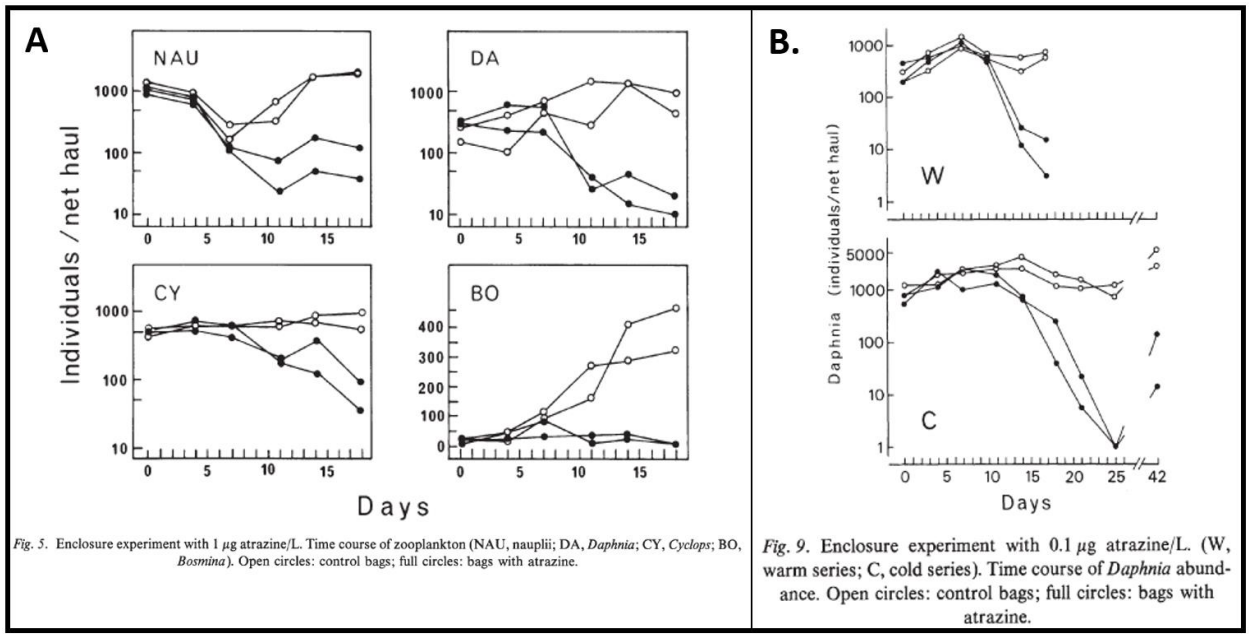


Fig. 5. Enclosure experiment with 1 µg atrazine/L. Time course of zooplankton (NAU, nauplii; DA, *Daphnia*; CY, *Cyclops*; BO, *Bosmina*). Open circles: control bags; full circles: bags with atrazine.

Fig. 9. Enclosure experiment with 0.1 µg atrazine/L. (W, warm series; C, cold series). Time course of *Daphnia* abundance. Open circles: control bags; full circles: bags with atrazine.

Figure 2.2. Excerpts of Figure 5 (A) and Figure 9 (B) from Lampert *et al.* (1989)

EPA's previous interpretation considered another possible explanation of the reduction in zooplankton and dissolved oxygen. Atrazine exposure may have reduced the phytoplankton community biomass (measured as chlorophyll *a*), which could have led to reduced oxygen generation, a decreased food source for the zooplankton, and increased oxygen demand from microbial decomposition of the zoo- and phytoplankton. The expanded translation and review of Fleckner (1988) and Lampert *et al.* (1989) has provided further evidence from all four test concentrations that casts uncertainty on this interpretation.

First, Lampert *et al.* (1989) only reports the response of chlorophyll *a* for the 0.1 and 1 µg/L treatment groups (**Figure 2.3**). Although there was an apparent drop in chlorophyll *a* for the 1 µg/L group compared to the control, it was not clearly evident in the 0.1 µg/L group. If the drop in dissolved oxygen was driven primarily by reduced phytoplankton density and microbial decomposition of dead phytoplankton (*e.g.*, no impact of EtOH), then consistent results would be expected across all experiments. However, the drop in oxygen for the "warm series" (**Figure 2.1B**) treatment group should have also been observed in the control given that chlorophyll *a*

decreased in both the treatment group and the control over the course of the experiment and there were no differences in chlorophyll *a* concentrations between the treatment group or the control (**Figure 2.3B**).

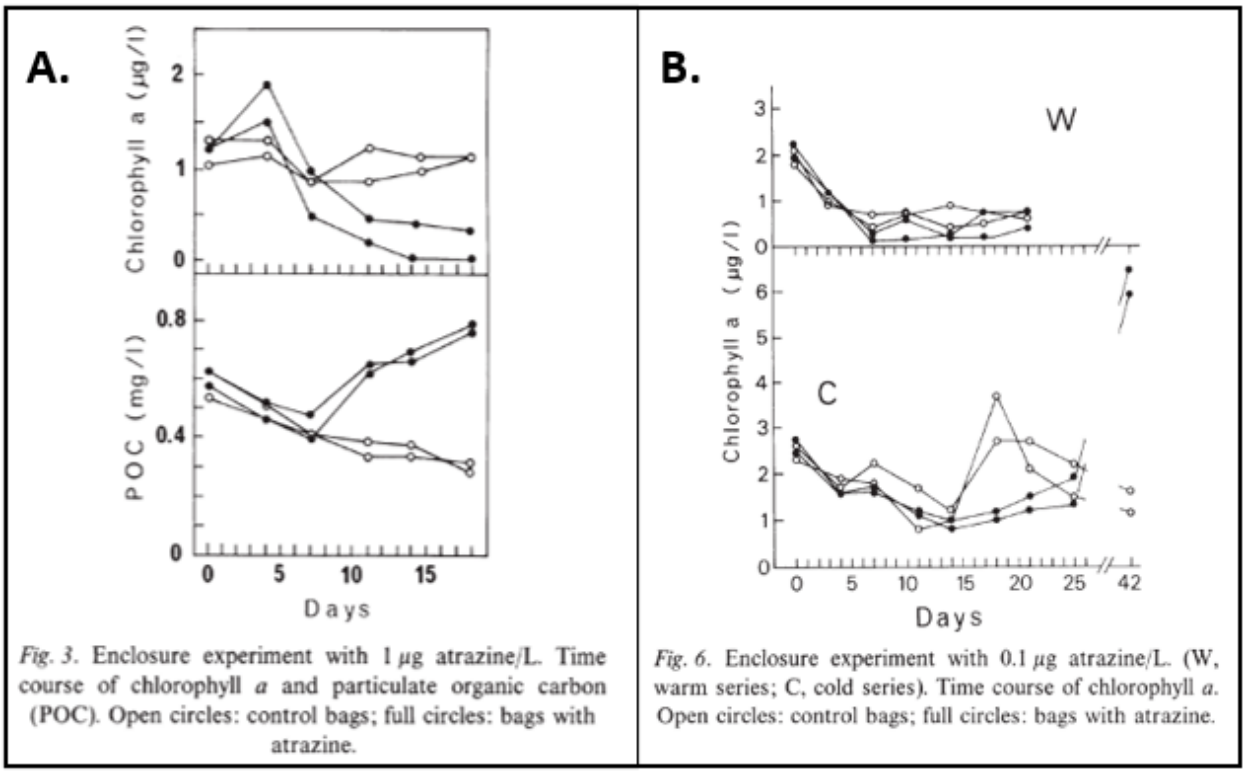


Fig. 3. Enclosure experiment with 1 µg atrazine/L. Time course of chlorophyll *a* and particulate organic carbon (POC). Open circles: control bags; full circles: bags with atrazine.

Fig. 6. Enclosure experiment with 0.1 µg atrazine/L. (W, warm series; C, cold series). Time course of chlorophyll *a*. Open circles: control bags; full circles: bags with atrazine.

Figure 2.3. Excerpts of Figure 3 (A) and Figure 6 (B) from Lampert *et al.* (1989)

Second, as presented in Fleckner (1988) there is also no clear pattern in the response of chlorophyll *a* across all the tested concentrations (**Figure 2.4**) that correspond to changes in dissolved oxygen and zooplankton. For example, there isn not a clear difference in chlorophyll *a* when exposed at the highest treatment group (100 µg/L) when compared to the control or the lake (**Figure 2.4a**). During this experiment (100 µg/L), chlorophyll *a* concentration decreased in the control, the treatment, and the lake at relatively similar levels over the course of the experiment. In contrast to the relatively similar responses in chlorophyll *a* dropping among the control, treatment, and lake, the drops in dissolved oxygen and percent oxygen saturation were only observed in the treatment group (**Figure 2.5)**. This suggests that a factor other than, or in addition to, phytoplankton played a role in the observed drop in dissolved oxygen in the treatment group. Because of these patterns, EPA believes that the solvent may be a contributing factor in the declining oxygen levels within the treated cosms. Differing responses for some treatment groups or concentration studies could be related to the different initial phytoplankton

communities since each test concentration was tested in a different season[24]; however, it is reasonable to consider the relationship among the treatment groups given that the communities are from the same source water and there is a three order of magnitude difference in test concentrations. Nonetheless, the clear lack of consistent response within an experiment (*i.e.*, each treatment group) calls into question the idea that a shift in phytoplankton biomass alone led to the sharp drop in dissolved oxygen that was observed in all the treatment groups.

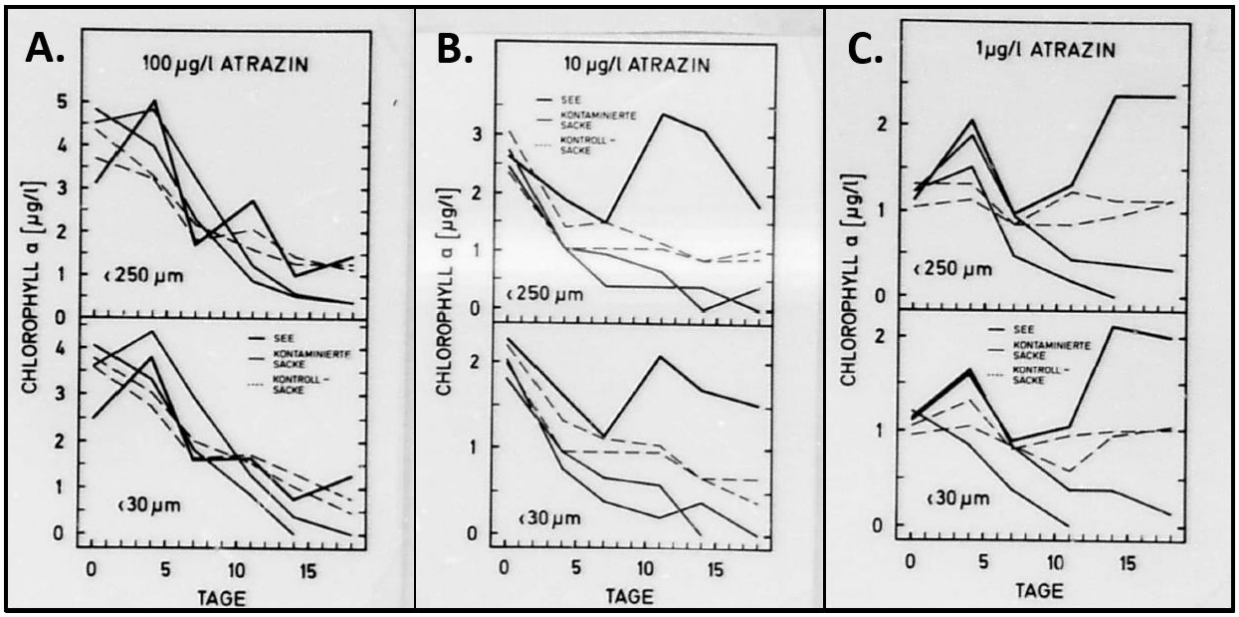


Figure 2.4. Chlorophyll *a* for Atrazine Experiments at 100 (A), 10 (B), and 1 (C) µg/L Atrazine (from Fleckner, 1988, results presented for two different size fractions of the phytoplankton community). "Tage" = Days; "See" = Lake (concentration in the lake water at the concurrent time point; thick solid line); "kontaminierten sacken" = contaminated [atrazine] sack [bag/mesocosm]; (thin solid lines); "kontroll sacken" = control sack [bag/mesocosm](dashed lines).

[24] From Fleckner (1989): " *Da die drei Versuche nacheinander bei vergleicbbaren Witterungsverhiltnissen (100 µg/1: 25.6.1981 bis 13.7.1981; 10 µg/1: 16.7.1981 bis 3.8.1981; 1 µg/1: 6.8.1981 bia 24.8.1981)…"* Translation*: "Since the three tests were carried out one after the other under comparable weather conditions (100 µg/l: June 25, 1981 to July 13, 1981; 10 µg/l: July 16, 1981 to August 3, 1981; 1 µg/l: August 6, 1981 to August 24, 1981)…*". Lampert *et al.* (1989) states that the 0.1 µg/l tests were carried out in June/July and September 1982 and again in August/September 1984 (results were not presented in Lampert *et al*., 1989).

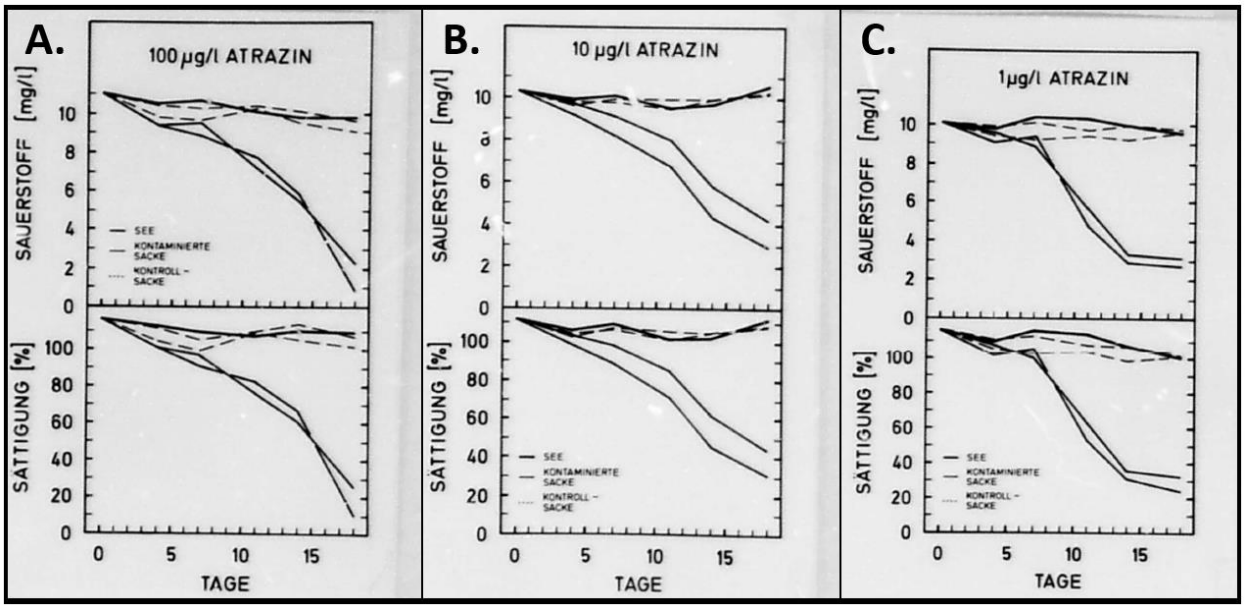


Figure 2.5. Dissolved oxygen (top panel) and Saturation (bottom panel) for Atrazine Experiments at 100 (A), 10 (B), and 1 (C) µg/L Atrazine (from Fleckner, 1988). "Sauerstoff " = Dissolved oxygen; "Sattigung" = saturation; "Tage" = Days; "See" = Lake (concentration in the lake water at the concurrent time point; thick solid line); "kontaminierten sacken" = contaminated [atrazine] sack [bag/mesocosm]; (thin solid lines); "kontroll sacken" = control sack [bag/mesocosm](dashed lines).

In contrast, to chlorophyll *a*, the sharp drop in dissolved oxygen among all atrazine-treated groups (**Figure 2.1** and **Figure 2.5**) corresponds with a sharp drop in at least one component of the zooplankton community among all treated groups (**Figure 2.2** and **Figure 2.6**). The one exception among the experimental results with atrazine exposure is that from a separate experiment conducted 2 years after the rest at a 0.1 µg/L concentration. Lampert *et al.* (1989) mentioned that there were no effects observed, and Fleckner (1988) reported results that graphically show that there were no clear treatment-related effects on dissolved oxygen, phytoplankton (chlorophyll *a* and photosynthetic rate), or zooplankton abundance. There is not an obvious reason for this result if EtOH and zooplankton were the cause of the declines in dissolved oxygen discussed above; however, as mentioned before there is uncertainty about the use of EtOH and perhaps the procedure was changed.

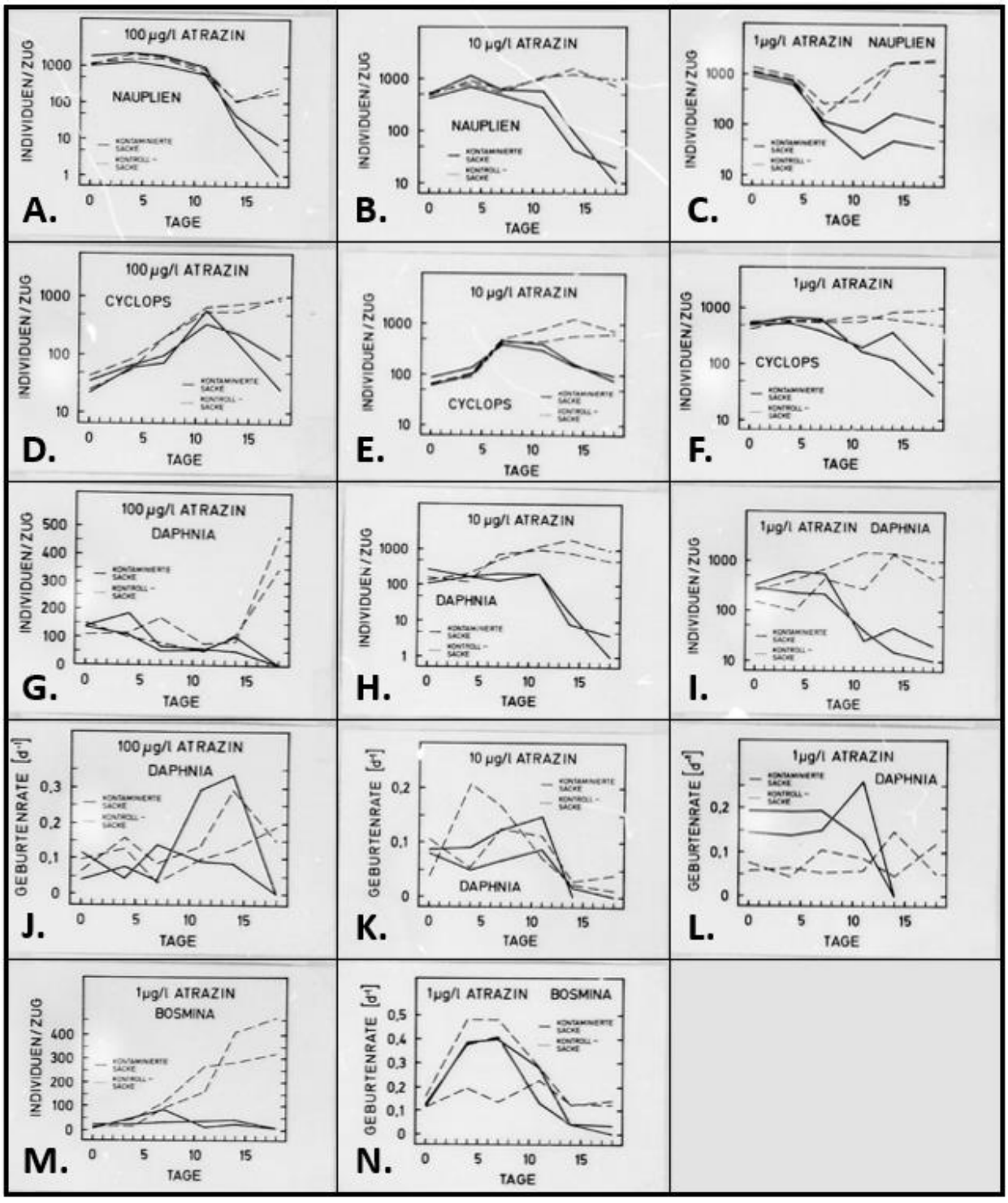


Figure 2.6. Zooplankton Abundance for Atrazine Experiments (from Fleckner, 1988). "Zug" = net haul (from Lampert *et al.*, 1989); "Geburtienrate" = birth rate; "Tage" = Days; "kontaminierten sacken" = contaminated [atrazine] sack [bag/mesocosm]; (solid lines); "kontroll sacken" = control sack [bag/mesocosm](dashed lines).

The dissertation (Fleckner, 1988) reported similar experiments in the same lake with exposure of phytoplankton communities to 2,4-D (100 and 500 µg/L; the 100 µg/L was conducted twice – once in Spring and once in Fall) that showed similar sharp drops in dissolved oxygen (**Figure 2.7**) as were observed in the atrazine experiments. Notably, 2,4-D was also dissolved in EtOH in this experiment[25]. As discussed for the atrazine experiments, there was no clear or consistent impact on phytoplankton biomass compared to changes in dissolved oxygen (**Figure 2.7** and **Figure 2.8**). In the 2,4-D experiments, there is less indication that zooplankton mortality may have contributed to the drops in dissolved oxygen (**Figure 2.9.1 to Figure 2.9.4**). Although there was a drop in daphnids in the 100 µg/L treatment compared to the control in the Fall experiment, there was not as clear of a drop in any of the zooplankton in the 500 µg/L treatment relative to the control or the Spring experiment in the 100 µg/L. Unlike most of the other experiments, there was an apparent recovery of dissolved oxygen levels in the 500 µg/L treatment, which is difficult to explain given the other information available. Without a clear use of a solvent control, these results again suggest the potential confounding influence of EtOH on the interpretation of the experimental results, and support EPA's conclusions discussed above for the atrazine experiments.

[25] From Fleckner (1988) " *Zur Applikation in die Sacke mußten die Herbizide in ca. 5 mL Ethanol aufgelost und mit destilliertem Wasser auf 100 mL aufgefullt werden. Mit einer Pipette wurden dann gleichmaßig wahrend des Fullvorgangs jeweils kleine Volumina der Losungen in die diagonal zueinander stehenden Tanks eingebracht*." Translation: "*For application into the sacks [bags], the herbicides had to be dissolved in about 5mL of ethanol and filled up to 100mL with distilled water. With a pipette, small volumes of the solutions were continuously introduced into the diagonally adjacent tanks during the filling process*."

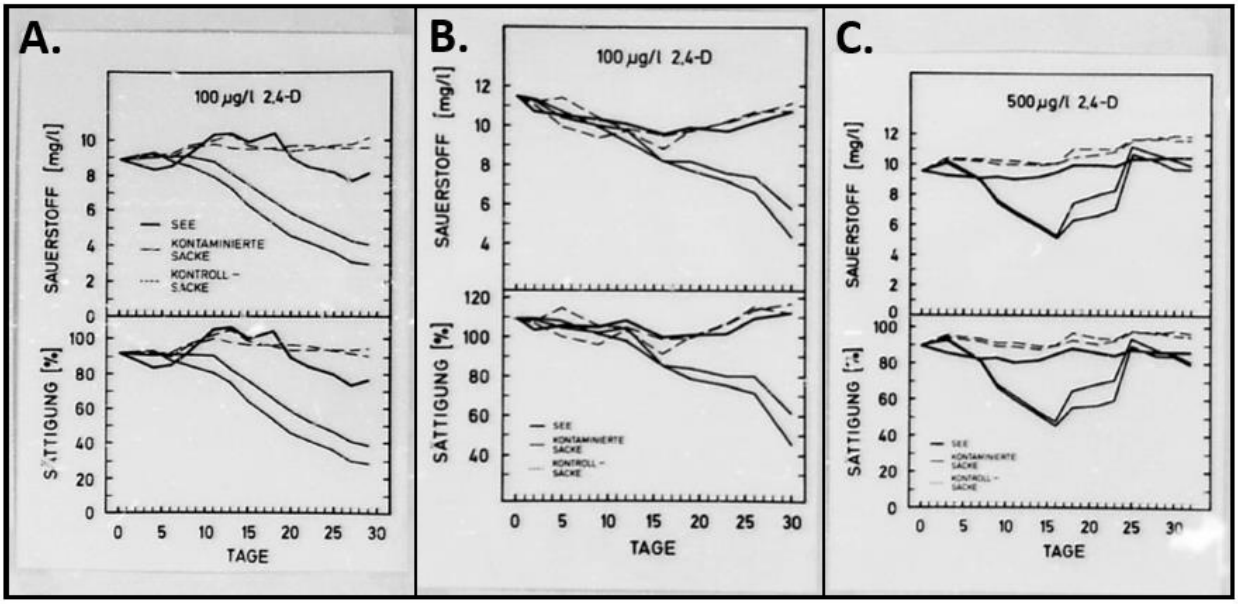


Figure 2.7. Dissolved Oxygen (top panel) and Saturation (bottom panel) for 2,4-D Experiments (from Fleckner, 1988). "Sauerstoff" = Dissolved oxygen; "Sattigung" = saturation; "Tage" = Days; "See" = Lake (concentration in the lake water at the concurrent time point; thick solid line); "kontaminierten sacken" = contaminated [2,4-D] sack [bag/mesocosm]; (thin solid lines); "kontroll sacken" = control sack [bag/mesocosm](dashed lines). The 100 µg/L experiment was conducted twice (once in Fall [left panel] and once in Spring [right panel]).

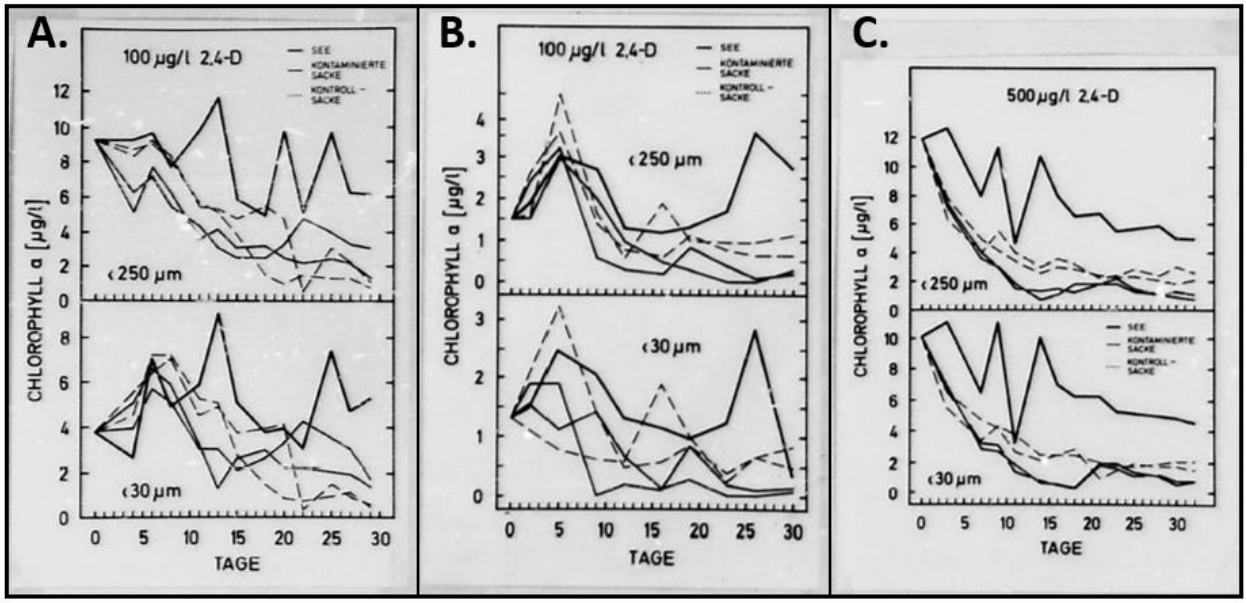


Figure 2.8. Chlorophyll *a* for 2,4-D Experiments (from Fleckner, 1988, results presented for two different size fractions of the phytoplankton community). "Tage" = Days; "See" = Lake (concentration in the lake water at the concurrent time point; thick solid line); "kontaminierten sacken" = contaminated [2,4-D] sack [bag/mesocosm]; (thin solid lines); "kontroll sacken" = control sack [bag/mesocosm](dashed lines). The 100 µg/L experiment was conducted twice (once in Fall [left panel] and once in Spring [right panel]).

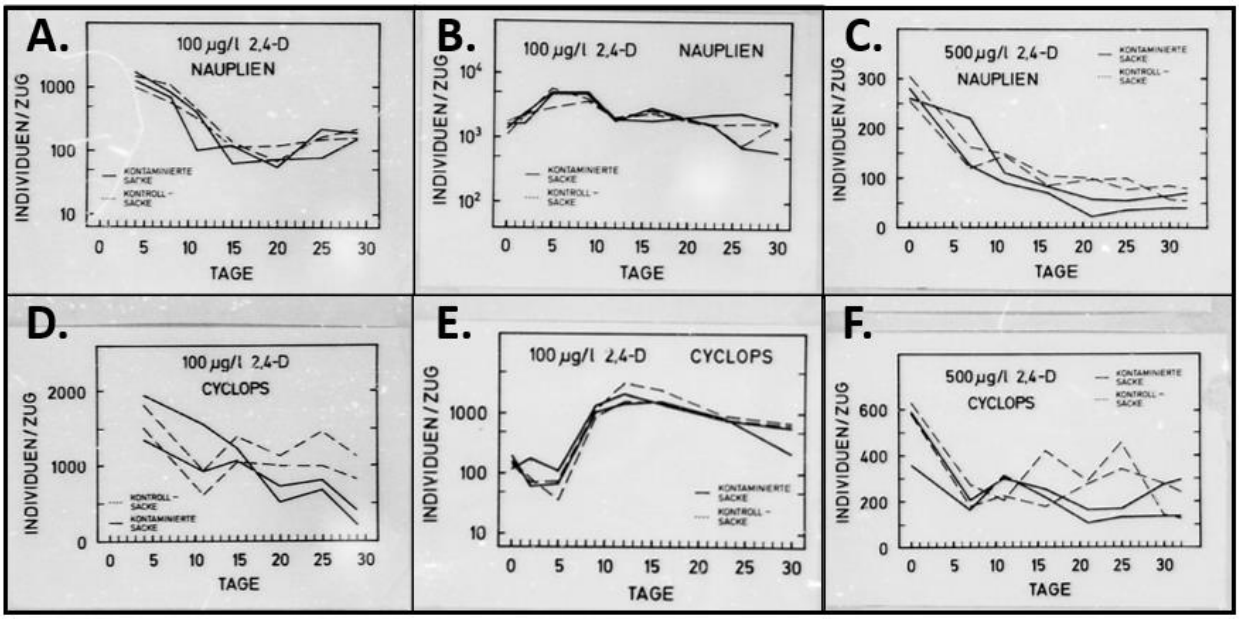


Figure 2.9.1. Copepod Abundance for 2,4-D Experiments (from Fleckner, 1988) "Zug" = net haul (from Lampert *et al.*, 1989); "Geburtienrat"e = birth rate; "Tage" = Days; "See" = Lake (concentration in the lake water at the concurrent time point; thick solid line); "kontaminierten sacken" = contaminated [2,4-D] sack [bag/mesocosm]; (thin solid lines); "kontroll sacken" = control sack [bag/mesocosm](dashed lines). The 100 µg/L experiment was conducted twice (once in Fall [left panel] and once in Spring [right panel]).

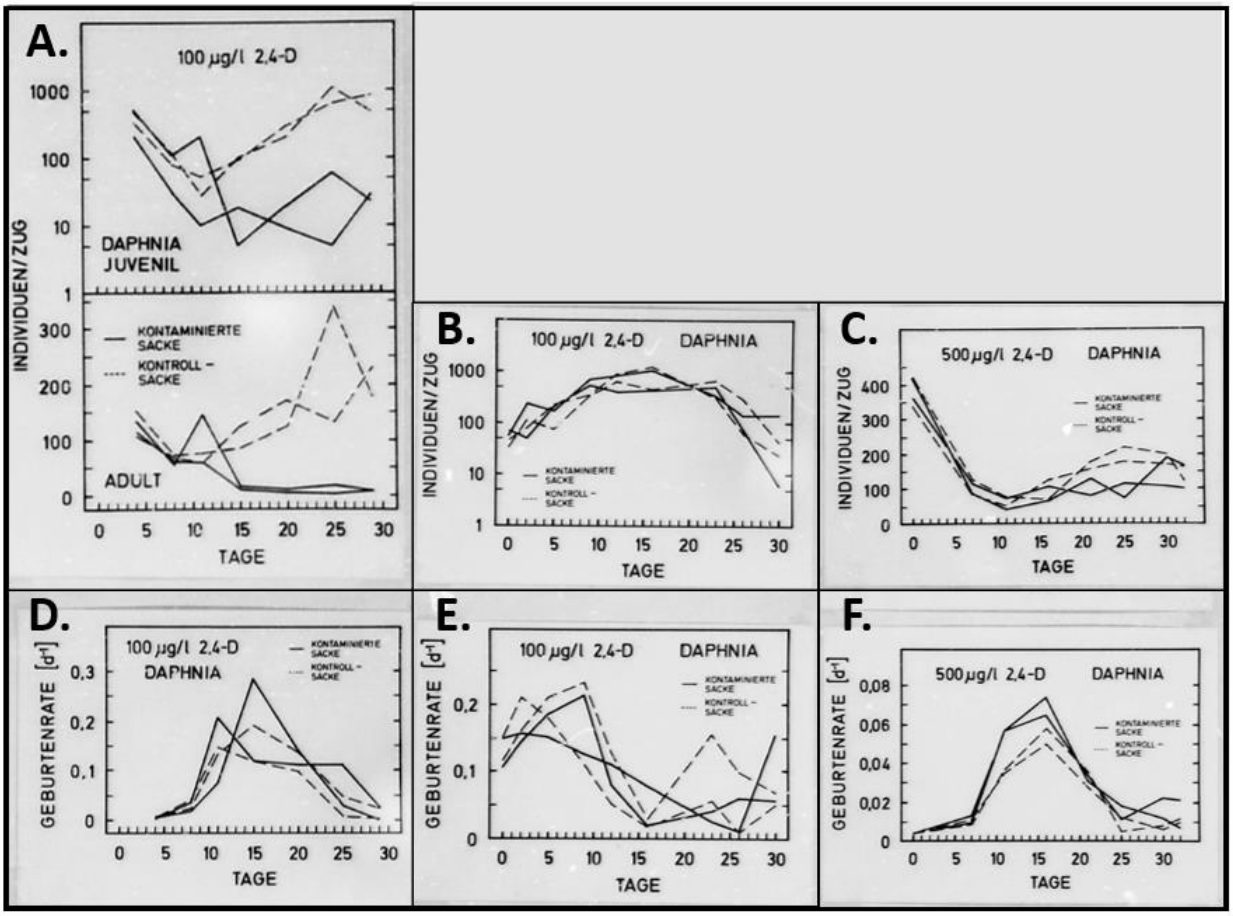


Figure 2.9.2. Daphnia Abundance for 2,4-D Experiments (from Fleckner, 1988) "Zug" = net haul (from Lampert *et al.*, 1989); "Geburtienrat"e = birth rate; "Tage" = Days; "See" = Lake (concentration in the lake water at the concurrent time point; thick solid line); "kontaminierten sacken" = contaminated [2,4-D] sack [bag/mesocosm]; (thin solid lines); "kontroll sacken" = control sack [bag/mesocosm](dashed lines). The 100 µg/L experiment was conducted twice (once in Fall [left panel] and once in Spring [right panel]).

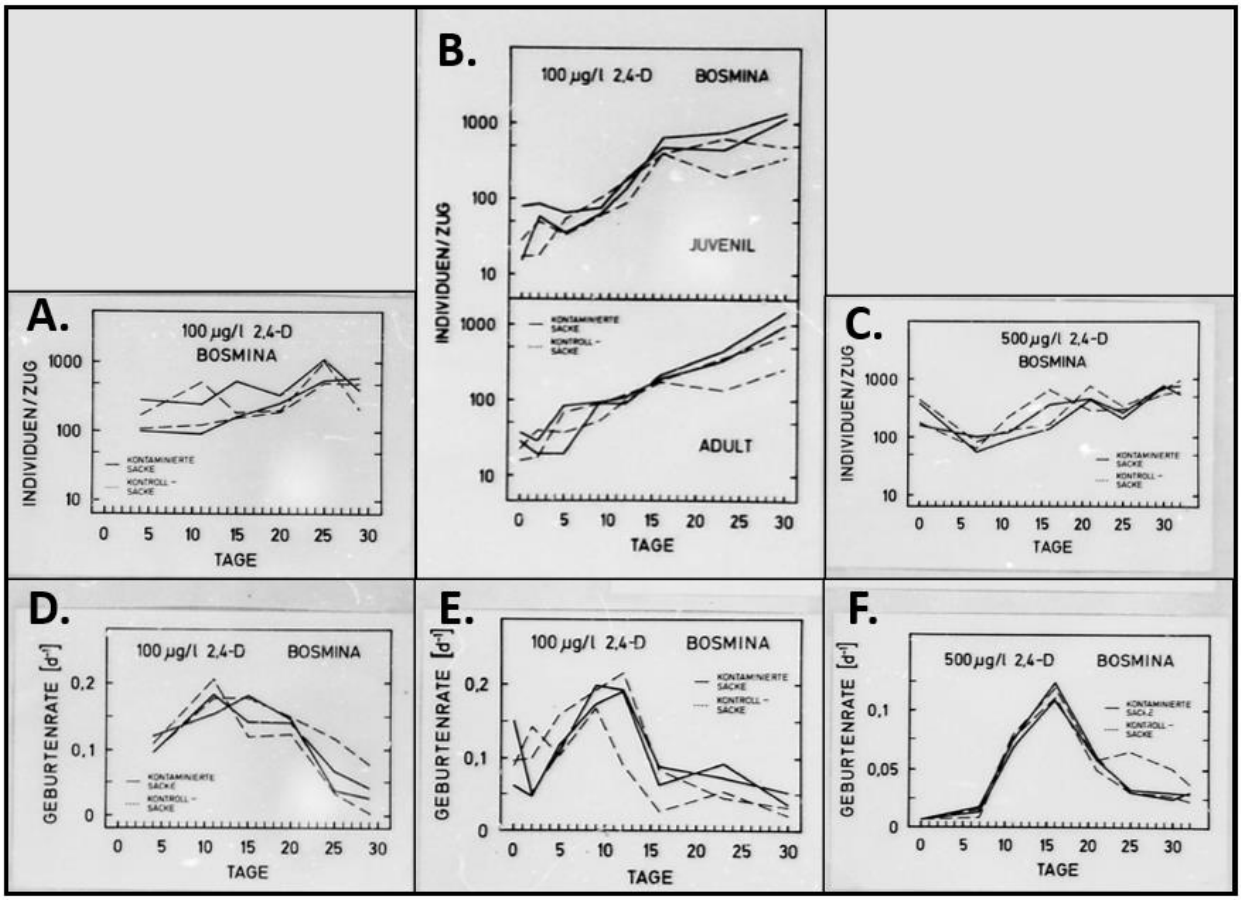


Figure 2.9.3. Bosmina Abundance for 2,4-D Experiments (from Fleckner, 1988) "Zug" = net haul (from Lampert *et al.*, 1989); "Geburtienrat"e = birth rate; "Tage" = Days; "See" = Lake (concentration in the lake water at the concurrent time point; thick solid line); "kontaminierten sacken" = contaminated [2,4-D] sack [bag/mesocosm]; (thin solid lines); "kontroll sacken" = control sack [bag/mesocosm](dashed lines). The 100 µg/L experiment was conducted twice (once in Fall [left panel] and once in Spring [right panel]).

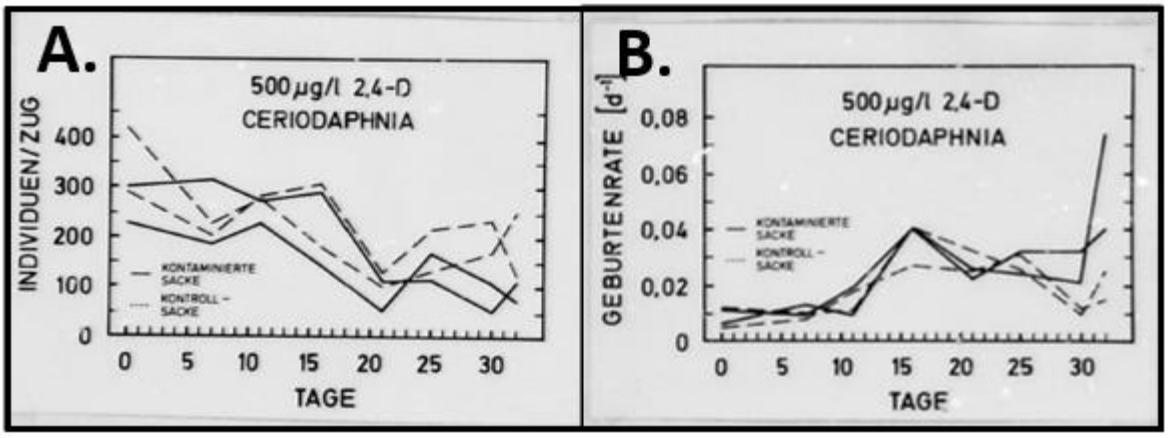


Figure 2.9.4. Ceriodaphnia Abundance for 2,4-D Experiments (from Fleckner, 1988) "Zug" = net haul (from Lampert *et al.,* 1989); "Geburtienrat"e = birth rate; "Tage" = Days; "See" = Lake (concentration in the lake water at the concurrent time point; thick solid line); "kontaminierten sacken" = contaminated [2,4-D] sack [bag/mesocosm]; (thin solid lines); "kontroll sacken" = control sack [bag/mesocosm](dashed lines). The 100 µg/L experiment was conducted twice (once in Fall [left panel] and once in Spring [right panel]).

2.5.1 EPA's 2023 Conclusions

There are several sources of uncertainty with this study and potentially confounding factors that when considered in combination, impact interpretation of the results. Fleckner (1988) indicates that EtOH was used, the open literature publication (Lampert *et al.*, 1989) states that a solvent was not used. There is also uncertainty about the amount of solvent that may have been used and it is unclear if the experiment accounted for the potential effects of EtOH because there is a lack of clarity on the composition of the control mesocosm tanks. Because these studies do not provide detail regarding the use of a solvent control, there is not an effective means of determining whether there was a clear treatment-related response within or across test concentrations. Lastly, treatment levels were not tested concurrently (*note*: a treatment level consisted of four concurrently tested cosms: two of the same nominal treatment level and two controls), and as a result, each used zoo- and phytoplankton communities obtained from the lake at different times. After consideration of additional information from Fleckner (1988), EPA considers the interpretation of the results from this study to be confounded by several factors that could cause changes in dissolved oxygen other than due directly to exposure to atrazine. More specifically, dissolved oxygen levels may have been impacted by degradation of EtOH and zooplankton (*i.e.*, oxygen consumption through bacterial consumption of EtOH or decomposition of dead organic matter). Although the causal factors in this study are unclear, there are consistent changes among the atrazine treatment levels including sharp drops in dissolved oxygen and

zooplankton abundance, both which could be associated with drops in dissolved oxygen levels. Similar studies with 2,4-D further suggest that EtOH was a potential confounding factor given sharp declines in dissolved oxygen without a clear association with changes to phytoplankton abundance. Given the compounding uncertainties associated with the conduct (*e.g.*, lack of a solvent control) and confounding factors associated with this study (*e.g.*, potential impact of EtOH), potential impacts on the phytoplankton community from exposure to atrazine cannot be reliably distinguished. **Therefore, EPA considers this study invalid for purposes of the cosm database.**

CHAPTER 3. UNIVERSITY OF KANSAS – 1979 EXPERIMENT

3.1 Overview

A series of experiments were conducted at the University of Kansas from the late 1970s to the early 1980s. Those involving atrazine occurred in 1979 (discussed in this chapter) and from 1981-1983 (discussed in **Chapter 4** next). This overview section will first discuss the connection between the two experiments before providing specific details about the 1979 experiment. Specific details about the 1981-1983 experiment can be found in the next chapter.

The 1979 experiment and 1981-1983 experiment used many of the same experimental ponds (**Figure 3.1**). As further discussed in the *Experimental Design and Execution Sections* (**Section 3.2 and 4.2**), the ponds were drained and refilled prior to each experiment. Plankton, three to four fish species, and potted macrophytes were added to each pond during each experiment. For non-potted macrophytes, Carney (1983) stated that "Since their construction in 1977 [ponds 11-16] and 1979 [ponds 21-26], the ponds had developed similar though sparse macrophyte communities. Therefore, it was deemed unnecessary to actively seed them with macrophyte propagules" for the 1981-1983 experiment. The origin of the macrophytes is unknown but based on this statement, EPA assumes that they were not fully removed and replaced between experiments. While seasons in Kansas likely reduced the macrophyte populations each year, new spring populations would still be based on macrophytes from the prior populations, and thus potentially be selected for based on the prior year's experiment. There is no information about the addition of other taxa (*e.g.*, macroinvertebrates, amphibians) and because the ponds were not covered, EPA assumes that some species may have naturally colonized the ponds.

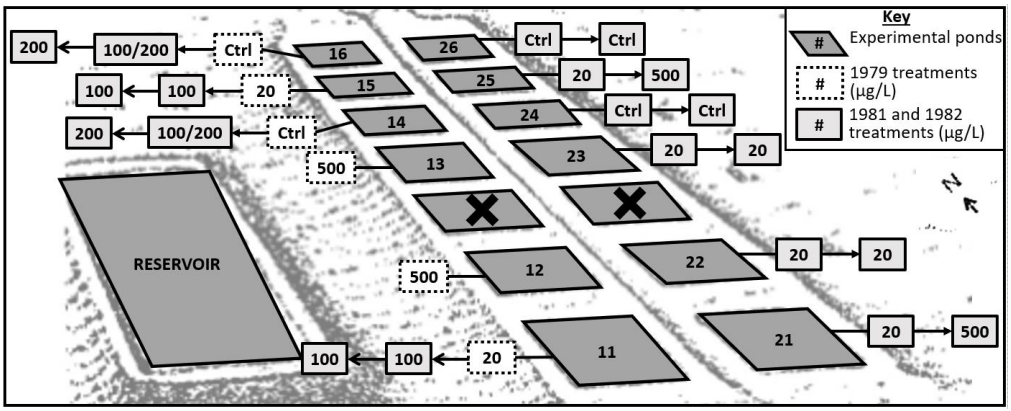


Figure 3.1. The University of Kansas experimental facility, as depicted in several publications associated with the two groups presented in this White Paper, with additions to increase clarity. Numbers in the ponds were assigned by the researchers. The two sets of boxes for the 1981-1983 experiment represent the 1981 treatments and 1982 redosing (and sometimes changes). In 1983, the dosing was the same as 1982. The 1981 dosing for pond 14 and 16 is denoted as "100/200" because it is unknown which pond received which treatment. *Note: The documents do not describe what happened to the ponds between 1979 and 1981.*

The 2012 SAP had concerns with the following five studies that are associated with the two University of Kansas experiments: Carney (1983), deNoyelles *et al*. (1982), deNoyelles *et al*. (1989), Dewey (1986), and Kettle *et al*. (1987) (USEPA, 2012b).

The 1979 experiment is discussed in deNoyelles *et al*. (1982), deNoyelles *et al*. (1989), and Kettle *et al*. (1987), as well as deNoyelles and Kettle (1980), Kettle (1982), and Larsen *et al*. (1986), which will also be included here. These publications include journal articles, a report, and a thesis. All of these publications discuss methods and results associated with the 1979 experiment, with some discussing different parts than others. There may be more publications associated with the 1979 experiment but EPA believes that those listed above contain the primary information regarding the conduct and results of these studies as they relate to the purpose of the cosm database. Below, the studies are discussed collectively with separation only when relevant (*e.g*., specific methods, results, concerns).

3.2 Experimental Design and Execution

In July 1979, experimental ponds (each 0.045 ha) were drained and refilled to a depth of 2.1 m (470,000 L) with "natural water" and plankton from an adjacent small reservoir (0.33 ha; maintained by well water). Five days later, three fish species were added: 50 Bluegill Sunfish (*Lepomis macrochirus;* average length: 85 mm), 20 Channel Catfish (*Ictalurus punctatus*; 116

mm), seven Gizzard Shad (*Dorosoma cepedianum*; 185 mm). Grass Carp (*Ctenopharyngodon idella*) were not included in the 1979 experiment. Kettle *et al*. (1987) notes that these fish were from local ponds and reservoirs. Their prior exposure history was not noted. There is no other information about the addition of other species (aside from potted *Najas* plants mentioned below), but several species of macrophytes, macroinvertebrates, and amphibians were present and were likely from natural colonization due to the lack of covering and the water source (*e.g.*, established reservoir). The density of these organisms was not mentioned.

On July 24, atrazine treatments were applied, which included 0 (control), 20, and 500 µg/L, each replicated twice. The atrazine used was "commercial grade" (40.8% active ingredient; liquid atrazine; two registration numbers were mentioned: #1990-318 and #1990-381 – the former likely an error and latter likely correct) and the concentrations were based on the percentage of active ingredient. This was the only application of atrazine for the duration of the experiment and the documents do not describe how it was applied. There were no other manipulations until 3 December 1979 when the ponds were drained, and the experiment was terminated. Water levels were not maintained and by 3 December 1979, the water level had declined by 20 to 40 cm.

Monitoring of the ponds began just before the atrazine treatment (July 1979) and continued for the next 136 days. Monitoring was most frequent during the first 63 days, with monthly sampling occuring thereafter. Abiotic conditions monitored included: Temperature, light profiles, dissolved oxygen (DO), turbidity, conductivity, pH, total dissolved solids (TDS), and total alkalinity. Temperature, light, and DO were measured at discrete depths, while the other parameters were measured with a column sampler. Atrazine concentrations in the water column were measured as well. Two days after atrazine treatments were applied, measured atrazine concentrations in the 20 µg/L treatment were 95-96% of the nominal and were 94-104% of the nominal in the 500 µg/L treatment. By Day 92, measured atrazine concentrations in the 20 µg/L treatment were 64-68% of the nominal and in the 500 µg/L, were 70-78% of the nominal.

Biotic monitoring occurred over the same period and included: Phytoplankton, filamentous algae, macrophytes, zooplankton, benthic invertebrates, fish, and possibly amphibians. Only primary producers will be discussed here because those are the groups relevant to the CE-LOC. Various water column samples were taken to assess phytoplankton in a variety of ways. For

species enumeration, samples were preserved with acid Lugol's solution and later counted with an inverted microscope. Distributional changes of several of the more common species were followed throughout the experiment. Algal biomass was estimated with a fluorometer (*i.e.*, chlorophyll *a*) or using a counter to determine mean counts, mean volume and finally, biomass (*i.e.*, particle size analysis). Carbon uptake was measured by incubating a set amount of water from a sample with radiolabeled sodium bicarbonate ($NaH^{14}CO_3$) under various conditions [*e.g.*, in the pond (*in situ*), in the laboratory] and then processing the samples for liquid scintillation counting.

The studies say that filamentous algae and macrophytes (*Najas* and *Chara* spp) were rare throughout the experiment and that they were assessed visually weekly. Some studies mentioned using qualitative rake hauls (for species identification) and aerial analysis of photographs as well.

In addition to the above, short-term *in situ* and laboratory experiments were conducted using phytoplankton, macrophytes, zooplankton, and macroinvertebrates. For the current evaluation, only the *in situ* macrophyte experiment is relevant because they are part of aquatic plant communities. The macrophyte experiment included adding potted *Najas* to the bottom of each pond in plastic trays (two trays/pond) and checking the plants for mortality on Days 14, 31, and 63. However, it is important to note that the studies do not document when these macrophytes were added, so the duration of exposure is unknown. The documents do not say if these are in addition to the *Najas* mentioned above or if they were the *Najas* mentioned above.

None of the studies assessed herein mention any statistical analyses in the methods sections. deNoyelles *et al*. (1982) mentions a Student-Newman-Keuls (S-N-K) test in the results section. EPA's screening and scoring criteria did not include a requirement for statistical analyses and, in their absence, EPA's best professional judgement was used, which considered results characteristics such as magnitude, duration, replication, variability, and recovery.

3.3 EPA's Use as of 2016

In the 2016 Refined Ecological Risk Assessment (USEPA, 2016), EPA included two endpoints from the University of Kansas 1979 experiment (**Table 3.1**). One endpoint (#52) represents the 20 µg/L treatment and the other endpoint (#3) represents the 500 µg/L treatment. Both were

scored as "Effect" to the aquatic plant community. Note that the cosm database associated with 2016 Refined Ecological Risk Assessment did not associate deNoyelles and Kettle (1980), Kettle (1982), Kettle *et al*. (1987), or Larsen *et al*. (1986) with the 1979 experiment endpoints, despite them being associated with the 1979 experiment (see **Section 3.1**). As a result, they are missing from the reference column in the table below.

Table 3.1. A summary of the cosm endpoints associated with this group that appeared in Appendix G.2 of the 2016 Refined Ecological Risk Assessment. These endpoints were used to evaluate the potential effects of atrazine on aquatic plant communities.

References	Endpoint Number	Duration (days)	Nominal Conc. (µg/L)	Plant Group	Results and Recovery	Effect/No-Effect Conclusion
deNoyelles *et al.* (1982); deNoyelles *et al*. (1989)	52	63[A]	20	Phyto	Decrease (50% decline) in ^{14}C-uptake and biomass of phytoplankton. Recovery: > 3 wks for biomass and ^{14}C-uptake	Effect
deNoyelles *et al*. (1982); deNoyelles *et al*. (1989)	3	63[B]	500	Phyto and Macro	Decrease (> 90% decline) in phytoplankton ^{14}C-uptake and biomass. 100% decline in SAV[C]. Change (observed after 14 days) in species composition of phytoplankton. Recover: > 63 d (no recovery reported for shift in species composition; recovery in phytoplankton biomass and production occurred > 3 weeks)	Effect

[A] Although full exposure period was 136 d, atrazine exposure concentrations are available for only 63 days.
[B] From 136 day study; according to study authors, ponds were intensely monitored for first 63 days, then sampled monthly thereafter until day 136; however, exposure concentration information is not available past 63 days.
[C] Submerged aquatic vegetation
*Note: The durations here are incorrect. Corrections have been made in **Table 3.5**.

3.4 Stakeholders' Major Concerns and Criticisms

The publications associated with the 1979 University of Kansas experiment have been in the cosm database since the 2003 Atrazine Interim Registration Eligibility Decision (IRED) (USEPA, 2003). For the 2007 SAP Meeting, the University of Kansas endpoints were included in an appendix of the White Paper that contained the database but otherwise, there was no discussion about the 1979 University of Kansas experiment in the White Paper, by public commenters, or by the SAP (USEPA, 2007). For the 2009 SAP Meeting, EPA indicated a change in the scoring of endpoint #52 (20 µg/L) in the White Paper (USEPA, 2009a). The public did not comment on the 1979 University of Kansas experiment. The 2009 SAP commented on the

effect/no-effect conclusion for endpoint #52 (USEPA, 2009b). For the 2012 SAP, EPA indicated that endpoint #52 would be scored as "Effect" (USEPA, 2012a), to which Syngenta suggested during the public comment period the rescoring of endpoint #52 to "No Effect" (Syngenta, 2012a). The SAP echoed Syngenta's suggestion about rescoring endpoint #52 and expressed concerns with the use of fish in the studies (USEPA, 2012b). The same concerns regarding the scoring were echoed in comments from Syngenta on the 2016 Refined Ecological Risk Assessment (Syngenta, 2016) and again in comments on the 2022 Proposed Revisions to the Atrazine Interim Registration Review Decision (Syngenta, 2022). In addition, concerns about having multiple endpoints per concentration were also brought up by Syngenta in 2016 and 2022. The specific concerns brought about during each of these events is discussed next.

3.4.1 The 2009 SAP Meeting

In the White Paper submitted for the 2009 SAP meeting, EPA indicated that endpoint #52 would be changed from a Brock score of 2 (*i.e.*, that the effect was slight/transient) to a score of 3 (*i.e.*, significant effect followed by return to control levels during an observation period of less than 56 days) (USEPA, 2009a) (**Table 3.2**). The change in the Brock score is significant given the eventual change to a binary scoring (*i.e.*, effect /no effect) system.

Table 3.2. Information from Table III-1 from the 2009 White Paper with the caption "Summary of Microcosm/Mesocosm Data Point Changes"

Reference	Endpoint Number	Change	Basis for Change
DeNoyelles *et al*. (1989)	52	Brock score changed from 2 to 3	A 50% decline in phytoplankton and biomass production was observed at a nominal atrazine concentration of 20 µg/L compared to the control, with recovery occuring at 3 weeks. The Brock score for this data point was initially identified as 2 meaning that the effect was "slight" and/or "transient". Given that a significant decline in biomass was observed (50%) and that recovery of biomass occurred at 3 weeks, the Brock score for this data point was changed to 3.

There were no relevant comments from the public about the 1979 experiment, but the 2009 SAP said the following in their meeting minutes (USEPA, 2009b):

> *Similar observations of functional redundancy in mesocosms were observed in a series of studies in lentic mesocosms exposed to atrazine for 136 days at three concentrations (1 µg/L, 20 µg/L, and 50 µg/L) at Kansas University [University of Kansas] (DeNoyelles et*

> *al. 1989). Although laboratory effects were demonstrated in short-term assays at test concentrations as low as 1 µg/L atrazine, overall measures of phytoplankton biomass were not observed even at the 20 µg/L level due to replacement of sensitive species by more resistant species. In fact, algal biomass in the 20 µg/L treatment was significantly higher at the end of the 136-d study. The 50 µg/L concentration significantly reduced algal biomass and altered algal succession dynamics which were statistically and ecologically significant. However, this concentration, as the authors indicated, is not environmentally relevant to aquatic systems and only occurs under edge-of-field conditions (deNoyelles et al. 1989). Therefore, functional redundancy must be considered as a mitigation factor when extrapolating single species aquatic plant data to predict effects on plant communities in higher order systems such as aquatic mesocosms.*

The 1979 experiment lasted 136 days but it was not part of a series of experiments. Additionally, the treatments were 0, 20, and 500 µg/L, not 1, 20 and 50 µg/L. Therefore, the 2009 SAP comment is not entirely clear.

3.4.2 The 2012 SAP Meeting

In the White Paper submitted for the 2012 SAP meeting, EPA mentioned again that endpoint #52 was changed from a Brock score of 2 to 3, and that this change would result in an "Effect" call with the new binary scoring approach (USEPA, 2012a). All studies were included in Appendix D of the 2012 White Paper, which was the same as what appeared in the 2016 Refined Ecological Risk Assessment (**Table 3.1** above).

During the public comment period, Syngenta suggested that endpoint #52 should be scored as "No Effect" based on the following summary (Syngenta, 2012a):

> *deNoyelles et al. (1982) stated that phytoplankton biomass and photosynthesis were reduced in the 20 µg/L cosms on Day 2 of the first (1979) study but returned to control levels by Day 7. The inference of effects on Day 2 was based on analysis of changes between Day -1 and Day 2 using the Student-Newman-Keuls test. The raw data presented by Kettle (1982) show that a phytoplankton bloom developed in one of the two control cosms, while in the other control cosm phytoplankton biomass and photosynthesis*

> *remained at or below the levels of the 20 µg/L cosms. CSI's independent analysis of the raw data using Dunnett's Test indicated that no significant differences occurred at 20 µg/L even on Day 2. Because of the variability among controls, the inference of even a transient atrazine effect on phytoplankton in the 20 µg/L cosms in the 1979 study cannot be supported. A later publication synthesizing the results of this study (Larsen et al. 1986) stated that "no effects on algal photosynthesis occurred at the 20 µg/L treatment level."*
>
> *… Considering data from all study years together, a reasonable weight-of-evidence interpretation is that the cosm phytoplankton showed little or no consistent response to 20 µg/L atrazine, and CSI assigned a binary effect score of "0" for ID #52.*

Syngenta concluded that "The cosm studies at the University of Kansas at Lawrence were the most ambitious ever conducted with atrazine, in terms of the size and complexity of the experimental ecosystems" and ended up scoring endpoint #52 as "No Effect" and endpoint #3 as "Effect".

As detailed in **Section 3.1**, the 2012 SAP commented on three studies associated with the 1979 experiment: deNoyelles *et al*. (1982), Kettle *et al*. (1987), and deNoyelles *et al*. (1989)[26]. The SAP comments on deNoyelles *et al*. (1982) and Kettle *et al*. (1987) are below (USEPA, 2012b):

> *deNoyelles et al. (1982) – This study showed basically no effects on biomass and C-14 uptake in phytoplankton at an atrazine concentration of 20 µg/L. This is revealed by the overlapping confidence intervals in Fig. 1. In fact, the greatest proportional differences occurred when the 20 µg/L concentration stimulated primary productivity compared to the control. Figure 2 shows stimulation of 3 species of algae at 20 µg/L. In addition, this study contained gizzard shad at a total of 7 fish/mesocosm, or 70/acre…in addition to bluegill and channel catfish. There was no accounting of survival of gizzard shad which are very difficult to handle in transfer and stocking. Differential survival would have large indirect effects due to differences in the zooplankton community. Effects noted at 1*

[26] The comment on deNoyelles *et al*. (1989) can be found in **Chapter 4, Section 4.4.2** because the comment was only about the 1981-1983 study.

> *µg/L were short-term studies where control pond water was treated with atrazine in lab bioassays. No macrophyte data were cited. These data should not be assigned a 20 µg/L effect.*
>
> *Kettle et al. (1987) – The pertinent reference should be the original 1980 Master's Thesis which led to the 1987 paper. The 1987 paper reports a negative effect of atrazine on bluegill reproductive success. It suffers from the same design flaws as Dewey et al. (1985) such as presence of gizzard shad. With no information on differential survival of gizzard shad the results on bluegill reproduction are suspect. Loss of gizzard shad from the controls would lead to increased numbers of zooplankton and higher survival of young bluegill. Note: there was zero grass carp survival in the controls as indicated by deNoyelles et al. (1989), which resulted in high macrophyte biomass that served as refugia for young bluegills and allowed them to avoid predation by channel catfish*. [The mention of Grass Carp is incorrect. Grass Carp were not present in the 1979 experiment and were not discussed in Kettle et al. (1987)].

The panel also noted that these publications represent "studies discussed by the 2009 SAP as having flawed methodology, which affected interpretation of the results" and specifically said the following about the University of Kansas experiments:

> *Studies conducted at the University of Kansas from 1979-1991. These studies were considered "ecotoxicological classics" based on the hypotheses tested, study complexity and ecological relevance. However, they were not conducted under any semblance of Good Laboratory Practices (GLPs). Note: The University of Kansas study is accounted for five times in EPA's analysis, which may bias the data in Fig. 16 of the White Paper for atrazine effects at 20 µg/L.*

Note: Studies were not required to be conducted under GLP but rather were required to meet EPA's minimum screening criteria developed after the 2009 SAP.

3.4.3 The 2016 Refined Ecological Risk Assessment

In the 2016 Refined Ecological Risk Assessment (USEPA, 2016), EPA reiterated change to "Effect" for endpoint #52 and then for the 2016 reevaluation, said the following about the University of Kansas experiments (1979 and 1981-1983 2016 reevaluation was combined):

> ***deNoyelles et al. 1982, Carney and deNoyelles 1986, Dewey et al. 1986, Kettle et al. 1987, deNoyelles et al. 1989 (Endpoints 1, 2, 3, 4, 5, 41, 42, 52):***
> *The endpoints identified from the various reported results from these studies were re-reviewed as recommended by the 2012 SAP. The main focus of the review was to identify first the endpoints where there was agreement between EPA's endpoint classifications and the 2012 SAP and Giddings 2012 [Syngenta, 2012a here] classifications. The review identified agreement for all endpoints that were 100 µg/L or higher (endpoints 1, 3, 4, 5, 41, and 42). Therefore there was disagreement on endpoints 2 and 52, each of which was reported from an independent mesocosm study testing the effects of 20 µg/L for 365 and 63 days, respectively. Reported effects for endpoint 2 included only the first year of the 805-day study where no recovery was reported, and there were biologically significant decreases in floating and submerged plant cover (40% decline in Typha; 50% decline in SAV [submerged aquatic vegetation]; 50% decline in Najas). Reported effects for endpoint 52 were a 50% decline in 14C-uptake and the biomass of phytoplankton, with recovery to control levels taking longer than 3 weeks.*

Ultimately, EPA made no changes to the 1979 University of Kansas endpoints in the cosm database (**Table 3.1**).

In the public comments received after the publication of the 2016 Refined Ecological Risk Assessment, Syngenta reiterated much of what they said in their comments from the 2012 SAP Meeting, including the same suggested changes. Syngenta also said the following regarding splitting endpoints within an experiment (Syngenta, 2016):

> *Furthermore, while the database includes separate endpoints for periphyton, phytoplankton and macrophytes from some studies (e.g., deNoyelles et al. 1982; Seguin et*

> *al. 2001a,b), this has not been applied consistently across all studies, and we have split the other data points accordingly.*

Essentially, Syngenta increased the number of endpoints to account for cases where two or more taxa groups were present (*e.g*., one endpoint for one concentration with both phytoplankton and periphyton was changed to two endpoints for one concentration with one for phytoplankton and one for periphyton). This was done because it appeared that EPA had done this inconsistently in the cosm database.

Syngenta also included an extensive review of both the 1979 and the 1981-1983 University of Kansas experiments and concluded:

> *… the experimental design suffered from several complications that interfere with interpretation of the results: (1) ponds were reused for different treatments from year to year, so the results for any given year could have been affected by previous treatments; (2) water levels were allowed to fall by as much as 50% during the summer, with various consequences for water chemistry, atrazine concentrations, and shoreline development; and (3) grass carp, whose impact on the ecosystem was similar to that of atrazine, were introduced into the ponds* [1981-1983 experiment only].
>
> *From this evaluation, it appears that atrazine treatments of 100 µg/L [1981-1983 experiment only] and higher resulted in loss of macrophytes with secondary consequences for macrophyte-dependent invertebrates and the fish that fed on those invertebrates. Atrazine treatments of 20 µg/L may have caused partial reductions in macrophytes.*

Finally, Syngenta contacted Dr. deNoyelles to discuss the experiments and provided details about the conversation in their comment. The question that is most relevant here was, “Are there unpublished data that might shed more light on the magnitude of impact at 20 µg/L?” to which Syngenta wrote:

> *Not in Dr. deNoyelles' opinion. The main effect at 20 µg/L was a shift in phytoplankton communities to greater resistance. There was an effect on macrophytes at 20 µg/L in the presence of grass carp, but no effect in the absence of grass carp [1981-1983 experiment only]. The development of resistance in phytoplankton was confirmed in other KS ponds that had been exposed to high concentrations of triazine herbicides. We spent a few minutes on the question: Is the change in phytoplankton communities a good thing (indicating resilience in the ecosystem) or a bad thing (implying potential for indirect food-web effects)? In our opinion, it could be interpreted either way.*

Another substantive comment came from the Triazine Network (2016), who gave summaries for deNoyelles *et al*. (1982), Kettle *et al*. (1987), and deNoyelles *et al*. (1989), which included details from EPA's data evaluation records (DERs), the 2009 and 2012 SAP meeting, Syngenta (2012a), Triazine Network (2015), Moore *et al*. (2016), and finally EPAs 2016 Refined Ecological Risk Assessment (USEPA, 2016). There were no topics or concerns raised beyond those discussed above that were specific to the University of Kansas 1979 experiment.

3.4.4 Post-Risk Assessment to Present

After issuance of the proposed revisions to the Interim Decision (USEPA, 2022b; 2022c), EPA again received several comments about the cosm database. In Syngenta's comment, similar suggestions were provided regarding the effect/no-effect conclusions for the University of Kansas endpoints (Syngenta, 2022). Syngenta also discussed the splitting of endpoints further by noting:

> *In a letter to the Triazine Network ([US]EPA 2022a), EPA stated, "A major difference between recommendations made in Giddings and those made at the 2012 SAP meeting is that Giddings 'split' cosm study endpoints, which the SAP did not recommend, as not all panelists agreed that splitting endpoints is sound science. Splitting endpoints looks at effects on individual parts of the community rather than on the entire community (i.e., one effect/no effect score per test concentration). By splitting endpoints, Giddings counts 'no effect' endpoints multiple times for components of the community that are less sensitive to atrazine."*

> *We have reconsidered this question in light of the origins of the database. While in most cases the original* [EPA] *data compilation assigned a separate data point to the response of each community in a particular study (the* [University of] *Kansas studies are a prominent example), the practice was inconsistent and should have been normalized before the cosm data began to be used in a formal statistical analysis such as LOC calculation. In light of EPA's latest considerations and objectives, we agree that a single effect score based on the response of the overall plant community is consistent with the risk assessment endpoint and protection goal for aquatic plants. Results from a single study should be represented in the LOC analysis by a single point to avoid over-representing studies with endpoints for multiple plant communities. The early intention of separating analyses for phytoplankton, periphyton, and macrophytes (e.g., Giddings et al. 2002a* [Syngenta, 2002 here], *EPA 2003a* [Gonzalez-Valero et al., 2003 here]*) is no longer a consideration, although the "Plant Group" field remains in the database (EPA 2016a).*
>
> *In the G-22 database [the updated database created by Jeffery Giddings on behalf of Syngenta], data points for effects on multiple plant communities from a single treatment group in a single study were combined into a single point representing the response of the whole plant community to the associated exposure...*

Syngenta later stated in their review of the University of Kansas experiments:

> *From the beginning (EPA 2003a [*Gonzalez-Valero et al., 2003 here*]), ID #2 [*1981-1983 experiment*] represented results for macrophytes and ID #52 represented results for phytoplankton at 20 µg/L. In each case, data were amalgamated across studies from 1979-1983, with citations to 7 publications… In the G-22 database the 2 data points were merged into a single point (ID #2) representing the responses of the overall plant community to 20 µg/L and scored "no-effect."*

It is important to note that based on the comments provided, there seems to be some confusion about who is splitting endpoints and when, which will be discussed further in **Section 3.5**. Also

important to note is that endpoint #52 (1979 experiment) and #2 (1981-1983 experiment) still appear in the G-22 database (*i.e.*, they were not merged).

The score given by EPA in the 2016 Refined Ecological Risk Assessment, by Syngenta in their comment on the 2016 risk assessment, and by Syngenta in their 2022 comment on the proposed revisions to the Interim Decision were summarized by Syngenta in the table titled "Table SI-1(mod)" (**Table 3.3**).

Table 3.3. Data from Syngenta's Table SI-1 (mod) table. For brevity, some columns that are not needed for this evaluation have been removed (*e.g.*, Test System description). For the binary scores, 0 equals "No Effect" and 1 equals "Effect".

Endpoint Number	Reference	Conc (ppb)	Plant Community Response	Binary Scores (EPA 2016)	Binary Scores (Syngenta 2016)	Binary Score (Syngenta 2022)
52	deNoyelles *et al.*,1982, 1989	20	Slight effects on phytoplankton in 1979 study, no effects in 1981 and 1982 (see text)	1	0	0 (1979)
3	deNoyelles *et al.*,1982, 1989	500	Biomass and photosynthesis reduced	1	1	1 (1979)

The Triazine Network also provided comments, but none were focused solely on the 1979 University of Kansas experiment (Triazine Network, 2022).

3.5 EPA's 2023 Reevaluation

After consideration of all of the studies' limitations, the determinative concerns will be discussed here, including those brought up by past SAPs and public commenters. These include the fish in the ponds, the splitting of endpoints (within a treatment), and the effect/no-effect conclusions for endpoints.

3.5.1 Fish in Ponds

The 1979 University of Kansas experiment only had Bluegill Sunfish, Gizzard Shad, and Channel Catfish. There were no Grass Carp present in the 1979 experiment.

In the meeting minutes, the 2012 SAP said the following about including fish in cosm experiments: "cosms incorporating fish should be of a sufficient size as to allow robust growth of

fish without excessive intra-specific competition that will substantially alter plant communities (*e.g.*, 2 gm biomass/m^3; see Touart 1988)" (USEPA, 2012b).

According to Touart (1988), "Densities within the range of 2-5 g/cubic meter are appropriate." Therefore, based on this, the experimental ponds (470 m^3) used in the University of Kansas experiments could support 940 to 2350 grams of fish biomass.

Unfortunately, the publications associated with the 1979 experiment do not provide any data on the initial mass of the fish. However, one publication from the 1981-1983 experiment (Huggins, 1990) provides the mean initial starting mass of the fish for the 1981-1983 experiment, which can be used as a proxy (**Table 3.4**). Based on the information in Huggins (1990), the total starting biomass was approximately 602 g in 1981, which is below the lower range calculated based on Touart (1988). The fish in 1979 were longer than those in 1981, but it is very unlikely that a difference in 2-3 cm would result in a total biomass that exceeded the upper end, which is almost four times the 1981-1983 experiment estimate. Additionally, there were three less Gizzard Shad in 1979, which were the fish with the largest mass. Therefore, the biomass of the fish was very likely within the range presented in the citation cited by the 2012 SAP (i.e., Touart, 1988).

Table 3.4. The fish species added at the beginning of the 1979 and 1981-1983 University of Kansas experiments, including the common and scientific names, total added, initial mean length, initial mean wet mass, and the estimated biomass. The total added and length (mean or range) were mentioned in various publications, but the mean wet mass could only be found for 1981 in Huggins (1990).

Species	Total Fish Added	Mean Length (cm)	Mean Wet Mass (g)	Estimated Biomass (g)
1979 Experiment				
Bluegill Sunfish (*Lepomis macrochirus)*	50	8.5	Unknown	Unknown
Channel Catfish (*Ictalurus punctatus)*	20	11.6		
Gizzard Shad (*Dorosoma cepedianum)*	7	18.5		
Total	77	-		
1981-1983 Experiment[A]				
Bluegill Sunfish (*Lepomis macrochirus)*	50	5.2	2.0	100
Channel Catfish (*Ictalurus punctatus)*	20	9.4	7.8	156
Gizzard Shad (*Dorosoma cepedianum)*	10	15.2	34.6	346
Total	*80*	-	-	*602*

[A] Grass Carp were not included in the 1979 experiment. Therefore, Grass Carp data from the 1981-1983 experiment is not included here because that comparison is not needed for this reevaluation.

After the initial stocking of the fish, it is expected that there will be changes in the total biomass due to natural growth and treatment effects, as that is the goal of these long-term experiments. However, the natural growth and the potential impacts of atrazine on growth could result in exceeding the biomass limits and differing community impacts. In the 1979 experiment, the results that are used to determine the effect/no-effect conclusion (see **Section 3.5.3**) occurred within the first month of the experiment. Therefore, it is unlikely that the biomass due to natural growth exceeded the upper range calculated based on Touart (1988). However, there were treatment effects on growth with control fish being larger than those in the 500 µg/L treatment (reduced by 26% for Bluegill Sunfish, 16% for Channel Catfish, and 31% for Gizzard Shad; no difference at 20 µg/L), which could have resulted in differing impacts on the communities (discussed further below).

In addition to growth, natural and/or impaired reproduction can alter total biomass and there were differences in reproduction at the end of the experiment. The only fish to reproduce was the Bluegill Sunfish with a mean number of fry in the controls of 1376, whereas the means in the 20 and 500 µg/L treatments were not dose responsive with 59 and 53 fry, respectively. The various

authors state the this was not due to number of females or direct toxicity but could have been due to resources (food and shelter), especially given the reduction in macrophytes in the treated ponds (see **Section 3.5.3**).

Finally, there were concerns regarding the fish survival, especially if it varied among treatments. For the originally stocked fish, Kettle (1982) states that, "There was no difference in mortality among ponds for any of the original fish stocked. Approximately 80% of the original *Lepomis* stocked in the ponds had survived the experiment and were retrieved at the end compared to more than 96% of the *Dorosoma* and *Ictalurus*." This is echoed in other publications associated with the 1979 experiment. So differential survival among or within treatments is not a concern.

Imbalances between ponds due to growth and reproduction represent a potential confounding effect. The fish used in the experiment represent a plankton/insect predator (Bluegill Sunfish), benthic omnivore (Channel Catfish), and filtering omnivore (Gizzard Shad). The Bluegill Sunfish biomass was likely the biomass that varied the most because of both growth and reproduction. The higher Bluegill Sunfish biomass in the controls could have resulted in a reduction in zooplankton. This could then subsequently result in an increase in phytoplankton in the controls that would not be directly caused by atrazine. However, zooplankton groups were either greater in the controls or unaffected. Based on feeding method, the other two species could have directly impacted the aquatic plant community. However, reductions in these fish species would result in increases in the parts of the aquatic plant community that they feed on, which is opposite of what was observed in the 500 µg/L treatment (discussed in **Section 3.5.3**). While other indirect effects could have occurred (*e.g.*, nutrient increases due to waste, physical disturbances), EPA has concluded that the effects to the aquatic plant community can largely be attributed to atrazine and that the changes in fish biomass were those that would be expected in cosm experiments.

Given the above information, EPA has decided to keep the University of Kansas 1979 endpoints in the database. The endpoints help expand the breadth of communities represented in the cosm database and contribute to the knowledge about how atrazine affects the diverse aquatic communities found around the nation.

3.5.2 Splitting Endpoints

Another concern expressed was EPA's inconsistency of having more than one endpoint for an experimental treatment (*e.g.*, >1 endpoint for a single concentration to represent different parts of the aquatic plant community – one for phytoplankton and one for macrophytes). This is opposed to the goal of having one endpoint per concentration in an experiment to represent the whole aquatic plant community. There seems to be some confusion here due to EPA's "Plant Group" column (and potentially due to errors made by EPA, which are discussed in **Chapter 4, Section 4.5**). For example, endpoints #52 and #2 are both associated with a 20 µg/L treatment with phytoplankton and macrophytes, respectively, for the "Plant Group". However, the "Duration" and "Comment" columns indicate that endpoint #52 is for the 1979 experiment, while endpoint #2 is for the 1981-1983 experiment (**Chapter 4**). There are also two 500 µg/L endpoints (#3 and #1) associated with the University of Kansas experiments, but they too are associated with the different experiments (*i.e.*, #3 for 1979 and #1 for 1981-1983). Therefore, EPA did not split endpoints associated with the two treatments in the 1979 experiment and are solely associated with this experiment. The 1981-1983 endpoints are discussed further in **Chapter 4**.

3.5.3 Effect/No-Effect Conclusion

With the information above, the two endpoints (*i.e.*, #52 and #3) associated with the 1979 Kanas experiment will remain. Both Syngenta and past SAPs commented on the effect/no-effect conclusion for #52, which is for the 20 µg/L treatment, with both suggesting a "No Effect" conclusion. For EPA's 2023 reevaluation, results across the 1979 experiment publications mentioned in **Section 3.1** were considered. It is important to note that some results show considerable variability and that multiple publications indicated that filamentous algae and macrophytes were rare (hence only the potted *Najas* is discussed). Additionally, the publications associated with the 1979 experiment provided very little indication of statistical significance, although statistical analyses were not a requirement here and differences can be judged by the magnitude of the effects. The statistical significance mentioned below represents what was available in the text of the available publications.

20 µg/L treatment (#52)

Phytoplankton – There were no declines in biomass (based on chlorophyll *a*) and it was instead often higher than the control (**Figure 3.2A**). Biomass (based on particle counts) and carbon-14

uptake significantly (p<0.05) declined by day 2 but returned to control levels by day 7 and remained at or above control levels thereafter (**Figure 3.2B and C**). In both cases, there was considerable overlap with the control treatment. Species distribution changes were minimal (**Figure 3.3 and 3.4**).

Macrophytes – Text in various publications states that the planted *Najas* biomass was visually reduced compared to the control but did survive. Some indicate 50%, others 60%, and others 90%. Some publications indicate that these results were verified via rake hauls and/or aerial photographs. However, no figures or tables presented the macrophyte results.

After EPA's 2023 reevaluation, this endpoint has been scored as "**No Effect**" given the transient effect and considerable overlap of the 20 µg/L treatment with the control in the phytoplankton results. Macrophyte results were not consider sufficient enough to make an effect/no-effect conclusion due to the lack of quantification and presentation.

500 µg/L treatment (#3)

Phytoplankton –Biomass (based on chlorophyll *a*) declined after dosing and returned to control levels by Day 15 (**Figure 3.2A**). Biomass (based on particle counts) significantly ($p < 0.05$) declined after dosing as well and returned to control levels by Day 24 (**Figure 3.2B**). Biomass (via particle counts) also declined again later in the experiment. Carbon-14 uptake significantly ($p < 0.05$) declined early on and like biomass (via particle counts), it returned to control levels before declining again near the end of the experiment (**Figure 3.2C).** Species distributions were dramatically altered by atrazine with many species showing rapid initial declines in the first week, but some later increased (**Figure 3.3 and 3.4**).

Macrophytes – Based on various accounts, 90 – 100% of the planted *Najas* died in the 500 µg/L, which was verified by rake hauls and aerial photographs. However, similar to the 20 µg/L treatment, there were no figures or tables presenting these results.

After EPA's 2023 reevaluation, this endpoint has been scored as "**Effect**" given the sharp and prolonged decline in phytoplankton in the 500 µg/L treatment. The macrophyte results were not considered here due to the lack of quantification and presentation.

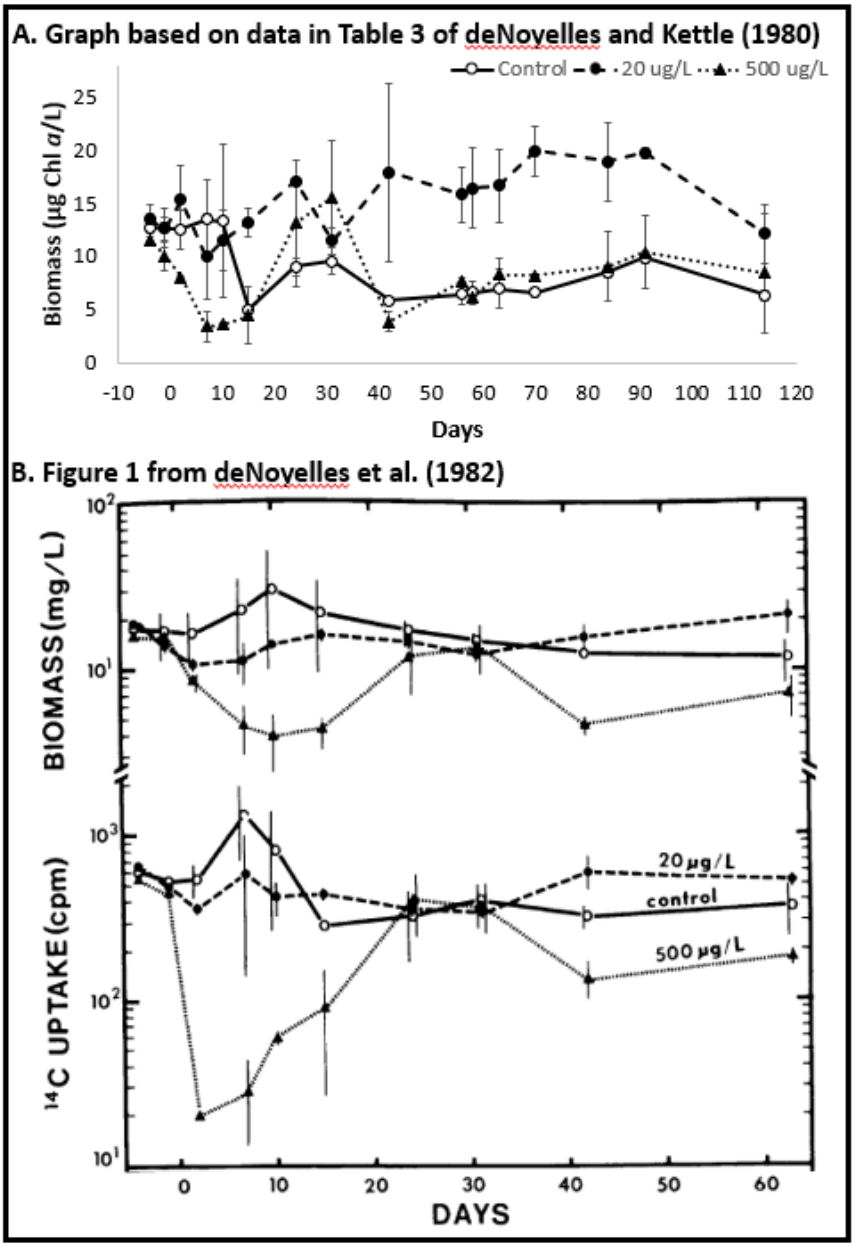


Figure 3.2. Phytoplankton biomass and carbon-14 results. A. Biomass measured via chlorophyll *a* fluorescence. deNoyelles and Kettle (1980) says, "Due to the effects of atrazine on algal chlorophyll fluorescence these values require some interpretation before being used as algal biomass estimates." B (top). Biomass measured via Coulter counter particle counts. B (bottom). Carbon-14 uptake in counts per minute (CPM; amount of radioactivity detected) for samples incubated in the laboratory for 4 h. Each value is the mean of 4 samples and is corrected for dark carbon-14 uptake by subtracting dark bottle CPM. For all, treatment means are plotted with vertical bars representing the range.

A. Table 6 from deNoyelles and Kettle (1980)

	Tetraëdron						Peridinium					
(days)	P_4	P_6	P_1	P_5	P_2	P_3	P_4	P_6	P_1	P_5	P_2	P_3
	-	50	-	2	2	-	271	198	286	314	193	305
	2	62	-	2	2	-	100	88	143	133	62	114
(2)	2	86	2	2	5	2	76	52	90	93	10	19
(7)	-	133	7	2	-	-	93	14	14	45	-	-
(10)	-	225	67	31	-	-	136	36	76	45	-	-
(15)	76	324	281	433	5	-	105	38	112	60	-	-
(24)	671	3392	640	3861	-	-	136	29	26	33	-	-
(31)	240	2303	566	5498	-	-	69	26	57	40	-	-
(42)	105	1352	847	12916	-	-	50	-	14	62	-	-
(63)	81	607	2235	6341	-	-	14	2	-	17	-	-
(84)	17	495	1297	2073	-	-	-	-	-	-	-	-
(114)	64	438	2292	471	7	-	-	-	-	-	-	-

B. Table 7 from deNoyelles and Kettle (1980)

		Mallomonas						Mallomonas spp.					
date	(days)	P_4	P_6	P_1	P_5	P_2	P_3	P_4	P_6	P_1	P_5	P_2	P_3
7-20		2	-	2	2	7	5	-	2	-	-	2	-
7-23		21	7	60	26	29	2	7	7	31	7	2	5
7-26	(2)	21	5	105	24	17	-	5	-	14	-	-	-
7-31	(7)	24	10	1449	88	-	-	-	-	405	12	-	-
8- 3	(10)	40	5	2	1019	-	-	7	33	224	167	12	-
8- 8	(15)	64	2	-	2372	-	7	55	55	657	39	155	31
8-17	(24)	10	5	-	-	2	2421	45	5	62	21	990	3156
8-24	(31)	-	-	2	-	39	1147	5	-	5	-	951	2754
9- 4	(42)	-	-	-	2	245	129	-	-	-	-	294	-
9-25	(63)	2	-	-	-	-	-	12	-	-	62	-	2
10-16	(84)	-	-	-	-	-	-	157	-	-	39	10	29
11-15	(114)	-	-	-	-	-	-	67	-	-	2	2	12

C. Table 8 from deNoyelles and Kettle (1980)

		Cryptomonas						Synedra					
Date	(days)	P_4	P_6	P_1	P_5	P_2	P_3	P_4	P_6	P_1	P_5	P_2	P_3
7-20		114	21	86	60	38	17	426	136	314	350	364	366
7-23		105	12	83	154	48	38	171	48	129	202	231	217
7-26	(2)	50	12	50	88	10	-	86	63	95	160	131	119
7-31	(7)	62	10	302	119	-	-	131	214	36	114	40	33
8- 3	(10)	112	12	516	288	2	-	21	252	150	157	26	33
8- 8	(15)	69	17	452	107	426	2	24	214	793	250	52	10
8-17	(24)	57	167	36	79	198	66	152	378	171	57	143	21
8-24	(31)	36	112	121	62	445	208	93	140	150	55	33	44
9- 4	(42)	40	38	447	224	419	583	64	98	202	39	5	133
9-25	(63)	74	50	129	217	312	163	5	67	12	262	74	-
10-16	(84)	250	76	69	271	240	95	5	110	5	148	81	5
11-15	(114)	317	12	93	226	754	24	881	48	10	1285	60	-

Figure 3.3. Distribution of phytoplankton taxa. Values in all panels are in cells/mL with blanks (-) below the detection limits of 2-10 cells/mL. "P" stands for pond, with P_4 and P_6 representing the control ponds, P_1 and P_5 representing the 20 µg/L ponds, and P_2 and P_3 representing the 500 µg/L ponds.

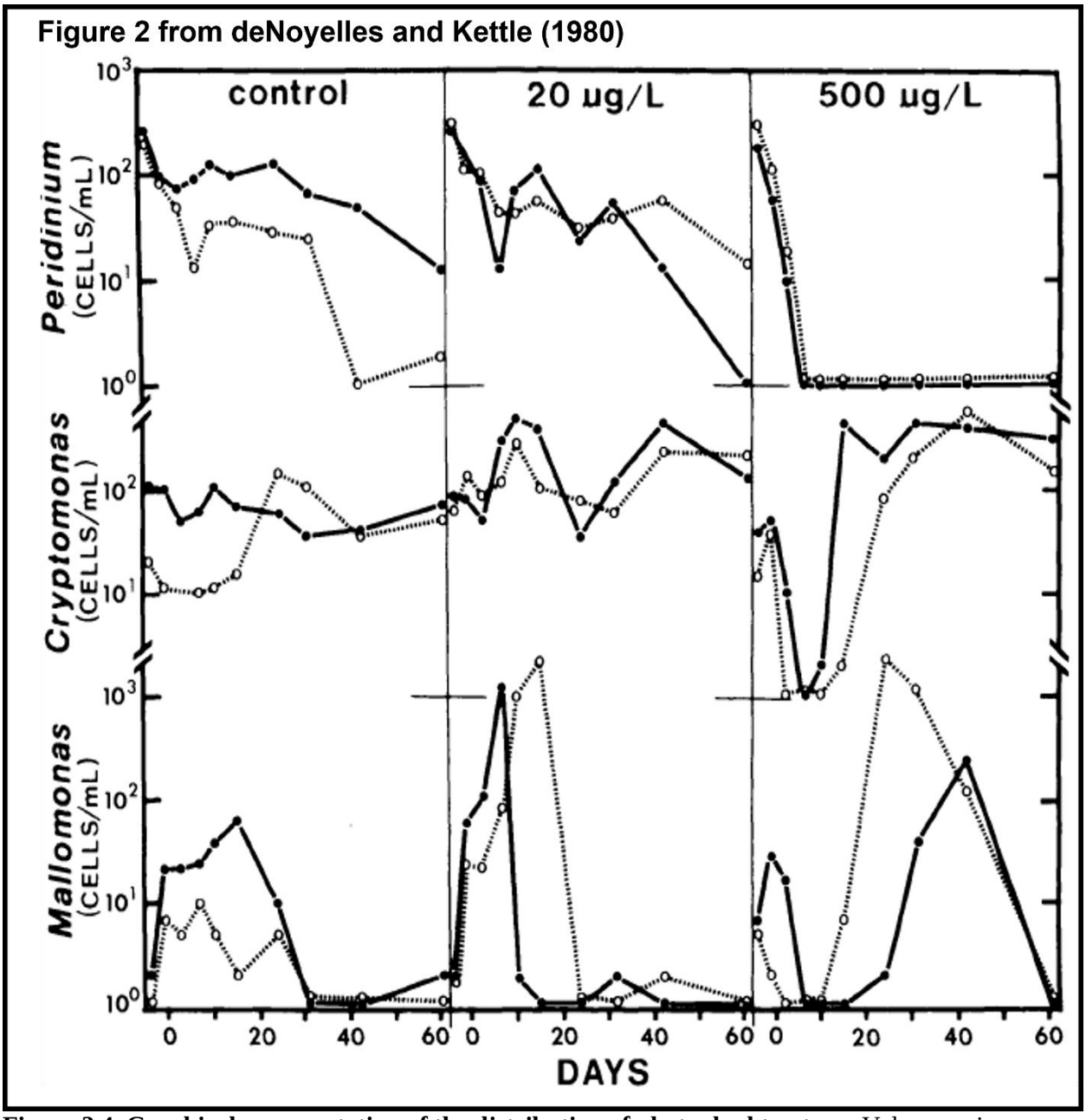


Figure 3.4. **Graphical representation of the distribution of phytoplankton taxa**. Values are in cells/mL and the detection limit was 2-10 cells/mL. The two lines in each panel represent the two replicate ponds in each treatment, hence there are no verticals bars representing variance.

3.5.4 EPA's 2023 Conclusions

While the studies have deficiencies, EPA has decided that the studies are sufficient to contribute to our knowledge about the effects of atrazine to aquatic plant communities under the conditions of the experiment. These studies contribute to our understanding of potential effects on natural aquatic plants communities exposed to atrazine when considered within the context of the larger collective body of experimental data from other cosm studies. Therefore, while there is

uncertainty in the results, it is reasonable to include these studies in the cosm database. **Based on the changes discussed above, EPA's database will retain the two original endpoints associated with the 1979 University of Kansas experiment with one scored as "No Effect" (*i.e.*, endpoint #52) and one as "Effect" (*i.e.*, endpoint #3; Table 3.5**).

Table 3.5. A summary of the cosm endpoints associated with this group that remain after the 2023 reevaluation. These endpoints will be used to evaluate the potential effects of atrazine on aquatic plant communities.

References	Endpoint Number	Duration (days)[B]	Nominal Conc. (µg/L)	Plant Group	Results and Recovery	Effect/No-Effect Conclusion
deNoyelles and Kettle (1980)[A]; deNoyelles *et al*. (1982); deNoyelles *et al*. (1989); Kettle (1982)[A]; Kettle *et al*. (1987)	52	92	20	Phyto Macro	No decrease, but at times increases, in phytoplankton biomass via chlorophyll *a*. Decrease in phytoplankton biomass via particle counts and ^{14}C-uptake early on with considerable overlap with the control. Increases in phytoplankton biomass via particle counts and ^{14}C-uptake near the end. Planted *Najas* visually reduced but no presentation of the results.	No effect
deNoyelles and Kettle (1980)[A]; deNoyelles *et al*. (1982); deNoyelles *et al*. (1989); Kettle (1982)[A]; Kettle *et al*. (1987)	3	92	500	Phyto Macro	Decreases (up to 90%) in phytoplankton ^{14}C-uptake and biomass via chlorophyll *a* and particle counts early on with recovery occuring >3 weeks. Lasting changes on phytoplankton species composition. Planted *Najas* visually reduced but no presentation of the results.	Effect

[A] Publications that were not part of the original 11 identified by the 2012 SAP but are associated with them.
[B] From 136 day study; according to study authors, ponds were intensely monitored for first 63 days, then sampled monthly thereafter until day 136; however, exposure concentration information is not available past 92 days. The duration was previously 63, which is incorrect because it was based on the last abiotic sample, not the last atrazine concentration sample.

CHAPTER 4. UNIVERSITY OF KANSAS – 1981-1983 EXPERIMENT

4.1 Overview

As mentioned in **Chapter 3**, experiments were completed in 1979 and 1981-1983 at the University of Kansas (see **Section 3.1** for more details). The 2012 SAP had concerns with the following five studies associated with the University of Kansas experiments: Carney (1983), deNoyelles *et al.* (1982), deNoyelles *et al*. (1989), Dewey (1986), and Kettle *et al*. (1987) (USEPA, 2012b).

The 1981-1983 experiment is discussed in Carney (1983), Dewey (1986), and deNoyelles *et al.* (1989), as well as deNoyelles and Kettle (1983), deNoyelles *et al*. (1994), Huggins (1990), Huggins *et al.* (1994), and Larsen *et al.* (1986), which will also be included here. These publications include theses, journal articles, a report, and book chapters. There may be more publications associated with the 1981-1983 experiment but EPA believes that those listed above contain the primary information regarding the conduct and results of these studies as they relate to the purpose of the cosm database. Below, the studies are discussed collectively with separation only when relevant (*e.g*., specific methods, results, concerns).

4.2 Experimental Design and Execution

In the spring or summer of 1981, experimental ponds (each 0.045 ha) were drained and filled to a depth of 2.1 m (470,000 L) from a reservoir maintained by well water. This reservoir was the source of the starting phytoplankton community. Since the late 1970s, the experimental ponds had developed similar but sparse macrophyte communities. Each pond also received ten Gizzard Shad (*Dorosoma cepedianum*; length: 30-40 cm), 50 Bluegill Sunfish (*Lepomis macrochirus;* length: 3-7 cm), and 20 Channel Catfish (*Ictalurus punctatus*; length: 10-15 cm). In May 1982, four Grass Carp (*Ctenopharyngodon idella*; length 18-23 cm) were also added. In each pond, the Grass Carp were excluded from a portion so that macrophytes in the absence of herbivore pressure could be assessed (~5 or 10% of the total pond area – different reports). These four fish species remained in the ponds until the end of the experiment in 1983. There is no other information about the addition of other species, but several species of macroinvertebrates and

amphibians were present and likely from natural colonization. The density of these organisms was not mentioned.

In 1981 after an unknown amount of time, atrazine treatments were applied and included: 0 (control; 2 replicates), 20 (2 replicates; “reagent grade”, RG), 20 (2; “commercial grade”, CG), 100 (2; RG), 100 (1; CG), and 200 µg/L (1; RG+CG) (**Figure 4.1**). The “commercial grade” atrazine was liquid with 41% active ingredient (two registration numbers were mentioned: #1990-318 and #1990-381 – latter likely correct). The reagent grade was 97% active ingredient. The publications do not indicate how these treatments were applied.

In 1982, pond water levels were brought back to 2.1 m and the treatments were redosed, except that the two 20 µg/L (CG) were changed to 500 µg/L (RG) and the 100 µg/L (CG) and 200 µg/L (RG+CG) were changed to 200 µg/L (RG) (**Figure 4.1**). Therefore, all treatments in 1982 were replicated twice, dosed with reagent grade, and included: 0 (control), 20, 100, 200, and 500 µg/L. The authors say that this was done because there were no differences between the reagent and commercial grade results. They do not present this analysis. The experiment then continued with those treatments until October 1983 with another dosing occuring in 1983 (**Figure 4.1**).

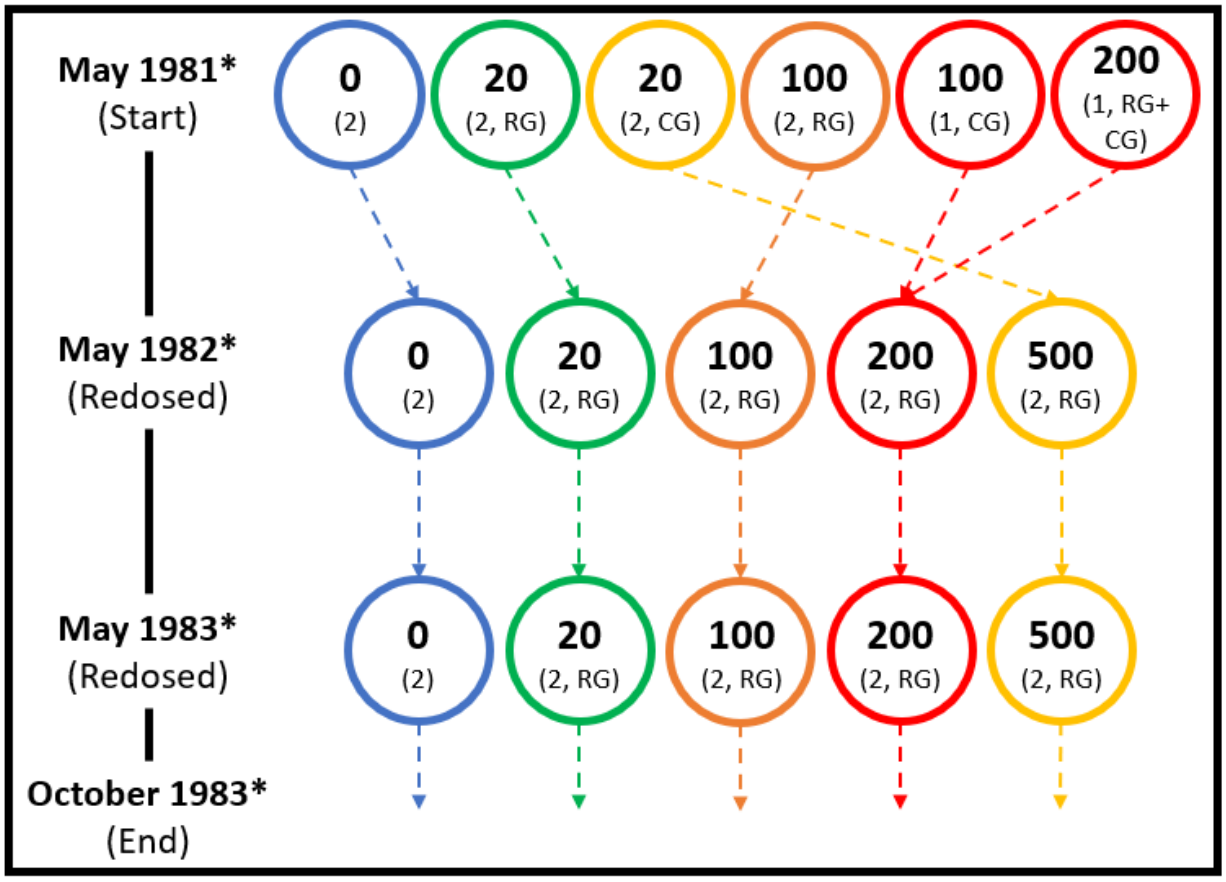


Figure 4.1. The experimental design of the 1981-1983 experiment. Within the circles, the top number is the concentration of the treatment in µg/L and the number below in parentheses is the number of replicates. "RG" stands for reagent grade and "CG" stands for commercial grade. **A few studies mention May 1981 as the start, but others mention June 1981. Some studies are silent about the timing of all the dosing. A few mention that the experiment lasted for 805 days until October 1983; however, that is not mathematically possible.*

Abiotic conditions, including temperature, total dissolved solids (TDS), pH, turbidity, total alkalinity, and atrazine concentrations were monitored over the 1982 season via integrated column samples. There is no mention of abiotic monitoring occuring in 1981, including atrazine concentrations (but see **Figure 4.2**). deNoyelles *et al*. (1989) states that "measured concentrations of atrazine differed by <10% of the nominal concentrations targeted. After 6 and 12 mo, measured concentrations declined to approximately 70 and 25% of the original measured concentrations, respectively" (see **Figure 4.2** for more details). deNoyelles *et al*. (1989) also says that most measurements (including the biotic measurements mentioned below) were taken weekly from May through September, including one month before atrazine was added, and then once or twice monthly thereafter through the following spring.

Biotic monitoring occurred over the same period and included: Phytoplankton, macrophytes, zooplankton, benthic invertebrates, fish, and possibly amphibians. The review here only discusses the results for primary producers because those are the groups relevant to the CE-LOC. For phytoplankton, Carney (1983) only states that planktonic chlorophyll *a* was measured over the 1982 seasons. The deNoyelles and Kettle (1983) publication includes a section in the methods about assessing phytoplankton resistance using fluorescence and carbon uptake; however, both involved dosing with atrazine to elicit a response, as opposed to the naïve measurement of fluorescence and carbon uptake and thus were not evaluated further. The deNoyelles *et al.* (1989) paper is vague in spots because it presents on both University of Kansas experiments, but it implies that the fluorescence and carbon uptake were assessed both with and without additional atrazine additions. In deNoyelles *et al.* (1989), the results are limited to only a few treatments and it is often hard to separate the two experiments.

For macrophytes, a rake was used to collect multiple samples per pond in September 1981 and June-September of 1982 and 1983. Each sample was rated visually from 0 to 4, with 0 being an empty rake and 4 being at maximum capacity. After visually assessing the samples, the individual species were separated. From 26 July to 31 August 1982, an *in situ* experiment using the macrophytes *Najas guadalupensis* and *Chara globularis* was completed by adding two baskets of plants to one tank from the 0, 20, 100, and 500 µg/L treatments. Periodically, the relative growth (*i.e.*, number of nodes/segment) of the plant segments was assessed. In September 1982 (and possibly August 1982/1983 and September 1983), two dominant emergent macrophytes (*Typha latifolia* and *Typha angustifolia*) were assessed by counting the total number of green and brown leaves within quadrats. The deNoyelles *et al*. (1989) publication also mentions that aerial photographs were used to assess emergent macrophytes.

The deNoyelles *et al*. (1989) publication indicates that the data were analyzed using analysis of variance (ANOVA). When there were multiple measurements over time, a repeated measures ANOVA was used. As a reminder, though, treatments were only replicated once or twice, which limits the ability to discriminate effects using ANOVAs.

4.3 EPA's Use as of 2016

In the 2016 Refined Ecological Risk Assessment (USEPA, 2016), EPA included six endpoints from the University of Kansas 1981-1983 experiment (**Table 4.1**). Endpoints #2, 4, 41, and 42 are for the first year of the study, whereas endpoints #5 and 1 are for the second year of the study. All were scored as "Effect". Note that the cosm database associated with the 2016 Refined Ecological Risk Assessment did not associate deNoyelles and Kettle (1983), Huggins (1994), Huggins *et al.* (1994), or Larsen *et al*. (1986) mentioned in **Section 4.1** with the 1981-1983 experiment endpoints, despite them being associated with the 1981-1983 experiment. As a result, they are missing from the reference column in the table. Other citations listed in the 2016 cosm database are incorrectly associated with the 1981-1983 experiment and are actually associated with the 1979 experiment.

Table 4.1. A summary of the cosm endpoints associated with this group that appeared in Appendix G.2 of the 2016 Refined Ecological Risk Assessment. These endpoints were used to evaluate the potential effects of atrazine on aquatic plant communities.

References	Endpoint Number	Duration (days)	Nominal Conc. (µg/L)	Plant Group	Results and Recovery	Effect/No-Effect Conclusion
Carney (1983); deNoyelles and Kettle (1980)[A]; deNoyelles *et al*. (1989); deNoyelles *et al*. (1994)[A]; Dewey (1986); Kettle *et al.* (1987)	2	365[B]	20	Macro	Decrease in cover by floating and submerged aquatic plants (40% decline in Typha; 50% decline in SAV; 50% decline in Najas). Recovery: > 1 yr (no recovery observed)	Effect
Carney (1983); deNoyelles *et al*. (1989)	4	365[C]	100	Macro	Decrease in cover by emerged and submerged aquatic plants (75% decline in Typha; 80% decline in SAV). Recovery: > 1 yr (no recovery observed)	Effect
deNoyelles *et al*. (1989)	41	360[D]	100	Phyto	Decrease (>90% decline) in ^{14}C-uptake and biomass of phytoplankton. Recovery: > 21 d	Effect
deNoyelles *et al*. (1989)	42	360[E]	200	Phyto	Decrease (>90% decline) in 14C-uptake and biomass of phytoplankton. Recovery: > 21 d	Effect
Carney (1983);	5	340[F]	200	Macro	75-80% decline in Typha; $\geq$90% decline in SAV[H].	Effect

References	Endpoint Number	Duration (days)	Nominal Conc. (µg/L)	Plant Group	Results and Recovery	Effect/No-Effect Conclusion
deNoyelles *et al.* (1989)					Recovery: > 1 yr (no recovery observed)	
Carney, (1983); deNoyelles *et al*. (1989); deNoyelles *et al*. (1994)[A]; Kettle *et al*. (1987)	1	365[G]	500	Macro	Decrease in cover by emerged, floating, and submerged aquatic plants (>90% decline in Typha; 100% decline in SAV). Recovery: > 1 yr (no recovery observed)[I]	Effect

[A] Publications that were not part of the original 11 identified by the 2012 SAP but are associated with them.
[B] From 805-d study; study concentrations from first year of study.
[C] From 805-d study; study concentrations from first year of study where ponds were dosed with atrazine at 100 µg/L.
[D] Effect observed immediately (within first 3 wks) in both 136-d and 805-d studies during the first year. Recovery observed ≥ 3 weeks.
[E] Effect observed immediately (within first 3 wks) in both 136-d and 805-d studies during the first year. Recovery observed ≥ 3 weeks.
[F] From 805-d study; study concentrations from second year of study.
[G] From 805-d study where ponds formerly treated with formulated product at 20 µg/L were increased to 500 µg/L; exposure concentrations derived from graph during the second year of the study.
[H] Submerged aquatic vegetation
[I] Results were missing so copied from Appendix D
*Note: The durations here are incorrect. Corrections have been made in **Table 4.4**.

4.4 Stakeholders' Major Concerns and Criticisms

The publications associated with the 1981-1983 University of Kansas experiment have been in the cosm database since the 2003 Atrazine Interim Registration Eligibility Decision (IRED) (USEPA, 2003). For the 2007 SAP Meeting, the University of Kansas endpoints were included in an appendix of the White Paper that contained the database (USEPA, 2007) but otherwise, there was no discussion about the 1981-1983 University of Kansas experiment in the White Paper, by public commenters, or by the SAP (USEPA, 2008). For the 2009 SAP Meeting, the SAP suggested that endpoint #2 (20 µg/L) be reevaluated (USEPA, 2009b). For the 2012 SAP Meeting, Syngenta suggested during the public comment period the rescoring of endpoint #2 to "No Effect" (Syngenta, 2012a). The SAP echoed the same suggestion about rescoring endpoint #2 and also indicated concerns with the presence of fish in the studies (USEPA, 2012b). The effect/no-effect conclusion concerns were echoed in comments on the 2016 Refined Ecological Risk Assessment and again in comments on the 2022 Proposed Revisions to the Atrazine Interim Registration Review Decision. In addition, concerns about having multiple endpoints per

concentration were also raised by Syngenta in 2016 and 2022 (Syngenta, 2016, 2022). Details of the specific concerns brought about during each event mentioned above are discussed below.

4.4.1 The 2009 SAP Meeting

In 2009, the SAP commented on the effect/no-effect conclusion of endpoint #2 and said the following (USEPA, 2009b):

> *Cosm study #2, deNoyelles et al. (1989), Dewey (1986), and Kettle et al. (1987). Multiple studies were cited in regards to the effects of 20 µg/L atrazine and were all given a Brock score of 5 [i.e., study produced clear effects without recovery for 56 days or more]. This panel member stated that these studies should be closely evaluated for several reasons. These studies were conducted in the same pond over multiple years. Each study must be evaluated on its own merits to determine if it belongs in EPA's dataset. The studies authored by Dewey (1986) and Kettle et al. (1987) reports effects on insect emergence and bluegill reproduction. The data is not presented with credible measures of critical plant variables such as algal and plant biomass. In addition, it appears that these two studies were conducted in ponds that contained grass carp that were stocked at approximately 30 fish per hectare. This is an extremely high biomass and likely led to severe fish grazing impacts on aquatic plants, which will lead to indirect impacts on insect emergence and predation on young bluegills by adult bluegills and young channel catfish; these effects are the result of grass carp in the 20 µg/L ponds and not by atrazine as documented in the review publication of deNoyelles et al. (1989). The studies reported by deNoyelles et al. (1989) were conducted at atrazine concentrations of 100 µg/L (cosm study #41) and 200 µg/L (cosm study #42) and given a Brock Score of 3. This paper is a synthesis of a series of studies conducted from 1978 to 1982. Embedded within these studies were some assessments based on 1-h and 4-h uptake of C^{14} and not actual assessments of changes in overall algal and macrophyte biomass. These short-term bioassays must be interpreted in relation to observations made in the mesocosm in respect to overall phytoplankton biomass.*

4.4.2 The 2012 SAP Meeting

During the public comment period, Syngenta suggested that endpoint #2 should be scored as "No

Effect" (Syngenta, 2012a). The justification they provided in their comment largely focused on Kettle *et al.* (1987), which did not discuss the 1981-1983 experiment. This is likely because EPA incorrectly associated this publication with endpoint #2 and this, in combination with the "Plant Group" column, created confusion surrounding the designation and splitting of endpoints. After the discussion of Kettle *et al.* (1987), Syngenta stated:

> *Many of the difficulties in interpretation of these studies were noted by the 2009 SAP (EPA 2009a). In particular, the SAP noted that evidence that macrophytes (ID #2) and phytoplankton ID #52) were affected at 20 µg/L is weak. The SAP stated, "the studies authored by Dewey (1986) and Kettle et al. (1987) report effects on insect emergence and bluegill reproduction. The data is not presented with credible measures of critical plant variables such as algal and plant biomass… These two studies were conducted in ponds that contained grass carp that were stocked at approximately 30 fish per hectare. This is an extremely high biomass and likely led to severe fish grazing impacts on aquatic plants…these effects are the result of grass carp in the 20 µg/L ponds and not by atrazine as documented in the review publication of DeNoyelles et al. (1989)."*
>
> *In light of the scant documentation of the inferred effects in the 1979 study and the absence of effects on macrophytes at 20 µg/L in the 1981 and 1982 studies, CSI assigned a binary effect score of "0" to ID #2. Whether or not the score of 1 is retained for the 1979 study, it would be appropriate to add two additional data points for 1981 and 1982, each with a score of 0 for effects on macrophytes.*

Syngenta concluded that "The cosm studies at the University of Kansas at Lawrence were the most ambitious ever conducted with atrazine, in terms of the size and complexity of the experimental ecosystems" and ended up scoring all endpoints as "Effect" except for endpoint #2.

As detailed in **Section 4.1**, the 2012 SAP commented on three studies associated with the 1981-1983 experiment: Carney (1983), Dewey (1986), and deNoyelles *et al.* (1989;). The SAP comments are below (USEPA, 2012b):

Carney (1983) – Authors cited exclosure experiments in control ponds. Grass carp were stocked at 20/acre, which is an order of magnitude greater than common guidance (2/acre). Macrophytes were totally denuded in control pond. Note: These two ponds may not have been the ponds examined by deNoyelles et al. (1982) or Dewey et al. (1985); however, this is the exact magnitude of the direct effect on macrophytes that one would expect at this extreme stocking level.

Dewey (1986) – This study should be excluded because it does not meet the "must not have other stressors present" criterion listed in Appendix D, p. 5. Grass carp, gizzard shad, channel catfish, and bluegill were present with no data on percent survival (especially differential survival of grass carp; however, Kettle et al. (1987) mentioned 80% survival of adult bluegill). Grass carp were stocked at 20/acre, which is an order of magnitude greater that common guidance (2/acre). Author indicated that decreased insect emergence was an indirect effect and not primary effect of atrazine. Macrophyte biomass was decreased by 90 and >95% in the 20 and 500 µg/L treatments, respectively, which makes no ecotoxicological sense based on the large amount of data for atrazine. Visual observations indicated that periphyton was affected at 100 µg/L but no mention of effects at 20 µg/L. Note: there was zero grass carp survival in the controls as indicated by DeNoyelles et al. 1989, which resulted in high macrophyte biomass and associated insect emergence rates.

deNoyelles et al. (1989) – Ponds were exposed to 20, 100, 200, and 500 µg/L of atrazine. The results indicate that effects occurred at an atrazine concentration of 20 µg/L. However, the paper explicitly states (Fig. 4) that there were no lasting effects on phytoplankton biomass up to 500 µg/L. Species shifts occurred, but they were replaced by tolerant species. Effects were noted in the laboratory in C-14 uptake experiments, but these are short-time bioassays that do not reflect what happens in the mesocosm itself. Four species of fish were present (bluegill, channel catfish, gizzard shad, and grass carp). Biomass of all species was increased in the presence of atrazine with two exceptions: one control pond that had zero gizzard shad survival and another control pond that had zero grass carp survival. Fish data and macrophyte data presented in Fig. 7 clearly show the effects of grass carp on macrophyte biomass. Macrophyte biomass in

> *the control ponds was high because grass carp were absent. This observation, not mentioned in Dewey et al. 1986 and Kettle et al. 1987, invalidates these studies as well, as increased insect emergence and bluegill survival were observed in the controls due to decreased predation of young of the year bluegill. In the treated ponds, there was high grass carp survival, macrophyte biomass was decreased due to fish grazing, and emergent insects and larval bluegill were higher due to refugia from predation by bluegills and channel catfish.*

The panel also noted that these publications represent "studies discussed by the 2009 SAP as having flawed methodology, which affected interpretation of the results" and specifically said the following about the University of Kansas experiment:

> *Studies conducted at the University of Kansas from 1979-1991. These studies were considered "ecotoxicological classics" based on the hypotheses tested, study complexity and ecological relevance. However, they were not conducted under any semblance of Good Laboratory Practices (GLPs). Note: The University of Kansas study is accounted for five times in EPA's analysis, which may bias the data in Fig. 16 of the White Paper for atrazine effects at 20 µg/L.*

Note: Studies were not required to be conducted under GLP but rather were required to meet EPA's minimum screening criteria developed after the 2009 SAP.

4.4.3 The 2016 Refined Ecological Risk Assessment

In the Addendum to the Problem Formulation (USEPA, 2013), EPA addressed the 2012 SAP's recommendation to limit the cosm database to realistic concentrations and durations. EPA selected a duration of 240 days for the maximum length of the study and EPA said:

> *The time restriction impacts endpoints 1, 2, 4, 5, 41, and 42 (Appendix A-1). These endpoints all originate from a series of multi-year experiments conducted at the University of Kansas from 1979-1991 (summarized in deNoyelles et al. 1982 and 1989). The effects noted in these studies were initially reported within the first few days to weeks following atrazine introduction into the mesocosms. However, as these effects were*

occurring throughout the study, these endpoints were not removed from the cosm endpoint database.

In the 2016 Refined Ecological Risk Assessment (USEPA, 2016), EPA reiterated the new duration limit's lack of impact on the University of Kansas endpoints and then reevaluated the group, which can be found in **Chapter 3, Section 3.4.3**. Ultimately, EPA made no changes to the endpoints in the cosm database (**Table 4.1**).

In the public comments received after the publication of the 2016 Refined Ecological Risk Assessment, Syngenta reiterated much of what they said in their comments from the 2012 SAP Meeting, including the same suggested changes (Syngenta, 2016). Syngenta also commented on the splitting of endpoints and provided an extensive review of the University of Kansas experiments (Syngenta, 2016) (see **Chapter 3, Section 3.4.3**).

Another substantive comment came from the Triazine Network (2016), who gave summaries for Carney and deNoyelles, 1986, Dewey, 1986 and deNoyelles *et al*. (1989), which included EPA's data evaluation records (DERs), the 2009 and 2012 SAP meeting, Syngenta (2012a), Triazine Network (2015), Moore *et al*. (2016), and finally EPAs 2016 Refined Ecological Risk Assessment (USEPA, 2016). There were no topics or concerns raised beyond those discussed above that were specific to the University of Kansas 1981-1983 experiment.

4.4.4 Post-Risk Assessment to Present

After issuance of the proposed revisions to the Interim Decision in 2022 (USEPA, 2022b, 2022c), EPA again received several comments about the cosm database. In Syngenta's comment, similar concerns/suggestions were provided regarding the effect/no-effect conclusions and they elaborated on the splitting of endpoints further (Syngenta, 2022), which can be found in **Chapter 3, Section 3.4.4**.

The score given by EPA in the 2016 Refined Ecological Risk Assessment, by Syngenta in their comment on the 2016 risk assessment, and by Syngenta in their 2022 comment on the proposed revisions to the Interim Decision were summarized by Syngenta in the table titled "Table SI-1(mod)" (**Table 4.2).**

Table 4.2. Data from Syngenta's Table SI-1 (mod) table. For brevity, some columns that are not needed for this evaluation have been removed (*e.g.*, Test System description). For the binary scores, 0 equals "No Effect" and 1 equals "Effect".

Endpoint Number	Reference	Conc (ppb)	Plant Community Response	Binary Scores (EPA 2016)	Binary Scores (Syngenta 2016)	Binary Score (Syngenta 2022)
2	deNoyelles *et al.* 1989, 1994; deNoyelles and Kettle 1980; Carney 1983; Kettle *et al.* 1987, Dewey 1986	20	The evidence that macrophytes were affected at 20 µg/L is weak and contradictory (see text)	1	0	0 (1981)
4	deNoyelles *et al.* 1989; Carney 1983	100	Abundance reduced, vascular species replaced by Chara.	1	1	1 (1981)
41	deNoyelles *et al.* 1989	100	Biomass and photosynthesis reduced	1	1	X (1981)[A]
5	deNoyelles *et al.* 1989; Carney 1983	200	Abundance reduced, vascular species replaced by Chara.	1	1	1 (1981)
42	deNoyelles *et al.* 1989	200	Biomass and photosynthesis reduced	1	1	X (1981)[A]
1	deNoyelles *et al.* 1989, 1994; Carney 1983; Kettle *et al.* 1987	500	Abundance reduced, vascular species replaced by Chara.	1	1	1 (1981)

[A] These endpoints were removed from the database by Syngenta due to the thought that EPA was inconsistently splitting endpoints.

The Triazine Network also provided a comment, but none were focused solely on the 1981-1983 University of Kansas experiment (Triazine Network, 2022).

4.5 EPA's 2023 Reevaluation

After consideration of all of the studies' limitations, the determinative concerns will be discussed here, including those brought up by past SAPs and public commenters. These include the experiment duration, fish in the ponds, the splitting of endpoints (within a treatment), treatment replication, and the effect/no-effect conclusions for endpoints. Some concerns brought up in the past are not discussed here because they pertained to parts of the study that are no longer under consideration (*e.g.*, concerns about Grass Carp).

4.5.1 Experiment Duration

As detailed in **Section 4.4.3**, EPA added a duration limit after the 2012 SAP recommendation, which removed any studies that were greater than 240 days. Regarding the 1981-1983 University of Kansas Experiment, EPA stated that, "The effects noted in these studies were initially reported

within the first few days to weeks following atrazine introduction into the mesocosms. However, as these effects were occurring throughout the study, these endpoints were not removed from the cosm endpoint database." Therefore, all six endpoints from the 1981-1983 University of Kansas experiment remained in the cosm database.

During the 2023 reevaluation, EPA realized that two endpoints from year 2 (1982) of the experiment remained in the cosm database:

Endpoint #	Treatment (µg/L)	Year of the Study
2	20	1981
4	100	1981
41	100	1981
42	200	1981
5	200	1982
1	500	1982

EPA considered keeping these endpoints [and adding others from year 2 (1982) and year 3 (1983)] if the atrazine degraded over the course of the year to approximately control levels before the ponds were refilled and redosed. However, as indicated by **Figure 4.2**, the atrazine concentration remained elevated in all ponds during the duration of the 3-year study, including in the 20 µg/L treatment.

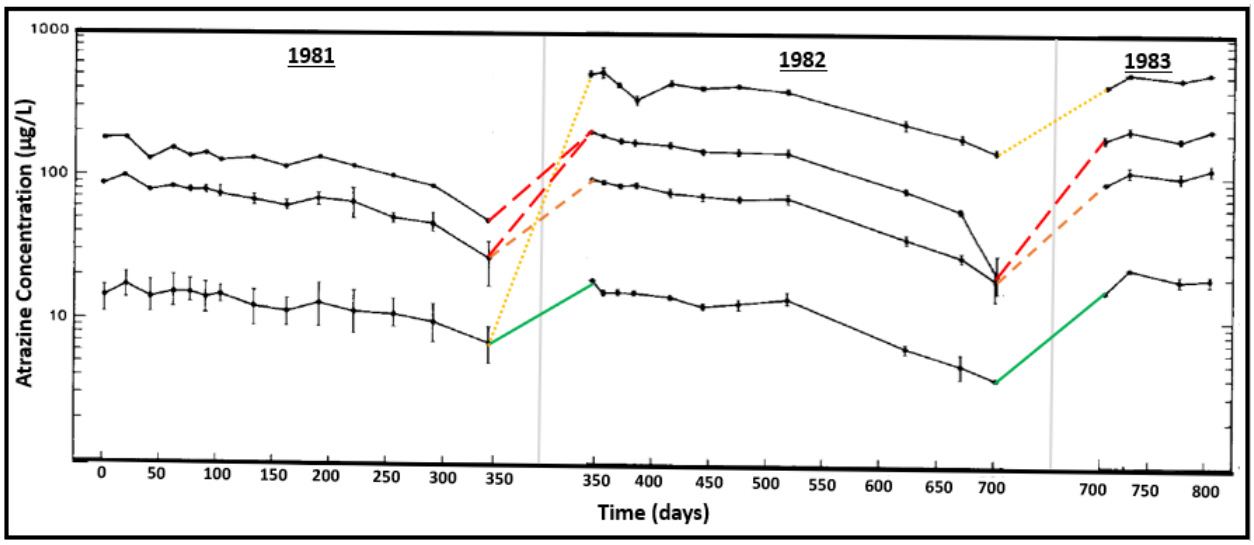


Figure 4.2. Concentrations of atrazine over time. The figure is adapted from Figure 2 in deNoyelles *et al.* (1989). Original caption reads, "Measured concentrations for the 805-d study. Values plotted as treatment means ± SE (see text for ponds per treatment during each year…. Atrazine was never detected in control ponds (detection limit of 2 µg/L)." Changes made here include splitting the graph at each year mark and adding the colored lines, which connect the treatments between years to show the ponds full concentration profile. The colors match Figure 4.1 but are: green solid line – 20 µg/L, yellow small dash line – 20 µg/L to 500 µg/L, orange medium dash line – 100 µg/L, and red large dashes – 100 and 200 µg/L to 200 µg/L. Other additions include the x and y axis labels and the years at the top. The ponds were redosed around day 350 and 700.

Based on these considerations, EPA is removing the year 2 (1982) endpoints (#5 and #1) from the cosm database because the community would have been exposed to atrazine for more than 240 days (EPA's duration cutoff). Some year 1 (1981) endpoints will remain in the database because effects occurred during the first few months of the experiment (*i.e.*, <240 days). Next, other potential impacts to year 1 endpoints are discussed.

4.5.2 Fish in Ponds

As detailed in **Section 3.5.1**, the 2012 SAP had concerns about the presence of fish in the ponds. Like the 1979 experiment, Bluegill Sunfish, Gizzard Shad, and Channel Catfish were present from 1981-1983. From 1982-1983, Grass Carp were also present and were perhaps the largest concern of the 2012 SAP. However, the removal of endpoints #5 and #1 due to the duration (see **Section 4.5.1**) eliminates the concerns surrounding Grass Carp because that portion of the experiment is no longer under consideration.

Concerns about the other three fish species could include the initial biomass, the increase in biomass over time, and differing biomass across treatments. According to the 2012 SAP and Touart (1988), fish densities should be within 2-5 g/cubic meter, or 940 to 2350 grams of fish biomass for the University of Kansas experiments. Huggins (1990) provides the mean initial starting mass of the fish for the 1981-1983 experiment (**Table 4.3**). Based on this information, the total starting biomass was approximately 602 g in the 1981, which is below the lower range calculated based on Touart (1988) and is therefore not a concern.

Table 4.3. The fish species added at the beginning of the 1981-1983 University of Kansas experiments, including the common and scientific names, total added, initial mean length, initial mean wet mass, and estimated biomass. The total added and length were mentioned in various publications, but the mean wet mass could only be found in Huggins (1990).

Species[A]	Total Fish Added	Mean Length (cm)	Mean Wet Mass (g)	Estimated Biomass (g)
Bluegill Sunfish (*Lepomis macrochirus)*	50	5.2	2.0	100
Channel Catfish (*Ictalurus punctatus)*	20	9.4	7.8	156
Gizzard Shad (*Dorosoma cepedianum)*	10	15.2	34.6	346
Total	80	-	-	602

[A] Grass Carp are not included here because only results from Year 1 (1981) are considered in the reevaluation (see **Section 4.5.1**) and Grass Carp were added in 1982.

Remaining concerns involve the change in total biomas due to the natural and/or impaired growth, reproduction, and survival of the fish. However, EPA did not come across a publication that indicated growth, reproduction, or survival results for 1981. This is likely because the measurements would not have been taken until the end of the experiment in 1983, which would not be representative of 1981. For example, the 2012 SAP noted that Gizzard Shad survival was variable in the controls, but this concern likely originated from a graph from deNoyelles *et al.* (1989; **Figure 4.3**), which shows Gizzard Shad biomass from 1983. It is important to note that the survival of stocked fish in the 1979 experiment was high and unaffected by the treatments and while that experiment was shorter, the 1981-1983 phytoplankton and macrophyte results discussed below occurred within the first few months and on a similar timeframe as the 1979 experiment.

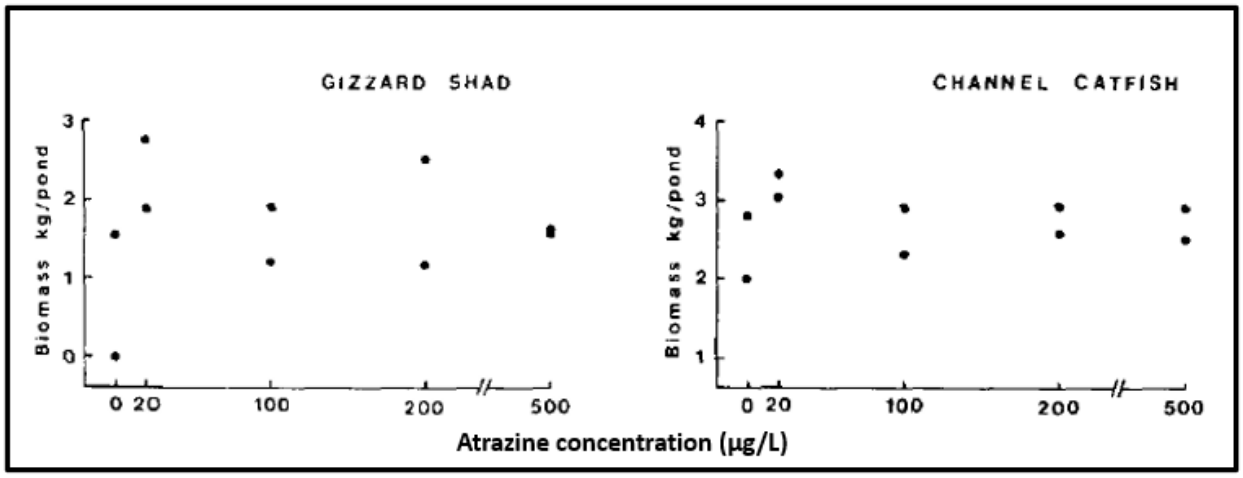


Figure 4.3. A subset of Figure 4 from deNoyelles et al. (1989). The caption reads, "Empirical examples of test results for effects of a pesticide on biological components of experimental pond ecosystems… that showed no response to pesticide additions to the experimental pond ecosystem." This data presents the final, unaffected biomass for both fish from 1983.

In 1979, there were impacts on fish growth and reproduction, but there were minimal impacts on zooplankton abundance and the effects on the aquatic plant community was opposite what one would think if fish were the cause. In 1981, there were minimal, if any, impacts on zooplankton.

Given the above information, EPA has decided to keep the University of Kansas 1981-1983 endpoints (1981 portion only) in the database. The endpoints help expand the breadth of communities represented in the cosm database and contribute to our knowledge about how atrazine affects the diverse aquatic communities found around the nation.

4.5.3 Splitting Endpoints

As explained in **Chapter 3**, another concern expressed was EPA's inconsistency of having more than one endpoint for an experimental treatment (*e.g.*, >1 endpoint for a single concentration to represent different parts of the aquatic plant community – one for phytoplankton and one for macrophytes). This is opposed to the goal of having one endpoint per concentration in an experiment to represent the whole aquatic plant community. There seems to be some confusion here due to EPA's "Plant Group" column (and potentially due to errors made by EPA).

As discussed in **Section 3.5.2**, endpoints #52 (1979) and #2 (1981-1983) and then #3 (1979) and #1 (1981-1983; removed in 2023 due to duration) are indeed separate endpoints as they represent

the 20 and 500 µg/L treatments, respectively, in the two different University of Kansas experiments.

The 1981-1983 endpoints that remain to be discussed here are #4, #5, #41, and #42. Endpoints #42 and #5 are associated with a 200 µg/L treatment, with #42 representing Year 1, and #5 representing Year 2. Therefore, they are separate endpoints but as mentioned in **Section 4.5.1**, endpoint #5 will be removed due to duration. Endpoint #42 is discussed further in **Section 4.5.4.**

The remaining endpoints, #4 and 41, are both associated with a 100 µg/L treatment and based on the comments associated with them, they are both for Year 1 of the 1981-1983 study. Endpoint #4 has a Plant Group of macrophytes, while endpoint #41 has a Plant Group of phytoplankton. This is likely one of the reasons for the confusion associated with EPA's splitting of endpoints within a treatment. This is an error in the database as there should only be one endpoint per treatment. **Therefore, endpoint #41 will be removed from the cosm database and there will only be one endpoint (#4) representing the 100 µg/L treatment.**

4.5.4 Treatment Replication

The final issue to discuss regarding the number of endpoints is treatment replication. While this issue was not brought up by past SAPs or during public comment, EPA noticed in the current evaluation that endpoint #42 was associated with the 200 µg/L treatment from Year 1 of the 1981-1983 experiment and this treatment only had a single replicate. **Therefore, endpoint #42 will be removed from the cosm database because having two or more replicates is a requirement (USEPA, 2011).**

4.5.5 Effect/No-Effect Conclusion

Based on the discussion above, two endpoints will remain in the cosm database (#2 and #4). Both Syngenta and past SAPs commented on the effect/no-effect conclusion for #2, which is for the 20 µg/L treatment, with both suggesting a "No Effect" call. For EPA's 2023 reevaluation, results across the publications mentioned in the **Section 4.1** were considered. It is important to note that some results show considerable variability.

Phytoplankton – As mentioned in **Section 4.2**, many of the phytoplankton measurements involved a second dosing of atrazine to elicit a response to measure tolerance. The deNoyelles *et al*. (1989) paper hints at there being an untreated set of samples by saying, "Atrazine tolerance for phytoplankton communities from the experimental ponds and other ponds was measured by incubating water samples containing phytoplankton for 24 h with and without a further addition of 100 µg/liter of atrazine." Based on the figure captions, the other publications only present treated samples. Figure 3 in deNoyelles *et al.* (1989) does not indicate that the samples were treated but presents results from 1979, which were not treated. However, for the 1981, only the 100 µg/L treatment (endpoint #4) was presented in Figure 3 (**Figure 4.4** here). The text states, "Phytoplankton declined in biomass and production at all concentration of atrazine during the first 3 wk of exposure… with a return to control pond levels thereafter". The study also indicates that the decline at 20 µg/L was significant ($p \leq 0.05$, Student-Newman-Keuls test), but not as severe as the complete cessation of carbon uptake at the higher concentrations.

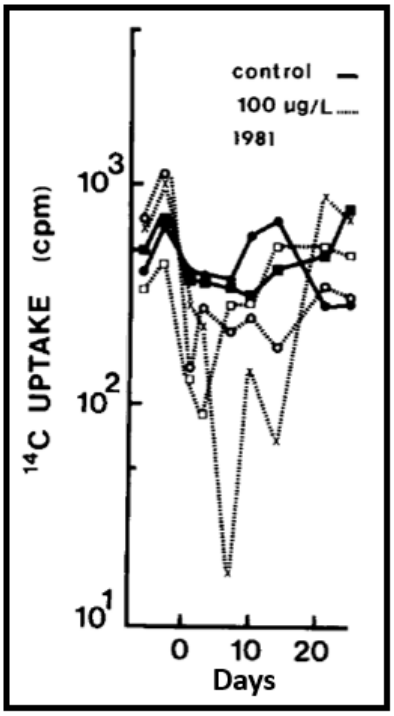


Figure 4.4. 1981 phytoplankton carbon-14 uptake results. The figure represents the 1981 portion of Figure 3 from deNoyelles *et al*. (1989). The caption reads, "Carbon-14 uptake by samples of phytoplankton communities from control (solid line) and atrazine treated (dotted line) ponds for… the first 25… d of exposure in years 1… of the 805-d study… Carbon-uptake is plotted as the mean C-14 counts per minute (cpm) from four samples from each pond." Omitted text is relevant to the 1979 and 1982 panels that are not shown here. There are three 100 µg/L replicates in the figure, which represent the two reagent grade replicates and one commercial grade replicate – deNoyelles et al. (1989) does not indicate which replicate is which.

Macrophytes – It is important to first note that the results do not distinguish between reagent grade (RG) and commercial grade (CG) treatments. This is likely because there were no differences, which was mentioned in several of the publications, and results were likely aggregated. Additionally, the only results presented from 1981 were from September. The macrophyte methods from the 1981-1983 experiment were more robust than the 1979 experiment (see **Section 4.2** for a description) and the results included graphical representation to assess the data variability.

20 µg/L treatment (endpoint #2; 2 reagent grade replicates + 2 commercial grade replicates) – By September 25, 1981, this treatment supported an extensive flora community composed mainly of vascular species, like the control (**Figure 4.5**).

100 µg/L treatment (endpoint #4; 2 reagent grade replicates + 1 commercial grade replicate) – By September 25, 1981, the total macrophyte cover was similar to the control and 20 µg/L treatments (**Figure 4.5A**), but the composition was mainly Charophytes (**Figure 4.5B**).

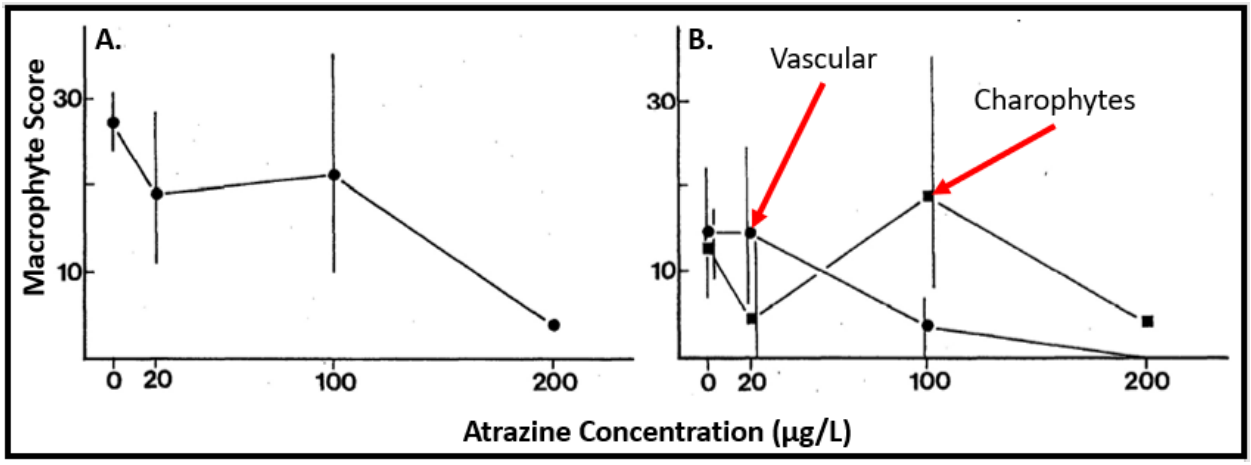


Figure 4.5. The 1981 macrophyte results. The figure is Figure 1 and 2 from Carney (1983). A. The caption reads, "Relationship between mean macrophyte score [1-4 given by authors] in replicate treated ponds and [nominal] atrazine concentrations on 25 September 1981. Vertical bars indicate range between ponds." B. The caption reads, "Mean score for types of macrophytes in replicate treated ponds vs. [nominal] atrazine concentrations on September 25, 1981. Circles represent vascular types and squares represent Charophytes. Vertical bars indicate range between ponds."

After EPA's 2023 reevaluation, endpoint #2 (1981 – 20 µg/L) has been scored as "**No Effect**" given the minimal presentation of the phytoplankton results and similarities with the control for

the macrophyte results. Endpoint #4 (1981 – 100 µg/L) has been scored as "**Effect**" given the change in carbon-14 uptake within the first month and the change in the macrophyte community composition approximately four months after dosing.

4.5.6 EPA's 2023 Conclusions

While the studies have deficiencies, EPA has decided that the studies are sufficient to contribute to our knowledge about the effects of atrazine to aquatic plant communities under the conditions of the experiment. These studies contribute to our understanding of potential effects on natural aquatic plants communities exposed to atrazine when considered within the context of the larger collective body of experimental data from other cosm studies. Therefore, while there is uncertainty in the results, it is reasonable to include these studies in the cosm database. **Based on the changes discussed above, EPA's database will have two endpoints (compared to six) for the 1981-1983 University of Kansas experiment with one "No Effect" (*i.e.*, endpoint #2) and one "Effect" endpoint (*i.e.*, endpoint #4)** (**Table 4.4**).

Table 4.4. A summary of the cosm endpoints associated with this group that remain after the 2023 reevaluation. These endpoints will be used to evaluate the potential effects of atrazine on aquatic plant communities.

References	Endpoint Number	Duration (days)	Nominal Conc. (µg/L)	Plant Group	Results and Recovery	Effect/No-Effect Conclusion
Carney (1983); deNoyelles and Kettle (1983)[A]; deNoyelles *et al*. (1989); deNoyelles *et al*. (1994)[A]; Dewey (1986); Huggins (1990)[A]; Huggins *et al*. (1994)[A]	2	350[B]	20	Phyto Macro	Potential decreases in phytoplankton ^{14}C-uptake but quantification or no presentation of the results. No effect on macrophyte abundance or composition.	No Effect
Carney (1983); deNoyelles and Kettle (1983)[A]; deNoyelles *et al*. (1989); deNoyelles *et al*. (1994)[A]; Dewey (1986); Huggins (1990)[A]; Huggins *et al*. (1994)[A]	4	350[B]	100	Phyto Macro	Decrease in phytoplankton ^{14}C-uptake with recovery occuring >15 days. No effect on macrophyte abundance after >3 months of exposure. Effects on macrophyte composition with charophytes dominating over vascular plants afer >3 months of exposure.	Effect

[A] Publications that were not part of the original 11 identified by the 2012 SAP but are associated with them.
[B] From first year of the 805-d study

CHAPTER 5. DETENBECK

5.1 Overview

Detenbeck (1996) was flagged by the 2012 SAP for removal from the cosm database (USEPA, 2012 b). The study has been included in the cosm database since 2003 (USEPA, 2003), and all of EPA's endpoints associated with the study were scored as "effects" from 2003 through 2022. The concerns raised by the 2012 SAP were considered by EPA in 2013 and 2016 (USEPA, 2013, 2016); however, the concerns regarding the inclusion of this study in the database were echoed in later public comments (*e.g.*, Syngenta, 2022). The discussion below provides description of the study design and reported results that are relevant to the raised concerns from the SAP and public and concludes with EPA's 2023 reevaluation.

5.2 Experimental Design and Execution

Detenbeck (1996) reports the results of an outdoor wetland mesocosm experiment that was conducted at EPA's Monticello Ecological Research Station (MERS) in Monticello, Minnesota, USA. The mesocosms consisted of four alternating pools (30 m × 2 m × 90 cm) and riffles (30 m × 2 m × 15 cm) with an approximate flow of 76 L/min and a total length of 230 m. The riffle sections had reported accumulated organic debris over a gravel substrate early in the study implementation. Several species of macrophytes were present via planting (P) and natural succession (N). Emergent plants consisted of *Typha* spp. (cattail; N), *T. latifolia* (cattail; P), *Phalaris arundinacea (*reed canary grass; N), *Zizania aquatica* (wild rice; P; 4th pools only). Submerged vegetation consisted of *Elodea canadensis* (N), *Potamogeton* spp (N), *Ceratophyllum demersum* (coontail; N). Floating vegetation consisted of *Lemna minor* (duckweed; N). Invertebrates and vertebrates (tadpoles and minnows; grazers) of unspecified origin and identity were also present.

Two mesocosms served as controls and two were treated with atrazine. The atrazine was commercial grade (85% active ingredient) and was dissolved in well water to make a stock solution. The treatments were given as a stepped exposure regime (*i.e.*, test cosms were given progressively increased concentrations over time): 15 µg/L (May 18–June 1; 15 days), 25 µg/L (June 2–July 15; 44 days), 50 µg/L (July 16–August 17; 33 days), and 75 µg/L (August 18–

September 4; 18 days), all reported as nominal concentration. Replicate water samples of the inlet and outlet of each cosm were taken weekly to measure atrazine.

To measure periphyton productivity, periphyton strips were placed into the cosms and allowed to colonize for 9 to 27 days (exact durations were not specified, however these occurred during each of the concentration treatment level tests). After the exposure, five strips from each mesocosm were placed into control water and five were placed into atrazine-treated water (latter was to test for tolerance, this separate evaluation was not considered in EPA's endpoint conclusion). Productivity was measured using the light-bottle/dark-bottle technique, except dark incubations were performed on the same bottles after the light incubations. Dissolved oxygen was measured using a YSI and biological oxygen demand (BOD) probe. Afterward, chlorophyll *a*, net primary production (NPP), respiration (R), gross primary production (GPP; exact method unknown), specific NPP (NPP/mg Chl a), and specific GPP were measured.

5.3 EPA's Use as of 2016

EPA included the study in the cosm database with effects limited to those related to periphyton plates that were added at the start of each study stage (**Table 5.1**). EPA considered other reported effects in the study unreliable because other endpoints were not discrete to individual exposure concentrations and durations were unclear. The 15 µg/L test (endpoint #22) started May 18th and continued to June 1st, reporting a 23% reduction in dissolved oxygen (DO) and statistically significant reduction in gross productivity relative to controls. The second stage (endpoint #23) increased atrazine concentrations to 25 µg/L from June 2nd through July 15th and resulted in statistically significant reductions in respiration and net primary productivity relative to controls. The third stage (endpoint # 24) tested 50 µg/L from July 16th through August 17th and resulted in a statistically significant reduction in net primary production. The final stage (endpoint #23) of the study tested 75 µg/L from August 18th through September 4th and resulted in statistically significant reductions in net primary productivity.

Table 5.1. A summary of the cosm endpoints associated with this group that appeared in Appendix G.2 of the 2016 Refined Ecological Risk Assessment. These endpoints were used to evaluate the potential effects of atrazine on aquatic plant communities.

References	Endpoint Number	Duration (days)[A]	Nominal Conc. (µg/L)	Plant Group	Results and Recovery	Effect/No-Effect Conclusion
Detenbeck *et al.* (1996)	22	15	15	Peri	23% decrease in dissolved oxygen (DO) and gross productivity ($p<0.05$ not reported). Increase in nutrients (15% increase in PO_4; 15% increase in ammonium). Recovery: Not reported	Effect
Detenbeck *et al.* (1996)	23	43	25	Peri	Decrease in periphyton respiration and net productivity ($p<0.05$) not reported). Recovery: Not reported	Effect
Detenbeck *et al.* (1996)	24	32	50	Peri	Decrease (reduction in net productivity ($p <0.05$)) not reported. Increase in nutrients (40% increase in PO_4; 100% increase in ammonium). Recovery: Not reported	Effect
Detenbeck *et al.* (1996)	25	17	79[B]	Peri	Decrease (reduction in net productivity ($p <0.05$)) not reported. Increase in nutrients (50% increase in PO_4; 250% increase in ammonium). Recovery: Not reported	Effect

[A] Atrazine detected in the control wetlands throughout study duration. Average concentration detected was 0.69 µg a.i./L.
[B] This is an error in the cosm database and should have been 75 µg/L.

5.4 Stakeholders' Major Concerns and Criticisms

5.4.1 The 2012 SAP Meeting

While this study had been a part of the cosm database since 2003, comments provided by the SAP and the public up to 2012 did not specifically raise concerns with the study. However, in 2012, the SAP provided a summary of several concerns it had with the study (USEPA, 2012b):

> *This study design involved steadily increasing doses of atrazine at 2-week intervals from 15 to 25 to 50 to 75 µg/L using two controls and two treatments. The authors concluded that there were effects of atrazine on gross primary production at the 15 µg/L level; however, no*

data were presented for the initial 2-week 15 µg/L exposure other than two reported dissolved oxygen concentrations. Gross photosynthesis is not reliable in this system due to the accumulation of large amounts of sediment and detritus that would result in high respiration rates. Hence the stressor in the early part of the study was not atrazine, but most likely accumulated decaying organic matter unrelated to the dosing. Neither chlorophyll a nor ash-free dry weight of periphyton was affected at any concentration. Elodea was not affected at concentrations up to 75 µg/L, but Ceratophyllum showed effects only at > 75 µg/L. The Panel recommended that this study be excluded from consideration.

This statement largely reflected public comments by Syngenta (2012a) which provided a review and cited three main reasons for recommending removal from the database:

- *"The step-wise exposure regimes prevent clear interpretation of exposure-response relationships;*
- *Effects at low exposure concentrations were not observed at higher exposures;*
- *The article presented only qualitative statements about effects with very little supporting data"*

Syngenta went on to say:

Gross productivity (GPP), respiration, chlorophyll, and dry weight were measured on periphyton communities colonizing artificial substrates (acetate strips) in each stream. The results were stated in the text but the data were not presented; effects are summarized in Table 2. The only parameter for which a consistent dose-response relationship was observed was the GPP/chlorophyll ratio, which was reduced at 79 [75] µg/L. Chlorophyll and dry weight were unaffected at all atrazine exposure levels, and GPP was reduced only at 15 µg/L but not at the higher concentrations. Respiration was reduced at 25 µg/L, increased at 79 [75] µg/L, and was unaffected at 15 µg/L and 50 µg/L. The only quantitative results were presented in Fig. 2 of the paper, and they indicated large variation in GPP between replicates during the 50 µg/L phase of the step-wise exposure.

Syngenta concluded "*that effects on periphyton were limited to a questionable (because not dose related) effect on GPP at 15 µg/L and an effect on GPP/chlorophyll (but not on GPP or chlorophyll alone) at 79 [75] µg/L. (We discount the reported effects on periphyton respiration because of the marked inconsistency of the dose-response relationship.)*" The report also provided discussion of the measured macrophyte effects and concluded that there were questionable effects at the highest dose levels.

Similar comments were provided in later comment submissions (*e.g.*, Syngenta, 2022; Triazine Network, 2022).

5.4.2 The 2016 Refined Ecological Risk Assessment

In 2016, EPA considered the 2012 SAP comments and the public comments that had been received on the Detenbeck (1996) study (USEPA, 2016). The 2016 reevaluation focused on the endpoints identified by EPA as of concern (*i.e.*, the periphyton plates). Since these plates were reportedly added then removed in short intervals associated with the individual dosing periods, EPA concluded that the concerns identified in the comments were not impacting the interpretation of the study results. Based on this conclusion, the study remained in the cosm database and the endpoints remained classified as effects.

5.5 EPA's 2023 Reevaluation

After consideration of all of the study's limitations, the determinative concerns will be discussed here, including those brought up by past SAPs and public commenters. Because of EPA's narrow focus on the reevaluation in 2016, the review missed an important aspect of the study conduct relevant to the concerns raised by the SAP. As discussed above, the study treatments were not independent from each other because every few weeks the concentration was increased and because of the stepwise approach, each new treatment occurred on a community with a prior exposure history. EPA's 2023 revaluation concludes that the exposure durations for observed effects were not clear; most effects reported (including for those for periphyton) are reflective of full duration of the treatments; and it is not clear if on some occasions the next sequential atrazine addition may have been made to the mesocosms before measurement of the effects were made.

Since the design for evaluating periphyton relied upon the colonization of periphyton strips placed into the cosms shortly after the atrazine dose increased, the source of the colonizing periphyton was from the cosms themselves. Therefore, the periphyton were not reflective of unexposed communities and may have reflected colonization by tolerant periphyton taxa. Also, the reported effects at the lowest exposure concentration were not observed at subsequent higher exposure concentrations.

Because of the stepwise exposure, the results from the 15 µg/L exposure were the only potentially useable results. However, issues were also identified with the 15 µg/L exposure. The methods were vague in terms of describing how long the exposures were relative to when the sampling took place. For example, periphyton exposures were 5 to 12 days, which did not match up to the durations of dosing and results were not provided in a manner to discern the different times of exposure. Additionally, none of the macrophyte results are useable for the 15 µg/L treatment because none of the sampling took place during that exposure. Finally, several factors lead to concern regarding the interpretation of reported results. The methods suggest that manipulations of the study design were continually happening throughout the experiment (*e.g.*, nutrient pots in and out, plants in and out), the results are not dose-dependent, and because the doses were not made at the same time the seasonal differences across the entire study (*e.g.*, light, temperature) and overall community development could have confounded the interpretation of any atrazine-related responses.

5.5.1 EPA's 2023 Conclusions

Based on the discussion above, EPA's 2023 conclusion is that Detenbeck (1996) should be excluded from the cosm database.

CHAPTER 6. KOSINSKI

6.1 Overview

Kosinski, 1984 was one of the 11 studies flagged by the 2012 SAP as warranting reanalysis (USEPA, 2012b). The group here includes Kosinski and Merkle (1984) and Kosinski (1984). These publications present different aspects of the results from the same two experiments conducted in the 1980s at Texas A&M University. The two cosm experiments discussed in the publications were methodologically identical; however, one study was conducted in 1981 and inoculated with water from a spring, while the other was conducted in 1982 and inoculated with water from a river. Kosinski and Merkle (1984) presents the algal community productivity (likely the combination of periphyton and phytoplankton) results from these two experiments, whereas Kosinski (1984) presents the periphyton community structure results. In this chapter, the studies are discussed collectively with separation only when relevant (*e.g.*, specific methods, results, concerns).

6.2 Experimental Design and Execution

The experiments conducted in 1981 and 1982 were methodologically identical, except for the source of the community. From April to May of each year, experiments were conducted in plastic-lined, outdoor, recirculating artificial stream cosms (2.4 m length x 12.6 cm width x 7 cm diameter; water depth: 4 cm; flow: 3 cm/sec). The water source used to fill the cosms was not provided. To establish biotic communities, each stream was inoculated with 100 mL of a homogenized water-algae suspension taken from one of two locations. In 1981, the inoculate was directly collected from Minter Spring (MS), which was a concrete standpipe in a pasture with water that was almost anoxic and "highly mineralized" (specific conductance: 54.6 mS/m; alkalinity: 71 mg $CaCO_3$/L; nitrate: 404 µg/L; soluble reactive phosphorus: 21 µg/L). Water in the spring had an aquatic community of tangled mats of cyanobacteria, epiphytic diatoms, and a few species of protozoans, rotifers, ostracods, and chironomids. In 1982, the inoculate was collected from Little Brazos River (LB), which was in an intensively farmed region (with atrazine use). The water from the river had a dissolved oxygen (DO) between 75 and 150%, was more mineralized and nutrient-rich than MS (specific conductance: 95.5 mS/m; alkalinity: 153 mg $CaCO_3$/L; nitrate: 1716 µg/L; soluble reactive phosphorus: 78 µg/L), and had an aquatic community of *Cladophora glomerulata* (green algae) and many diatoms, invertebrates, and fish

species. After the start of the experiment, “source water” was added automatically to each cosm to account for evaporation and then twice a week, 15% of the cosm water was discarded and replaced with “source water and algae” to input new algae and nutrients. The studies do not say whether this “source water” was the same as the unknown water used to fill the cosm or the water used to inoculate the cosms. The algae did come from MS or LB. Additionally, while the studies do not discuss any potential impacts this could have had on the taxa present in the mesocosms, the addition of new algae represents a confounding factor. The cosms were covered to provide protection against wind, rain, and excess evaporation.

The experimental design (**Figure 6.1**) included a three- or four-week colonization period within the cosms. During the colonization period, 16 cosms within each experiment were exposed to atrazine treatments (0.01 mg/kg; hereafter ‘10 µg/L’), and 16 others were maintained as controls with no atrazine added. The concentration in the atrazine treatment was maintained (without analytical verification) by dosing with atrazine whenever the 15% water replacements occurred. This exposure began what is referred to as the “chronic” exposure (Day 0 through Day 21 or 28). During the colonization period, samples of the community were taken. On Day 21 or 28, the cosms were divided into four separate “acute” exposure groups (0, 0.1, 1, or 10 mg/kg; or hereafter ‘0, 100, 1000, or 10,000 µg/L’) with each group replicated four times. Cosms were sampled and after three additional weeks within the “acute” exposure, the last community samples were taken (Day 42 or 49; **Figure 6.1**). The exact dosing method for the “chronic” and “acute” treatment was not provided. The atrazine used in the experiments formulated end-use product Aatrex™ 80 WP (90% active ingredient according to EPA Reg# 100-572) and all concentrations were reported based on the amount of active ingredient. A small number of water analyses were done to estimate the rate of atrazine degradation or dissipation (0 and 10,000 µg/L treatments only). The prior “chronic” treatment of these samples was not provided and the documents do not say water analyses were completed for both the MS and LB experiment. The treatments relevant to this evaluation (10 and 100 µg/L) were not measured.

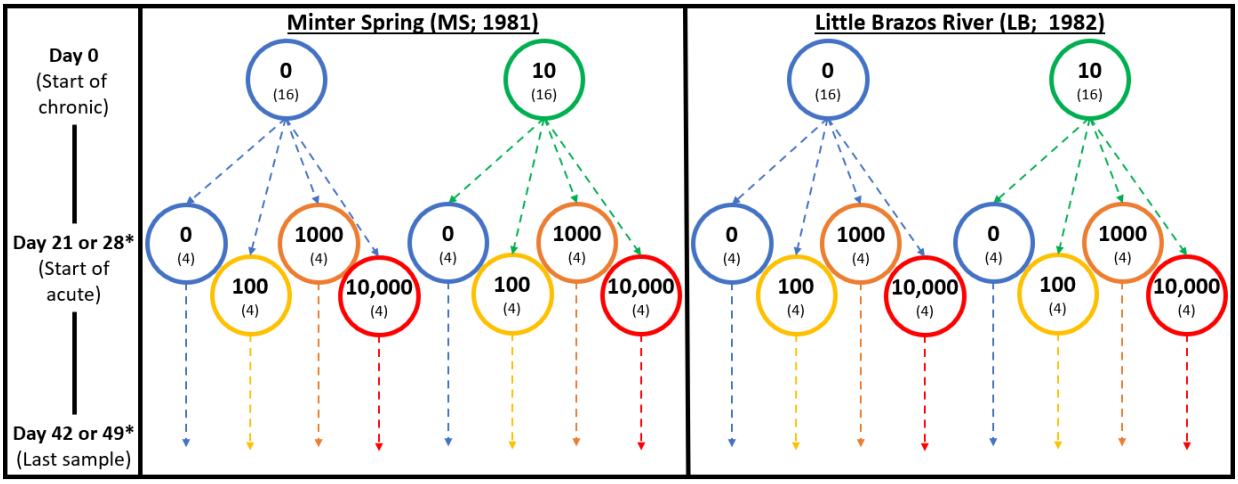


Figure 6.1. The experimental design of experiments presented in Kosinski and Merkle, 1984 and Kosinski, 1984. Within the circles, the top number is the atrazine concentration of the treatment in µg/L, and the number below in parentheses is the number of replicates. *The study text says "Acute herbicide exposure was determined by allowing the streams to colonize for 3 or 4 weeks" before applying the "acute" treatments. It does not specify with experiment lasted for which duration.

Kosinski and Merkle (1984) assessed algal community productivity by using the "open-water oxygen method (Odum, 1956; Owens, 1974)." This estimated the sum of photosynthesis (*i.e.*, gross primary production, GPP) and respiration, or net community productivity (NCP). The method relied upon dissolved oxygen (DO) sampling every 15 min from dawn to sunset, once per hour from sunset until midnight, and once the following dawn. Based on the reported results, DO measurements were taken at least 1 week before, 1 day before, the day of, 3 days after, 1 week after, 2 weeks after, and 3 weeks after the start of the "acute" treatment. Productivity was calculated by a computer program, which applied relevant correction factors (exact unknown). This program estimated productivity over time in terms of the sum of photosynthesis (*i.e.*, gross primary production, GPP) and respiration, or net community productivity (NCP)[27]. The pH was also recorded hourly to determine the highest pH for a rough estimate of cumulative CO_2 uptake. For the statistical analyses, an analysis of variance (ANOVA) was done on dawn-to-dusk AGPP, NCP, respiration, and pH values. Chi-square median tests were also performed on the dawn-to-dusk AGPP values. The level of statistical significance was set to an alpha of 0.05.

[27]In Kosinski and Merkle (1984), the authors state, "In some cases, especially during extreme suppression of productivity by herbicides, respiration in the light exceeded dark respiration, making computed 'GPP' negative. Because this is impossible, we have called NCP corrected for dark respiration apparent GPP (AGPP) and have reported negative values as they occurred."

Kosinski (1984) assessed the periphyton community structure by adding microscope slides to the mesocosms and sampling them approximately weekly (results show the same daily sampling events as the DO sampling). When sampled, the slides were scraped, the samples were preserved, and then later assessed using a microscope. Units counted varied with the growth form (*e.g.*, individuals vs colonies vs grids filled vs length). The volume of each of the counting units was determined by geometric approximation, and the results were expressed as μm^3 of biovolume/mm^2 of slide surface. For the MS community, samples from Week 1 and 3 are missing due to a lab accident and then a slide error necessitated combining the 0 and 10 µg/L results and the 1000 and 10,000 µg/L results. For the statistical analyses, univariate (ANOVA) and multivariate [MANOVA, Principal Components Analysis (PCAs)] analyses were performed on the various datasets (*e.g.*, most common species, biovolumes, Shannon-Wiener diversity indices). Data were transformed when needed, and significance was set at an alpha of 0.05.

6.3 EPA's Use as of 2016

In the 2016 Refined Ecological Risk Assessment (USEPA, 2016), EPA included two endpoints from the Kosinski publications (**Table 6.1**). One endpoint (#28) represents the 10 µg/L "chronic" treatment (**Figure 6.1**, green circles), while the other endpoint (#44) represents the subsequent 100 µg/L "acute" treatment (**Figure 6.1**, yellow circles)[28]. Both endpoints used in the assessment were scored as "Effect". In previous versions of the cosm study database, the 1000 and 10,000 µg/L treatments (**Figure 6.1**, orange and red circles respectively) were included; however, after the 2012 SAP's recommendation to limit the endpoints to realistic exposure conditions, EPA set a concentration limit of 500 µg/L and removed the 1000 and 10,000 µg/L Kosinski endpoints.

[28] In both publications, the "chronic" and "acute" results are presented separately, but the authors might have aggregated the preceding "chronic" treatments when assessing the "acute" results. Therefore, endpoint #44 might represent cosms with prior exposure to both the 0 and 10 µg/L "chronic" treatment. Interactions between the "chronic" and "acute" treatments were minimal and lacked a pattern.

Table 6.1. A summary of the cosm endpoints associated with this group that appeared in Appendix G.2 of the 2016 Refined Ecological Risk Assessment. These endpoints were used to evaluate the potential effects of atrazine on aquatic plant communities.

References	Endpoint Number	Duration	Nominal Conc. (µg/L)	Plant Group	Results and Recovery	Effect/No-Effect Conclusion
Kosinski (1984) Kosinski and Merkle (1984)[A]	28	21	10	Peri	40% decrease in apparent primary productivity[B]; slight decrease in biovolume. Recovery: >21 d (primary production); <7 days (biovolume)	Effect[C]
Kosinski (1984); Kosinski and Merkle (1984)[A]	44	21	100	Peri	30% decrease in apparent primary productivity[B]; no affect on biovolume. Recovery: < 3 d	Effect[D]

[A] Publications that were not part of the original 11 identified by the 2012 SAP but are associated with them.
[B] In Kosinski and Merkle (1984), the authors state, "In some cases, especially during extreme suppression of productivity by herbicides, respiration in the light exceeded dark respiration, making computed 'GPP' negative. Because this is impossible, we have called NCP corrected for dark respiration apparent GPP (AGPP) and have reported negative values as they occurred."
[C] Atrazine dissipated from the streams at a half-life of 3.2 days. Effect occurred at day 7; magnitude of effect for primary productivity estimated using study figures. Results from the two publications (Kosinski 1984 and Kosinski and Merkle 1984) were combined because they appeared to be from the same set of experiments.
[D] Effect occurred at day 0 and recovery occurred by day 3.

6.4 Stakeholders' Major Concerns and Criticisms

Both of the Kosinski publications have been in the cosm database since the 2003 Atrazine Interim Registration Eligibility Decision (IRED) (USEPA, 2003). For the 2007 SAP Meeting, the Kosinski endpoints were included in an appendix of the White Paper that contained the database (USEPA, 2007) but otherwise, there was no discussion about the Kosinski studies in the White Paper or by public commenters or the SAP. With the 2009 and 2012 SAP Meeting, both the SAP and public commenters identified concerns about the number of endpoints and effect/no-effect conclusions made by EPA regarding the Kosinski publications (Syngenta, 2012a, 201b; USEPA, 2009, 2012b). The concerns were echoed in comments on the 2016 Refined Ecological Risk Assessment (Syngenta, 2016) and again in comments on the 2022 Proposed Revisions to the Atrazine Interim Registration Review Decision (Syngenta, 2022). Details of the specific concerns brought about during each event mentioned above are discussed below.

6.4.1 The 2009 SAP Meeting

In comments submitted for the 2009 SAP meeting, Syngenta (2007) stated that:

Based on the acute treatments, the authors reported that AGPP in the 10-µg/L atrazine treatment was not significantly different than controls up to two weeks following exposure, and was significantly higher (~50% higher) than control treatments after 21 days of exposure [Figure 2 within Kosinski and Merkle (1984)]. Since a significant decrease in AGPP indicates potential adverse effects to primary producers, an increase in AGPP suggests an improvement in primary production. Therefore, based on the author's reported findings, exposure to 10-µg/L atrazine for 21 days did not adversely affect AGPP in these artificial streams.

Given the characteristics of this study, the 4-level effect score assigned to this exposure scenario by Brock et al. (2000) grossly overestimates impacts at these exposure durations and concentrations.

Note: Because there was no 10 µg/L "acute" treatment and the only treatment to cause a significant increase in AGPP was 100 µg/L, it is assumed that Syngenta meant the 100 µg/L treatment above.

In the minutes from the SAP meeting (USEPA, 2009b), the Panel stated that one panel member provided an analysis of several cosm studies and said the following about the Kosinski studies:

Cosm study #28, Kosinski (1984). This study evaluated a concentration of 10 µg/L atrazine and was given a Brock score of 4. Notes regarding the study indicate that there was a 40% decrease in "apparent productivity but only a slight decrease in algal biovolume." In addition, the notes indicate that atrazine had a half-life of 3.2 days in the streams. This study should be re-evaluated because the algal sensitivity and atrazine half-life simply do not fit with the tremendous amount of information in the literature regarding both single species and cosm studies.

6.4.2 The 2012 SAP Meeting

In comments submitted for the 2012 SAP Meeting (Syngenta, 2012a), Syngenta identified two main issues with the Kosinski endpoints: 1) The endpoints should be split to represent the two experiments [Minter Spring (MS; 1981) and Little Brazos River (LB; 1982)]; and 2) The

effect/no-effect conclusions for those endpoints should be adjusted based on the following effects assessment:

> *In the chronic exposure to 10 µg/L, the responses of the periphyton differed between sites and years. In cosms initiated with water and algae from Minter Spring (1981), productivity was significantly reduced on day 3 and day 14, but not on day 7 or day 21; thus the effect never occurred on consecutive sample events and would be considered slight according to the evaluation criteria presented in Section 3.0. A significant effect on biovolume was observed only on day 3. A reduction in abundance of Rhopalodia gibberula was reported, but the supporting data were not presented and it cannot be determined when the effect occurred. R. gibberula was unaffected in 21-d exposures to 100, 1000, and 10000 µg/L. Because effects of 10 µg/L in the first study were slight, transient, and (in the case of R. gibberula) not dose-related, CSI assigned a binary score of "0" (#ID #28a).*
>
> *In cosms initiated with water and algae from Little Brazos River (1982), significant differences in productivity between treated and controls streams were observed on both pre-treatment days (i.e., before 10 µg/L atrazine was added), and again on days 7, 14, and 21. The occurrence of significant differences before atrazine treatment may have indicated that the subsequent differences were not treatment-related. However, because treated and control streams were not significantly different on days 0 and 3 but were different on days 7, 14, and 21, CSI conservatively assigned a binary score of "1" for this exposure (ID #28).*
>
> *In the pulsed exposure experiments at 100 µg/L, the only significant effect was a slight reduction in productivity on Day 0 of the Little Brazos River experiment. There was no indication of effects on individual species. CSI therefore assigned a binary score of "0" at 100 µg/L for both experiments (#ID 44, 44a).*
>
> *EPA's cosm database includes 4 data points, all scored 1. CSI concludes that 4 additional points (8 points total) should be included to represent the two experiments (Minter Spring 1981, Little Brazos River 1982) separately.*

In paragraph two above, Syngenta incorrectly says that the effects occurred before atrazine treatments. All graphs in the Kosinski publications are in relation to the timing of the "acute" treatments. So -1 wk and -1 d in the table that presents "chronic" results (**Figure 6.2** in **Section 6.5**) is actually -1 wk and -1 d before "acute" treatments, but during the "chronic" treatments.

Syngenta's rationale for splitting the endpoints was that the current endpoints (#28 and #44) represented combined results of experiments conducted at different times or under different conditions, "*The experiments …. conducted using water and algae from two sites in different years, with different results. The results from Minter Springs site in 1981 should be reported separately from the results of the Little Brazos site in 1982."*

In terms of the effect/no-effect conclusion, Syngenta concluded the following for the 10 µg/L treatment:

> *Periphyton productivity was reduced in one experiment, but only slight and intermittent effects were observed in the other experiment. A reduction in the abundance of one diatom species, Rhopalodia gibberula, was reported, but this species was unaffected by exposure to 100, 1000, and 10,000 µg/L. The effect on periphyton productivity at 10 µg/L in the Little Brazos River experiment represents the lowest reliable (though conservatively interpreted, as discussed in Section 4.3) effect concentration in the atrazine cosm database.*

As detailed in **Section 6.1**, the 2012 SAP commented on Kosinski (1984) and said the following (USEPA, 2012b):

> *The 10 µg/L atrazine concentration listed for this study in Attachment 3, Appendix D, should be 100 µg/L. Although some streams were colonized (pre-treated at 10 µg/L), there are insufficient data to assign effects at this level. The abstract of Kosinski and Merkle (1984) states succinctly, "There was little evidence that exposure to 0.01 mg/kg [10 µg/L] herbicide during colonization modified the response of the algae to any of the herbicides."*

Note: EPA is unsure why the 2012 SAP suggested the 10 µg/L to 100 µg/L edit to Appendix D as the rows associated with the Kosinski studies in that appendix are correct. Additionally, the statement in the abstract of Kosinski and Merkle (1984) is in relation to how the colonization during the "chronic" treatment influenced the "acute" treatment response (*i.e.*, an interaction), not on solely the "chronic" treatment response.

The panel also noted that it was one of the "studies discussed by the 2009 SAP as having flawed methodology, which affected interpretation of the results" (see **Section 6.4.1**)

6.4.3 The 2016 Refined Ecological Risk Assessment

In the Addendum to the Problem Formulation (USEPA, 2013), EPA indicated that two endpoints from the Kosinski studies would be removed because of the new initial endpoint concentration restriction of 500 µg/L. These were the 1000 (#29) and 10,000 (#30) µg/L endpoints.

In the 2016 Refined Ecological Risk Assessment (USEPA, 2016), EPA reiterated the removal of the endpoints with high initial test concentrations and provided the following reevaluation:

> *These studies reported the results from a 21-day study testing 10, 100, 1000 and 10,000 µg/L of atrazine in recirculating artificial streams. The highest two test concentrations are excluded from the current EPA Cosm Effects Database (Appendix B) because they are above the expected environmental concentrations (maximum 500 µg/L). The results from the 10 µg/L test concentrations indicated a statistically significant 40% decrease in primary productivity and a slight decrease in biovolume. The 100 µg/L test concentration reported a statistically significant decrease (30%) in primary productivity and no change in biovolume.*

Ultimately, EPA made no changes to the Kosinski endpoints in the cosm database besides the removal of the 1000 (#29) and 10,000 (#30) endpoints.

After publication of the 2016 Refined Ecological Risk Assessment, EPA received comments from the registrants, crop groups, and members of the public. The most substantial comment was

from Syngenta, which reiterated much of what they said in their comments from the 2012 SAP Meeting, including the same evaluation of the results, suggested changes, and rationale (Syngenta, 2016; see **Section 6.4.2** section above).

Another comment came from the Triazine Network (2016), which gave summaries for the Kosinski publications, which included EPA's data evaluation records (DERs), the 2009 and 2012 SAP meeting, Syngenta (2012a), Triazine Network (2015), Moore *et al*. (2016), and finally EPAs 2016 Refined Ecological Risk Assessment. Note that the Triazine Network did not mention the splitting of endpoints.

6.4.4 Post-Risk Assessment to Present

After issuance of the Proposed Revisions to the Interim Decision in 2022 (USEPA, 2022b; 2022c), EPA again received several comments about the cosm database. In Syngenta's comment, similar suggestions were provided including the splitting of endpoints and changes to the effect/no-effect conclusions (Syngenta, 2022).

The score given by EPA in the 2016 Refined Ecological Risk Assessment, Syngenta's score in their comment on the 2016 risk assessment, and Syngenta's 2022 score in the comment on the proposed revisions to the Interim Decision were summarized by Syngenta in the table titled "Table SI-1(mod)" (**Table 6.2).**

Table 6.2. Data from Syngenta's Table SI-1 (mod) table. For brevity, some columns that are not needed for this evaluation have been removed (*e.g.,* Test System description). For the binary scores, 0 equals "No Effect" and 1 equals "Effect".

Endpoint Number	Reference	Conc (ppb)	Plant Community Response[A]	Binary Scores (EPA 2016)	Binary Scores (Syngenta 2016)	Binary Score (Syngenta 2022)
28	Kosinski 1984, Kosinski and Merkle 1984 (Little Brazos, 1982)	10	Significant reductions in productivity on all sample events except during the 4th week (K&M 1984); no effect on biovolume (K 1984)	1	1	1
44	Kosinski 1984, Kosinski and Merkle 1984 (Little Brazos, 1982)	100	Second experiment (performed in different year than 44a with inocula from different source, Little Brazos). Slight effect on productivity only on	1	0	0

Endpoint Number	Reference	Conc (ppb)	Plant Community Response[A]	Binary Scores (EPA 2016)	Binary Scores (Syngenta 2016)	Binary Score (Syngenta 2022)
			Day 1, no effect thereafter (K&M 1984); no effect on biovolume or community composition (K 1984)			
28a	Kosinski 1984, Kosinski and Merkle 1984 (Minter Spring, 1981)	10	No significant reduction in productivity until 3.5 weeks, no effect on consecutive sample events (K&M 1984); significant effect on biovolume only at 3.5 weeks, none thereafter (K 1984); reduction in abundance of *Rhopalodia gibberula* (K 1984), but data not presented and cannot determine when the effect occurred -- *R. gibberula* was not affected in 21-d exposures to 100, 1000, and 10000 µg/L	Not split	0	0
44a	Kosinski 1984, Kosinski and Merkle 1984 (Minter Spring, 1981)	100	First experiment (Minter Spring). No effect on productivity (K&M 1984); no effect on biovolume or community composition (K 1984)	Not split	0	0

[A] K 1984 = Kosinski (1984) and K&M 1984 = Kosinski and Merkle (1984)
* Note the 1000 and 10,000 µg/L endpoints were not included in this White Paper because they were removed from EPA's database.

The Triazine Network also provided a comment on these studies, but the only remark focused solely on the Kosinski studies was about the limited relevance due to the continuous exposure to 10 µg /L in the "chronic" treatment (Triazine Network, 2022).

6.5 EPA's 2023 Reevaluation

After consideration of all of the studies' limitations, the determinative concerns will be discussed here, including those brought up by past SAPs and public commenters. These include the number of endpoints associated with the Kosinski studies and the effect/no-effect conclusions for those endpoints. Each of these concerns are discussed below as part of EPA's 2023 reevaluation.

6.5.1 Splitting of Endpoints

As discussed above, EPA's database contained four endpoints and then two were removed due to high concentrations. This means that two remain relevant to the discussion of the Kosinski studies here:

#28 – Represented the 10 µg/L "chronic" treatment

#44 – Represented the 100 µg/L "acute" treatment

Syngenta suggested that EPA add two new endpoints to the database so that there was a set for each community (*i.e.*, MS and LB) tested.

In EPA's 2023 reevaluation of this group, EPA decided to add two new endpoints to the database to reflect this suggestion. The two experiments were completed in different years (1981 vs 1982), used different communities (spring vs river), and ultimately found different results (discussed next). This speaks to the importance of testing different communities to better understand how communities across the country will respond to atrazine exposure.

The endpoints that will now be associated with the Kosinski studies include:

#28a (new) – Represents the 1981 MS community 10 µg/L "chronic" treatment

#44a (new) – Represents the 1981 MS community 100 µg/L "acute" treatment

#28 (revised) – Represents the 1982 LB community 10 µg/L "chronic" treatment

#44 (revised) – Represents the 1982 LB community 100 µg/L "acute" treatment

6.5.2 Effect/No-Effect Conclusions

Both Syngenta and past SAPs commented on the effect/no-effect conclusion for all Kosinski endpoints. The last SAPs suggested changes to the 10 µg/L endpoint, while Syngenta's most recent comment in 2022 suggested that only one (#28, 10 µg/L; see **Table 6.2**) of the four endpoints be scored as effect.

For EPA's 2023 reevaluation, effects were considered relevant to the "chronic" treatment assessment if they occurred during the period before the "acute" treatment (*i.e.*, -1 wk and -1d results) and relevant to the "acute" treatment if they occurred after the start of the "acute" treatment (*i.e.*, 0 d, +3 d, +1 wk, +2 wk, and +3 wk results).

Before the discussion of the results below, it is important to note a few things that could influence the interpretation of the results. First, in Kosinski and Merkle (1984), there was no presentation of the pH or respiration (text notes that there were no effects) results, and very little discussion (and no presentation) of NCP results. Only the AGPP results were presented in a way that allowed for independent conclusions. Second, in both publications, the "chronic" and "acute" results are presented separately, but the authors might have aggregated the preceding "chronic" treatments when assessing the "acute" results because the results in the "acute" graph are not separated by "chronic" treatment (see **Figure 6.2B**). Third, Kosinski and Merkle (1984) states that "[C]ompared to productive natural streams in the literature, productivity in artificial streams was low." Lastly, the authors indicate that there was significant variation between streams within a treatment, which is not always evident in the figures below.

1981 MS community 10 µg/L "chronic" treatment (endpoint #28a)

There were no significant differences in AGPP (**Figure 6.2a**) or overall biovolume (**Figure 6.3**) prior to the "acute" treatment. According to **Figure 6.4**, there were significant effects on species-specific biovolume at Week -2 and -1 (*i.e*., during the "chronic" treatment). The species affected included: a cyanophyte [species #3. *Chroococcus sp*. (Week -2)] and three crysophyte species [species #59. *Synedra delicatissima* (Week -2), #56. *R. gibba ventricosa* (Week -1), and #57. *R. gibberula* (Week -1)]. The two significant timepoints do not share any species in common and therefore, are considered transient. Based on EPA's 2023 revaluation, this endpoint has been scored as "**No Effect**".

1981 MS community 100 µg/L "acute" treatment (endpoint #44a)

There were no significant differences in AGPP or respiration between the control (1) and the 100 µg/L (2) treatments (**Figure 6.2b**). There were also no significant effects on overall or species-specific biovolume (**Figure 6.3 and 6.4**). The biovolume results were difficult, if not impossible, to interpret for the MS community because of the author combined the 0 and 10 µg/L treatments. Based on EPA's 2023 reevaluation, this endpoint has been scored as "**No Effect**".

1982 LB community 10 µg/L "chronic" treatment (endpoint #28)

There was a significant effect on AGPP at Week -1 and Day -1 (**Figure 6.2a**) where AGPP was 45% and 35% lower in the 10 µg/L treatment, respectively. There was no significant difference in overall biovolume prior to the "acute" treatment (**Figure 6.3**). According to **Figure 6.4**, there were significant effects on species-specific biovolume at Week -2 and -1. The species affected included: 55. *Rhopalodia gibba* (Week -2), 56. *R. gibba ventricosa* (Week -2), and 12. *Phormidium minnesotense* (Week -1). These species are chrysophytes except the last one, which is a cyanophyte. There was no overlap between the two significant timepoints, but the MS and LB community shared one species – *R. gibba ventricosa*. Based on EPA's 2023 reevaluation, this endpoint has been scored as "**Effect**" due to the effects on AGPP.

1982 LB community 100 µg/L "acute" treatment (#44)

There was a significant reduction in AGPP in the 100 µg/L ("2" in the figure) treatment relative to the control ("1" in the figure) on Day 0 (**Figure 6.2b**). However, by Day 3 the two treatments were no longer significantly different. At Week 3, there was a significant increase in AGPP in the 100 µg/L treatment relative to the control. There were no significant effects on overall biovolume (**Figure 6.3**). For species-specific biovolume, the significant results were presented in a way that made it impossible to tell exactly which "acute" treatment was different from the control (**Figure 6.4**). Based on EPA's 2023 reevaluation, this endpoint has been scored as "**No Effect**" given the slight/transient nature of the effect on Day 0.

A. Chronic treatment

	Atrazine	
	MS	LB
A (-1 wk)	74	163**
	−1	90**
B (-1 d)	438	340**
	462	220**
C (0)	318	319
	308	314
D (+3 d)	245*	291
	55*	223
E (+1 wk)	993	315*
	906	187*
F (+2 wk)	857**	830**
	312**	467**
G (+3 wk)	883	1116**
	698	679**

* and ** Indicate a significant difference between the two values at the 0.05 or 0.01 level.

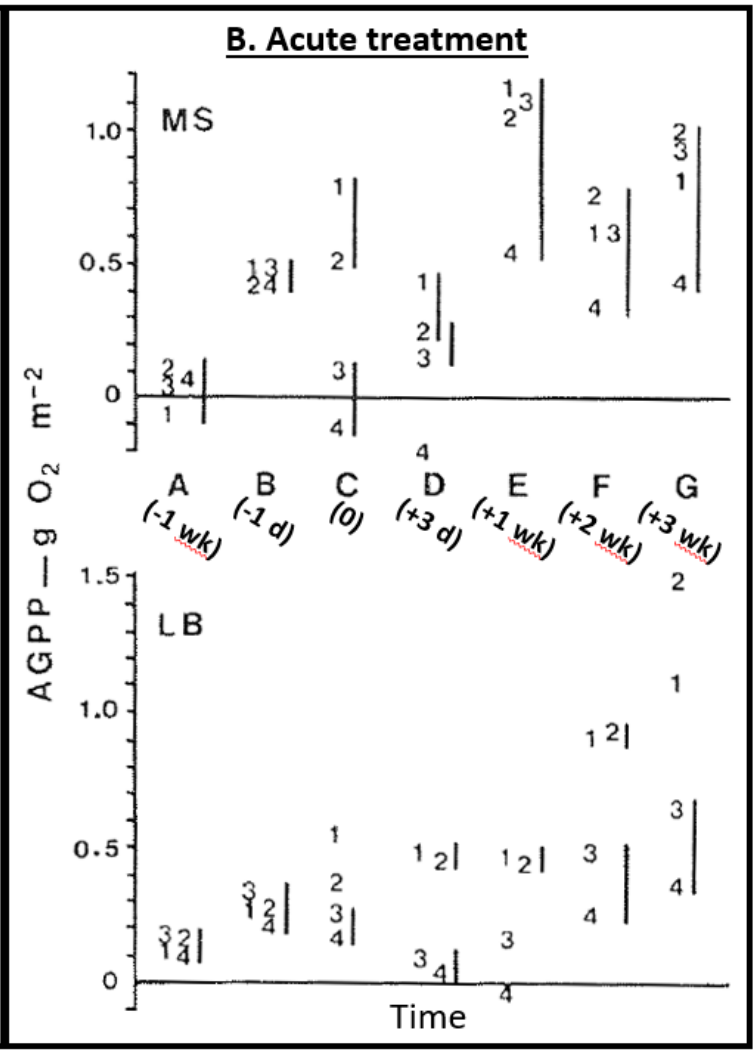
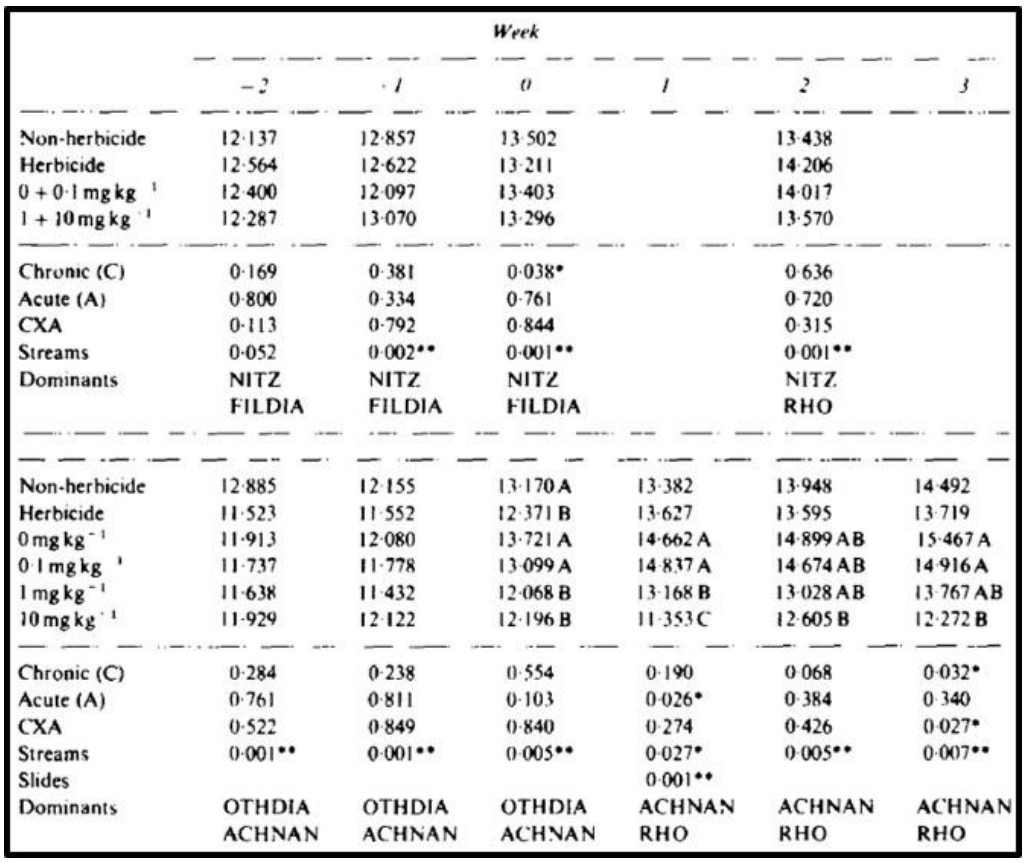


Figure 6.2. Phytoplankton apparent gross primary production (AGPP) results. A) Table 2 from Kosinski and Merkle (1984), which had the following caption, "Apparent gross primary productivity (AGPP) (mg O_2 m^{-2}) in the chronic [atrazine] treatments. The nonherbicide (0 mg kg^{-1} [or 0 µg/L]) and herbicide (0.1 mg kg^{-1} [or 10 µg/L]) treatments are the first and second values in each pair, respectively." B) Figure 2 from Kosinski and Merkle (1984), which had the following caption, "Response of AGPP to acute treatment in the atrazine experiments… Standard errors for an acute treatment were (left to right) 0.083, 0.102, 0.139, 0.081, 0.210, 0.164, 0.201 (1981), and 0.026, 0.040, 0.042, 0.063, 0.047, 0.098, 0.123 (1982)." In both panels, the meaning of A-G has been added directly to the panels and in both cases is in relation to the "acute" treatments. In panel B, 1 = 0 mg/kg (0 µg/L), 2 = 0.1 mg/kg (100 µg/L), 3 = 1 mg/kg (1000 µg/L), and 4 = 10 mg/kg (10,000 µg/L).



	Week					
	−2	−1	0	1	2	3
Non-herbicide	12·137	12·857	13·502		13·438	
Herbicide	12·564	12·622	13·211		14·206	
0 + 0·1 mg kg^{-1}	12·400	12·097	13·403		14·017	
1 + 10 mg kg^{-1}	12·287	13·070	13·296		13·570	
Chronic (C)	0·169	0·381	0·038*		0·636	
Acute (A)	0·800	0·334	0·761		0·720	
CXA	0·113	0·792	0·844		0·315	
Streams	0·052	0·002**	0·001**		0·001**	
Dominants	NITZ FILDIA	NITZ FILDIA	NITZ FILDIA		NITZ RHO	
Non-herbicide	12·885	12·155	13·170 A	13·382	13·948	14·492
Herbicide	11·523	11·552	12·371 B	13·627	13·595	13·719
0 mg kg^{-1}	11·913	12·080	13·721 A	14·662 A	14·899 AB	15·467 A
0·1 mg kg^{-1}	11·737	11·778	13·099 A	14·837 A	14·674 AB	14·916 A
1 mg kg^{-1}	11·638	11·432	12·068 B	13·168 B	13·028 AB	13·767 AB
10 mg kg^{-1}	11·929	12·122	12·196 B	11·353 C	12·605 B	12·272 B
Chronic (C)	0·284	0·238	0·554	0·190	0·068	0·032*
Acute (A)	0·761	0·811	0·103	0·026*	0·384	0·340
CXA	0·522	0·849	0·840	0·274	0·426	0·027*
Streams	0·001**	0·001**	0·005**	0·027*	0·005**	0·007**
Slides				0·001**		
Dominants	OTHDIA ACHNAN	OTHDIA ACHNAN	OTHDIA ACHNAN	ACHNAN RHO	ACHNAN RHO	ACHNAN RHO

Figure 6.3. An excerpt of phytoplankton biovolume results presented in Table 3 from Kosinski (1984). The original caption reads, "Total Log-Transformed Biovolume, Multivariate Results and Dominant Species Groups in the Atrazine Experiments. 1981 and 1982 Results are Above and Below the Double Line, respectively. See Text for Explanation" with a foot note of, "* Result significant at the 0.05 level, ** Result significant at the 0.01 level." The text adds, "Within each section, the upper part contains the values of In (1 + total biovolume in $\mu m^3/mm^2$). Means followed by different letters are significantly different by Duncan's multiple range test. The lower section gives the probabilities (computed from Pillai's trace) of the null hypothesis of no multivariate effect. Weeks are designated as weeks before (-) and after (+) the application of the acute treatments with data for week 0 collected the day after herbicide injection. "Non-herbicide" (NH) and "herbicide" (H) refer to the 0 [0] and 0.01mg/kg [10 µg/L] chronic treatments, while 0 [0], 0.1 [100], 1 [1000], and 10 mg/kg [10,000 µg/L] are the acute treatments. The dominant species groups are listed in the order of their dominance." MS data are missing for weeks 1 and 3 the 0 (0) and 0.1 mg/kg (100 µg/L) results and the 1 (1000) and 10 mg/kg (10,000 µg/L) results were combined (see Section 6.2).

Week	*Variable*	*Means (NH/H or 0/0·1/1/10)*	*Species affected*
−2	NFILBGS	1·867/4·804	3
−2	NAVSYN	1·245/5·384	59
−1	RHO	7·958/2·755	56, 57
0	RHO	9·841/3·472	57
−2	RHO	0·000/2·834	55, 56
−1	PHOR	6·748/1·968	12
0	PHOR	8·443/4·212	12, 13
0	NITZ	10·370/7·713	51
0	RHO	8·715/2·306	55
2	PHOR	8·542/5·054	12, 13
2	NITZ	11·318/6·602	53
0	PHOR	8·585/8·694/5·367/2·663	12, 13
0	RHO	9·984/4·769/5·826/1·462	55, 56
1	CNTRLES	4·635/2·945/2·624/1·609	37
1	ACHNAN	11·546/12·439/11·062/9·573	33
1	RHO	14·350/14·426/9·414/3·683	55, 56
1	OTHDIA	10·730/10·854/9·653/7·295	36
2	PHOR	10·970/5·997/3·773/6·755	12
2	SCENCLA	10·833/7·494/0·000/3·984	18
2	ACHNAN	11·836/12·109/11·184/11·039	33, 35
3	SCENCLA	9·632/8·614/0·000/0·000	18
3	RHO	15·142/14·417/11·396/1·549	55, 56
3	CNTRLES	4·120/2·683/0·000/0·000	37, 39

Figure 6.4. An excerpt of phytoplankton biovolume results presented in Table 4 from Kosinski (1984). The original caption reads, Statistically Significant Univariate Effects of Atrazine Treatment on Natural Log-Transformed Biovolume of Species Groups. 1981 [MS] and 1982 [LB] Results are Above and Below the Horizontal Line, Respectively. See Text for Explanation." The text adds, "two means on a line gives the NH [no herbicide; 0 mg/kg or 0 µg/L] and H [herbicide, 0.01 mg/kg or 10 µg/L] means and four means on a line gives the 0 [0], 0.1 [100], 1 [1000] and 10 mg/kg [10, 000 µg/L] means (both in the given order). 'Species' indicates the code number of the species (Table 1) most responsible for the effect, when this could be determined." MS data are missing for weeks 1 and 3 the 0 [0] and 0.1 mg/kg [100 µg/L] results and the 1 [1000] and 10 mg/kg [10,000 µg/L] results were combined (see Section 6.2).

6.5.3 EPA's 2023 Conclusions

While the studies have deficiencies, EPA has decided that the studies are sufficient to contribute to our knowledge about the effects of atrazine to aquatic plant communities under the conditions of the experiment. These studies contribute to our understanding of potential effects on natural aquatic plants communities exposed to atrazine when considered within the context of the larger collective body of experimental data from other cosm studies. Therefore, while there is uncertainty in the results, it is reasonable to include these studies in the cosm database. **Based on the changes discussed above, EPA's database will now have four endpoints for the Kosinski studies with one "Effect" (*i.e.*, endpoint #28) and three "No Effect" endpoints (*i.e.*, endpoints #28a; #44; #44a)** (**Table 6.3**). The duration for all endpoints is 21 days to represent the duration of the treatment. The "chronic" treatment lasted three or four weeks (three used

here) and involved a maintained concentration, while the subsequent "acute" treatment lasted three weeks and involved a single dose followed by three weeks of observation. Samples took place throughout both treatments.

Table 6.3. A summary of the cosm endpoints associated with this group that remain after the 2023 reevaluation. These endpoints will be used to evaluate the potential effects of atrazine on aquatic plant communities.

References	Endpoint Number	Duration (days)	Nominal Conc. (µg/L)	Plant Group	Results and Recovery	Effect/No-Effect Conclusion
Kosinski (1984; Kosinski and Merkle (1984)[A]	28[B]	21	10	Peri Phyto	45% and 35% decrease in apparent gross primary productivity (AGPP)[F] 1 week and 1 day prior to the "acute" treatment, respectively. No significant effect on periphyton community structure during the "chronic" treatment.	Effect
Kosinski (1984; Kosinski and Merkle (1984)[A]	44[C]	21	100	Peri Phyto	A slight and transient significant difference in apparent gross primary productivity (AGPP)[F] on Day 0 of the "acute" treatment with recovery occuring by Day 3. Significantly higher than the control at Week 3.	No Effect
Kosinski (1984; Kosinski and Merkle (1984)[A]	28a[D]	21	10	Peri Phyto	No significant effect on algal community productivity or periphyton community structure during the "chronic" treatment.	No Effect
Kosinski (1984; Kosinski and Merkle (1984)[A]	28b[E]	21	100	Peri Phyto	No significant effect on algal community productivity or periphyton community structure during the "acute" treatment.	No Effect

[A] Publications that were not part of the original 11 identified by the 2012 SAP but are associated with them.
[B] Results from the two publications were combined because they appeared to be from the same set of experiments. This endpoint represents the 1982 LB community experiment "chronic" treatment.
[C] Results from the two publications were combined because they appeared to be from the same set of experiments. This endpoint represents the 1982 LB community experiment "acute" treatment.
[D] Results from the two publications were combined because they appeared to be from the same set of experiments. This endpoint represents the 1981 MS community experiment "chronic" treatment.
[E] Results from the two publications were combined because they appeared to be from the same set of experiments. This endpoint represents the 1981 MS community experiment "acute" treatment.
[F] In Kosinski and Merkle (1984), the authors state, "In some cases, especially during extreme suppression of productivity by herbicides, respiration in the light exceeded dark respiration, making computed 'GPP' negative. Because this is impossible, we have called NCP corrected for dark respiration apparent GPP (AGPP) and have reported negative values as they occurred."

CHAPTER 7. SEGUIN – 2001 PUBLICATIONS

7.1 Overview

Seguin *et al*. (2001a) and Seguin *et al*. (2001b) were two of the 11 studies flagged by the 2012 SAP as warranting further review (USEPA, 2012b). The Seguin *et al.,* (2002) publication will be discussed in the following chapter. Both of the 2001 publications present results from freshwater outdoor lentic mesocosms located in France. The 2001b publication also presents experimental results from microcosms, but the concern from the 2012 SAP was specifically with the endpoints associated with the outdoor mesocosm studies. The 2001a publication presented outdoor mesocosm results associated with the periphyton community and the 2001b publication presented outdoor mesocosm results associated with the phytoplankton community. Previously, EPA has treated these two publications separately because the two publications were not explicitly linked. Upon further review and as detailed below, EPA has concluded that it is highly likely that the two publications present results of the different components of the algal community from the same outdoor mesocosm experiment; therefore, the two publications are presented together (Seguin *et al*., 2001a; Seguin *et al*., 2001b).

2012 SAP comment on Seguin *et al.* (2001a):

> *"Exposure to 2 and 30 µg/L of atrazine had a stimulatory effect on periphyton production, but this was incorrectly categorized as a negative effect."*

2012 SAP comment on Seguin *et al*. (2001b):

> *"No significant effects on periphyton biomass were observed at 30 µg/L of atrazine. The effects observed appear to be based on shifts in phytoplankton community. For example, as atrazine concentrations increased, the number of Chrysophysceae increased while the numbers of chlorophytes decreased. Acetonitrile was used as solvent, but amount added not listed. Rarely is this solvent used in ecotoxicological dosing*."

7.2 Experimental Design and Execution

Experimental design and execution of the outdoor mesocosms are described below as was presented in Seguin *et al*. (2001a) and Seguin *et al*. (2001b); therefore, many of the details are redundant since the description of the mesocosms and setup are the same. At the same time, some details are reported in one publication and are absent in the other. In general, the 2001b publication provided a greater level of detail[29]. Likewise, many of the details of the experiment described in Seguin *et al*. (2002; **Chapter 8**) are similar to Seguin *et al*. (2001a) and Seguin *et al*. (2001b), except that the periphyton community was not discussed.

Seguin et al. (2001a)

Mesocosms were constructed at the INRA (Institut National de la Recherche Agronomique, Rennes, France) experimental platform[30]. The mesocosms consisted of 15 identical circular tanks which were 3.2 m in diameter, 1.2 m high, and had capacity of approximately 5000 L with a funnel-shaped base for ease of draining. The mesocosms were filled with a 7 cm layer of natural sediment from a local uncontaminated pond, and then a mixture of tap water (not reported if dechlorinated) and pond water to a depth of 0.7 m (not stated if this was the same pond used to obtain sediment). Pond water contained phytoplankton and zooplankton and was pumped from and into the pelagic zone (not stated if this pond had atrazine contamination). Details regarding homogenization, transport and storage, physical and biological characteristics, precise pond and tap water mixture proportions, and tap water quality, are not provided. Water nutrient levels measured in the mesocosms showed an orthophosphate concentration of 0.0065 mg P/L and inorganic nitrogen level of 0.51 mg N/L. Temperature in the mesocosms ranged from 14.4°C to 24.8°C, with an average of 18°C. The timing and frequency of these measurements was not reported. The aquatic macrophyte *Glyceria maxima* was introduced in two concentric circles meant to mimic stillwater fish spawning habitat. Artificial substrata (glass slides) were placed in a holder and installed at constant depth 30 cm below water surface, around the middle of the water column. Number of slides and design of holder are not described. Colonization of the slides and equilibration of the aquatic ecosystem took place for 1 month prior to treatment.

[29] Seguin 2001a notes that a non-author assisted with translation from French.
[30] The location of the mesocosm was described by different names in Seguin *et al*. (2001a) and Seguin *et al*. (2001b), presumably meaning the INRA platform located on the ENSAR campus. https://international.institut-agro-rennes-angers.fr/history

Mesocosms were treated with nominal concentrations of 0 (untreated control), 2 µg atrazine/L, 30 µg atrazine/L, 2 µg nicosulfuron/L, or 30 µg nicosulfuron/L. Three replicates were used at each treatment level including controls. The form and application method of the atrazine, use of solvent, or analytical testing for atrazine was not described (but see Seguin *et al*., 2001b for more information).

Four randomly selected glass slides were collected on Days -1 (1 day pre-treatment), 1, 7, 17, and 37 or 40 days after treatment [*note*: it is unclear if the last measurements were made on two dates depending on the variable or if one of those dates (Day 37 vs 40) was a typo].

Periphyton was sampled by scraping algae from the surface of the four slides with a razor blade. Half of each sample was filtered through a Whatman GF/C glass fiber filter, then chlorophyll *a* was extracted and measured according to spectrophotometric methods. The rest of the sample was preserved in 5% formalin. A subsample of the preserved solution was counted (algal cell counts without species identification) by the Utermöhl[31] method with an inverted microscope. Another subsample was mounted in a high refractive index medium for microscopy, and about 400 frustules (*i.e.*, these are hard and porous cell wall or external layer of diatoms) per sample were identified (*note*: although details were not provided, EPA infers that this was the method used to identify diatom species in the samples and that the use of the term "frustule" in this context was a translation issue from French, which was performed by an assistant according to the acknowledgements). Frustule size of common diatoms were measured to determine if there were changes in cell morphology (size or valve distortion) that may have been caused by pesticide exposure. Periphyton biomass was estimated based on chlorophyll *a* measurement (calculations not described) and cell density. A Mann-Whitney test (p-values not stated) were used to compare the control and individual treatments on each sampling day.

Seguin et al. (2001b)

The mesocosm experiment was conducted in circular outdoor tanks at the ENSAR (Ecole Nationale Supérieure d'Agronomie de Rennes, France) campus[32]. Each tank was 3.2 m in

[31] Utermöhl method uses a chamber, into which a 5–50 mL sub-sample is placed and left to settle onto a coverslip, after which phytoplankton are counted using an inverted microscope. https://doi.org/10.1093/plankt/22.12.2255

[32] The location of the mesocosm was described by different names in Seguin *et al*. (2001a) and Seguin et al. (2001b), presumably meaning the INRA platform located on the ENSAR campus. https://international.institut-agro-rennes-angers.fr/history

diameter, 1.2 m high, and held about 5000 L with a funnel-shaped base. The tanks were sunk about 0.4 m into the ground to obtain stable temperatures. Nutrient concentrations in the mesocosms were 0.0065 mg P/L orthophosphate and 0.51 mg N/L inorganic nitrogen. Temperatures ranged from 14.4°C to 24.8°C with an average of 18°C. The timing and frequency of these measurements was not reported. The mesocosms were filled with 5 cm of natural sediment from a local uncontaminated pond. The tanks were filled with a mixture of tap water (not reported if dechlorinated) and water from a nearby pond at a depth of 0.7 m (not stated if this was the same pond used to obtain sediment). The pond water contained phytoplankton and zooplankton (not stated if this pond had atrazine contamination). Many methodological details were not provided, for example regarding homogenization, transport and storage, physical and biological characteristics, precise pond and tap water mixture proportions, and tap water quality. The aquatic macrophyte *Glyceria maxima* was introduced in two concentric circles with identical numbers of *Glyceria* in each mesocosm and were meant to mimic stillwater fish spawning habitat. The mesocosms were allowed to develop for 3 weeks before atrazine treatment.

Atrazine (assumed 98% purity[33]) was dissolved with acetonitrile, which was believed by the authors to be less toxic than acetone for fish. It is unclear why this would be important because the cosms were not suspended in a water body and there is no mention of fish being included[34]. The solvent concentration in the tanks (treatments and controls) was not explicitly provided for acetonitrile (but see preceding footnote). Three replicates were treated at each nominal treatment level: 0 µg/L (solvent control), 2 µg atrazine/L, 30 µg atrazine/L, 2 µg nicosulfuron/L, or 30 µg nicosulfuron/L. Analytical testing for atrazine was not described.

[33] The authors report that the atrazine was 98% purity and obtained from Greyhound Service, UK. The purity and source of atrazine were reported with the first of the four experimental types (single-species tests, indoor and outdoor microcosms, and the outdoor mesocosms) reported in this publication, so presumably it applies to all, including the outdoor mesocosm studies.

[34] In contrast, the first experiment described (single species tests) indicates that acetone was used at a concentration of 0.05% in treatments and controls. The next two experiments described (indoor and outdoor microcosms) indicate that there was a solvent control but there are no details, suggesting that the earlier details were not repeated and applicable to these experiments. The last experiment described (outdoor mesocosm) indicates that acetonitrile was used but does not state the concentration, again seeming to suggest that the earlier 0.05% applied to acetonitrile as well. Although EPA does not know for certain, EPA thinks this is a reasonable interpretation for the level of redundancy that would be provided in most open literature studies.

Phytoplankton was sampled with a homemade sampler from several points in each experimental unit and were pooled for analysis. One liter of sample was filtered through a Whatman GF/C filter for chlorophyll *a* determination (measured spectrophotometrically).

Effects of atrazine on photosynthetic efficiency was assessed by calculating photosynthesis-irradiance (P/I) curve parameters in one control and one 30 μg/L treatment mesocosm. Three parameters [α (*i.e.*, initial slope of the light saturation curve), β (*i.e.*, photoinhibition parameter), P_{max} (*i.e.*, the maximum biomass-specific production rate)], were calculated prior to treatment, immediately after treatment, and on Day 11. Photosynthetic efficiency was not considered for the cosm database because there was no replication for photosynthetic activity measurements (*i.e.*, measurements from independent cosms of the same treatment group and control).

Samples were taken pre-treatment, during exposure, and at the end of the experiment to identify and count phytoplankton cells. A 50 mL aliquot of 'algal suspension' was taken from each tank. Lugol's solution was added to kill and stain cells. Afterwards, cells were allowed to settle for 24 hours, and then were examined under a reverse-phase microscope for identification and enumeration. The abundance of the dominant species was estimated ±10%. The density of each dominant species (defined by study authors as >100 individuals/mL) in the treatment groups was compared to that of the controls using the Mann-Whitney test (p-values not reported). Compositional differences between treatment and control communities were described using the Bray-Curtis dissimilarity index (BCI; taxonomic resolution unknown). The BCI values were calculated for the mean densities in replicate communities, and significant differences in community composition between atrazine treatment and the control were assessed by applying the Mann-Whitney test to the within-control BCI versus the control-treatment BCI[35].

[35] The authors state "*Mann Whitney test (90%)*", which presumably means an alpha level was 0.1. The BCI discussion for the outdoor microcosms' states "*(p = 0.1; n = 6)*", which is consistent. There is some potentially confusing wording in the publication about the implementation of the Bray-Curtis dissimilarity index. The methods state that the Bray-Curtis dissimilarity index was used, but its implementation by the study authors refer to the BCI as a similarity index (presumably 1-dissimilarity) or just as an index thereafter. Another publication by these authors (Berard *et al.*, 1999) is cited for more information on the approach, and the discussion in that paper is consistent with presentation of the BCI data as a similarity (1-dissimilarity).

7.3 EPA's Use as of 2016

In the 2016 Refined Ecological Risk Assessment (USEPA, 2016), EPA included four endpoints that were based on the outdoor mesocosm studies in the 2001a and 2001b Seguin publications (**Table 7.1**). Periphyton endpoints (2001a publication) were scored as "No Effect" for 2 µg/L and 30 µg/L (endpoints #84 and #83, respectively). Phytoplankton endpoints (2001b publication) were scored as "Effect" for 2 µg/L and 30 µg/L (endpoints #86 and #85, respectively).

Table 7.1. A summary of the cosm endpoints associated with this group that appeared in Appendix G.2 of the 2016 Refined Ecological Risk Assessment. These endpoints were used to evaluate the potential effects of atrazine on aquatic plant communities.

References	Endpoint Number	Duration (days)	Nominal Conc. (µg/L)	Plant Group	Results and Recovery	Effect/No-Effect Conclusion
Seguin *et al.* (2001a)[A]	84	40	2	Peri	No effect Recovery: Not applicable	No Effect[B]
Seguin *et al.* (2001a)[A]	83	40	30	Peri	Increase (61%) in periphyton chlorophyll*a* concentration at Day 40. Recovery: Not reported	No Effect[C]
Seguin *et al.* (2001b)[A]	86	40	2	Phyto	Increased Chrysophyceae abundance on day 40 (unknown magnitude). Recovery: Not reported	Effect[D]
Seguin *et al.* (2001b)[A]	85	40	30	Phyto	Change in phytoplankton community structure (change in Bray-Curtis index starting on day 2 and increased Chrysophyceae abundance of unknown magnitude on day 40). Recovery: Not reported	Effect

[A] Also examined effects of nicosulfuron in separate treatments.
[B] Non-significant increase (14%) in periphyton chlorophyll *a* concentration at day 40
[C] Increased chlorophyll observed only at study termination.
[D] Effect on abundance observed at study termination.

7.4 Stakeholders' Major Concerns and Criticisms

This section is grouped and organized by common themes of major concerns and criticisms. It is intended to be a synthesis of overarching issues rather than a duplication of every statement previously made on these studies.

7.4.1 Combining Endpoints

Syngenta has consistently asserted that the outdoor mesocosm experimental results reported in Seguin *et al*. (2001a; periphyton) and Seguin *et al*. (2001b; phytoplankton) should be combined

on the premise that they are "*presumably from the same experiment*" and splitting or combining of different parts of the community should be consistent across the database (Syngenta, 2012a, 2016, 2022). They also point out that EPA stated that the response of the overall plant community (rather than separate macrophyte, periphyton, and phytoplankton communities) is consistent with the protection goal, and therefore EPA "*has maintained its approach of a single endpoint per test concentration in each experiment*."

7.4.2 Interpretation of the Seguin *et al*. (2001a) Periphyton 30 µg/L Results (#83)

Three main comments have been made about interpretation of the periphyton response at 30 µg/L. The first is from the Center for Biological Diversity (CBD, 2016; 2022) that the statistically significant increase in periphyton chlorophyll *a* should be considered an effect to the community. The second is from the 2012 SAP (USEPA, 2012b) stated that "*exposure to 2 and 30 µg/L of atrazine had a stimulatory effect on periphyton production, but this was incorrectly categorized as a negative effect*". The third is also from the 2012 SAP that stated "*Seguin et al. (2001b) No significant effects on periphyton biomass were observed at 30 µg/L of atrazine. The effects observed appear to be based on shifts in phytoplankton community."*

7.4.3 Interpretation of Periphyton and Phytoplankton 2 µg/L Results (#84 and 86)

Two main comments have been made about interpretation of the response at 2 µg/L. The first is that there should not be an "Effect" on phytoplankton at 2 µg/L on grounds that it is a "*slight, transient and/or temporally inconsistent effect*" or stated differently ""*2 µg/L did not elicit a response that justifies "effect" designation, nor prediction of* "*permanent or irreversible change in the aquatic plant community structure, function, and/or productivity would be expected*""(*e.g.*, Syngenta, 2012a, 2012b, 2016, 2022; Triazine network, 2012, 2016, 2022). EPA has previously scored the phytoplankton response at 2 µg/L as an effect. The second comment is about the response of the periphyton and is the same comment that the 2012 SAP made about the periphyton response at 30 µg/L (*i.e.*, the effect is stimulatory; *see* **Section 7.4.2**).

7.4.4 Solvent

One comment has been made about the solvent used. The 2012 SAP (USEPA, 2012b) pointed out that "*Acetonitrile was used as solvent, but amount added [was] not listed. Rarely is this*

solvent used in ecotoxicological dosing." but did not provide any recommendations or specific criticism. This critique was later reinforced by the Triazine network (2016).

7.5 EPA's 2023 Reevaluation

After consideration of all of the studies' limitations, the determinative concerns will be discussed here, including those brought up by past SAPs and public commenters.

7.5.1 Combining endpoints

As discussed above, EPA's 2016 cosm database contained four endpoints for the Seguin *et al.*(2001a; periphyton) and Seguin *et al.* (2001b; phytoplankton) publications:

#84 – Represented the 2 µg/L periphyton response
#83 – Represented the 30 µg/L periphyton response
#86 – Represented the 2 µg/L phytoplankton response
#85 – Represented the 30 µg/L phytoplankton response

Syngenta suggested that EPA combine #84 with #86 and #83 with #85 on grounds that they are presumably from the same experiment and to maintain consistency within the cosm database of splitting or lumping responses of different parts of the plant community (*e.g.*, periphyton, phytoplankton, and macrophytes).

EPA agrees that periphyton and phytoplankton results should be represented by a single endpoint if they are from the same experiment.

Although the Seguin *et al.* (2001a) and Seguin *et al.* (2001b) publications are not directly linked with each other or the subsequent publication (Seguin *et al.*, 2002) and there are some minor differences in wording, the basic description of the experimental setup is identical (*e.g.*, experimental location, mesocosm construction, replication, test concentrations, testing of both atrazine and nicosulfuron, and test duration) with the exception that the inclusion of the periphyton slides is not mentioned in the publication describing results for phytoplankton (Seguin *et al.*, 2001b). There are some minor apparent differences in the description of the timing. For example, Seguin *et al.* (2001a) says that mesocosms were allowed to develop for one

month and the Seguin *et al*. (2001b) publication says that mesocosms were allowed to develop for three weeks before exposure. The Seguin *et al*. (2001a) publication indicates that the experiment was conducted in the summer of 1998, while the Seguin *et al*. (2001b) publication is silent on the dates of the outdoor mesocosm experiment, but the other reported experiments in that paper were conducted in the same time frame (1997-1998). Another inconsistency is that the Seguin *et al*. (2001b) publication says both pesticides were dissolved in acetonitrile, whereas the Seguin *et al*. (2001a) publication is silent. Nonetheless, the strongest indication that the two publications are describing the same experiment is the identical and precise water quality conditions reportedly measured in the mesocosms (**Figure 7.1**).

A. Table 1 from Seguin et al. (2001a)

Table 1. Main water nutrient concentrations and temperature in the mesocosms.

Tableau 1. Principales caractéristiques de l'eau des mésocosmes.

orthophosphate (mg P/L)	0,0065
inorganic nitrogen (mg N/L)	0,51
mean temperature	18°C
minimum	14,4°C
maximum	24,8°C

B. Table 2 from Seguin et al. (2001b)

Table 2. Main water nutrient concentrations and temperature in the mesocosms

Orthophosphate (mg P/L)	0.0065
Inorganic nitrogen (mg N/L)	0.51
Mean temperature (°C)	18
minimum	14.4
maximum	24.8

Figure 7.1. Excerpts of tables from the 2001 Seguin studies that show the water quality conditions of the mesocosms

Based on EPA's 2023 reevaluation of this group, EPA has decided to combine endpoint #86 with #84 and endpoint #85 with #83 on grounds that there is a high likelihood that the periphyton and phytoplankton results are from the same experiment. To do this, EPA will be removing endpoints #86 and #85 from the cosm database and the following endpoints will now be associated with both Seguin *et al*. (2001a) and Seguin *et al*. (2001b):

#84– Represents the 2 µg/L periphyton and phytoplankton response

#83– Represents the 30 µg/L periphyton and phytoplankton response

7.5.2 Interpretation of 30 µg/L Results (now solely endpoint #83)

As discussed above, the two main comments about interpretation of the periphyton response at 30 µg/L are conflicting: one argues that the stimulatory effect on periphyton chlorophyll *a* is an effect and the other that this effect was incorrectly categorized as a negative effect.

The latter was either a misinterpretation of EPA's position or it was a typo. EPA previously considered there to be no effect on periphyton at 30 µg/L, as stated in all EPA documentation discussing this endpoint to date. Regarding the stimulatory effect, EPA does not consider stimulatory effects on biomass (chlorophyll *a* is a proxy for biomass) to be adverse effects in isolation. The previous position by EPA on this endpoint was based on the premise that the periphyton results were separate from those presented on phytoplankton. Certainly, an increase in the biomass of one component of the plant community and not another would be indicative of a shift in community structure, but there needs to be comparable data from another part of the plant community to make that inference. Given the linking of the Seguin *et al*. (2001a) and Seguin *et al*. (2001b) results, it can be argued that there may have been a shift in the structure of the overall non-macrophyte part of the plant community (periphyton + phytoplankton) at the end of the study (Day 40[36]) given that periphyton (**Figure 7.2A**) chlorophyll *a* in the atrazine treatment increased compared to the control, whereas there was no change in the chlorophyll *a* of the phytoplankton in the atrazine treatment compared to the control (**Figure 7.2B**). There is considerable uncertainty with this inference, if for no other reason than changes in chlorophyll *a* are a proxy for biomass but do not necessarily correlate 1:1 with algal abundance.

[36] The Figure 2 in Seguin *et al*. (2001a) shows plots data by calendar date rather than the number of Days of the experiment as in Figure 4 in Seguin *et al*. (2001b). Given the reported dates, Day 40 would be the final point in Figure 2 to the right of the calendar date 7/25 (*i.e*., 7/29).

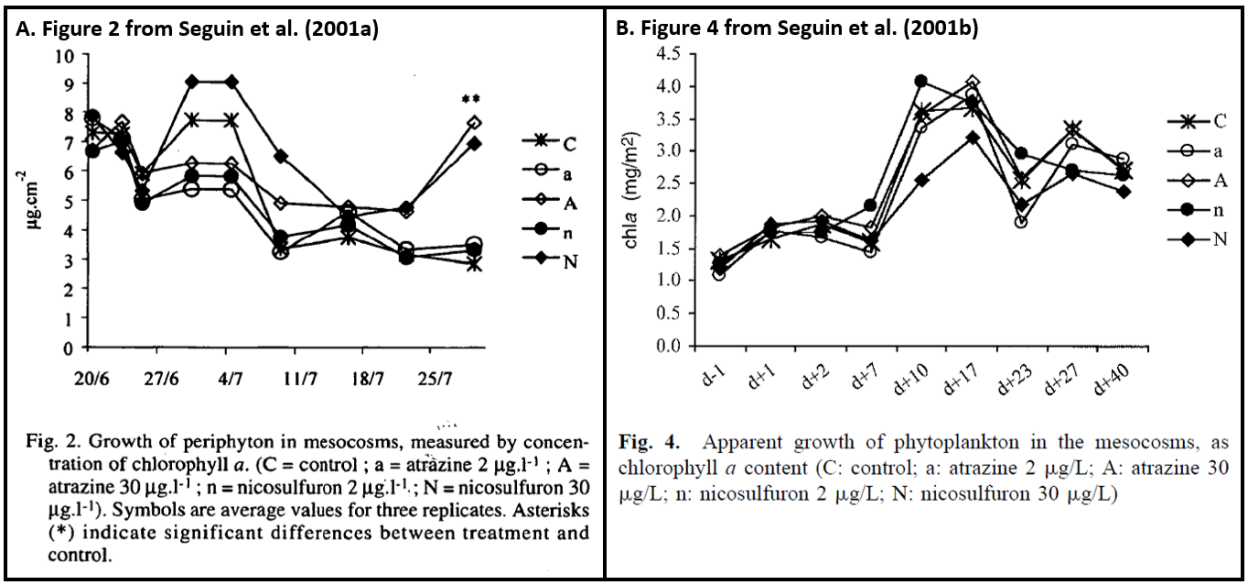


Fig. 2. Growth of periphyton in mesocosms, measured by concentration of chlorophyll *a*. (C = control ; a = atrazine 2 µg.l^{-1} ; A = atrazine 30 µg.l^{-1} ; n = nicosulfuron 2 µg.l^{-1} ; N = nicosulfuron 30 µg.l^{-1}). Symbols are average values for three replicates. Asterisks (*) indicate significant differences between treatment and control.

Fig. 4. Apparent growth of phytoplankton in the mesocosms, as chlorophyll *a* content (C: control; a: atrazine 2 µg/L; A: atrazine 30 µg/L; n: nicosulfuron 2 µg/L; N: nicosulfuron 30 µg/L)

Figure 7.2. A. Excerpt of Figure 2 from Seguin *et al.* (2001a). B. Excerpt of Figure 4 from Seguin *et al.* (2001b)

Another consideration is that the study authors indicate that the periphyton community consisted mainly of one species, *Achnanthidium minutissimum* (the exact percentage was not reported) with other species present at "*very low percentages*". This community composition dominated by a single species does somewhat limit the utility of the results for understanding community-level effects, yet may represent the diversity of some natural communities. Nonetheless, an effect has already been established on phytoplankton at 30 µg/L (change in community composition as measured by the Bray-Curtis indices[37]); therefore, with the combining of the Seguin *et al.* (2001a) and Seguin *et al.* (2001b) endpoints, there is an "Effect" at 30 µg/L independent of the interpretation of the meaning behind the stimulation of chlorophyll *a* in periphyton and the ecological relevance of a community dominated by a single species.

The third comment stated "*Seguin et al. (2001b) No significant effects on periphyton biomass were observed at 30 µg/L of atrazine. The effects observed appear to be based on shifts in phytoplankton community."* Based on the totality of the comment, EPA assumes that there is a typo and that "periphyton" should be "phytoplankton". EPA agrees that there were effects on phytoplankton, which is consistent with the previous "Effect" call for the 30 µg/L treatment group.

[37] See section 7.5.3 about reinterpretation of the potential effect in Chrysophyceae at Day 40.

Therefore, based on the 2023 reevaluation, endpoint #83 will represent the combined results of the Seguin *et al*. (2001a) and Seguin *et al*. (2001b) publications and be scored as **"Effect."**

7.5.3 Interpretation of 2 µg/L Results (now solely endpoint #84)

As discussed above, two main comments have been made about interpretation of the response at 2 µg/L: one that there was no effect on periphyton and the second that there was no effect on phytoplankton. In terms of whether there is an effect on periphyton, this was either a misinterpretation of EPA's position or it was a typo. EPA previously considered there to be no effect on periphyton at 2 µg/L, as stated in all EPA documentation discussing this endpoint to date.

With respect to phytoplankton, EPA has previously scored the phytoplankton response at 2 µg/L as an effect. Many of the previous critiques misunderstood or misinterpreted the reason that EPA scored the response as an effect. More specifically, many of the comments discuss effects observed in the Bray-Curtis Index and the density of Chlorophyceae as being transient and not clearly treatment related. For example, Syngenta (2012a) stated that "*Adverse effects on phytoplankton at 2 µg/L were therefore limited to a decrease in Chlorophytes on one isolated sample event (Day 17), and a change in community composition during the first week after atrazine treatment (Days 2 and 7) but not subsequently. The magnitude of the inferred effect on community composition is difficult to discern from the Bray-Curtis results presented (Figure 12), but it appears to be small. In light of the absence of other effects, CSI considered the reported effects at 2 µg/L to be slight and transient, and assigned a binary score of "0" to ID #86.*" EPA agrees with the characterization that those effects are transient (**Figure 7.3B and C**). Although that point may not have been stated directly in documentation such as the 2016 Refined Ecological Risk Assessment (USEPA, 2016), EPA's Data Evaluation Records (DER) was clear on this interpretation.

A. Figure 5

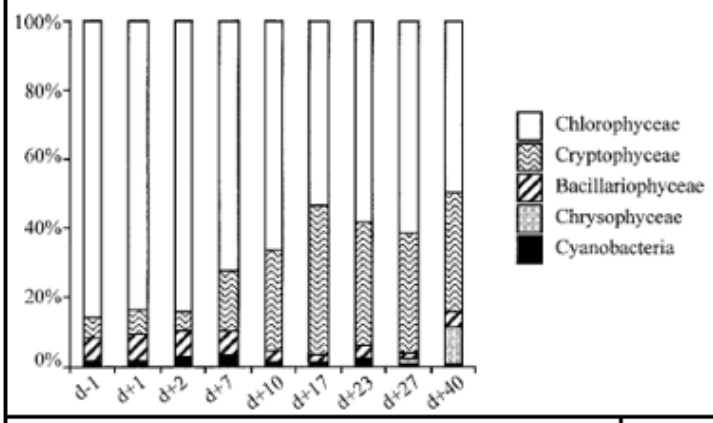


Fig. 5. Class-level taxonomic composition of algal communities in mesocosms at the different sampling periods (d−1 = day before the contamination; d+i = i days after the contamination date), expressed in % of the total phytoplankton identified

B. Figure 6

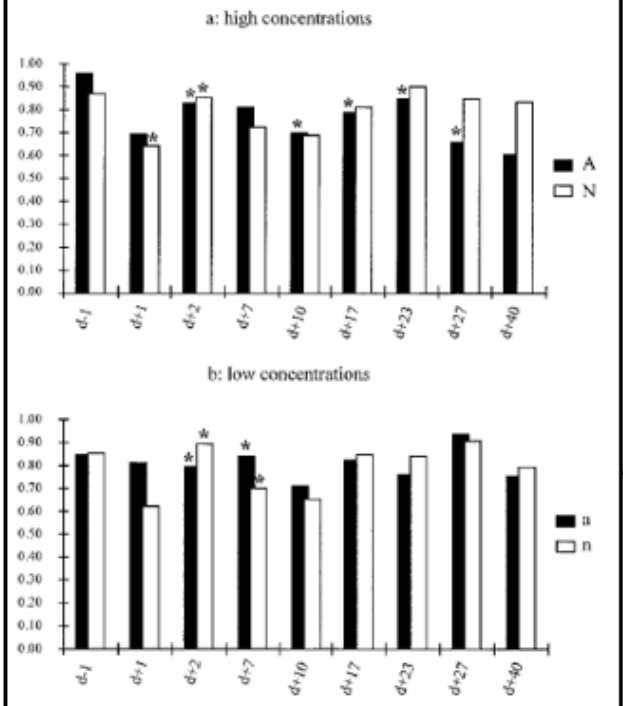


Fig. 6. Bray-Curtis indices of the different phytoplankton samples in the mesocosms. The BCI were calculated for the mean density of replicate communities, and the significance of difference between control and treated was tested with a Mann-Whitney test between indices calculated among controls (C-C) and indices calculated between controls and tests (C-A).*: (C-A/C-C) Mann-Whitney test (90 %)

C. Table 5

Table 5. Significative response of dominant taxonomic groups to 2 µg/L and 30 µg/L atrazine and 2 µg/L and 30 µg/L nicosulfuron in mesocosms (Mann-Whitney nonparametric test at each sampling)

Class	Atrazine 2 µg/L	Atrazine 30 µg/L	Nicosulfuron 2 µg/L	Nicosulfuron 30 µg/L
Bacillariophyceae	0	> d2	0	0
	0	> d10	0	> d10
Cryptophyceae	0	0	0	> d27
Chlorophyceae	< d17	< d17	0	< d17
	0	< d23	< d23	0
	0	0	0	> d40
Chrysophyceae	> d40	> d40	> d40	> d40

<: fewer density of individuals than in controls. >: greater density of individuals than in controls
0: no significant effect
Significant effects appeared after several days: d2 means 'day 2,' d10 means 'day 10,' etc

Not relevant to this reevaluation

Figure 7.3. Excerpt of page 205 of Seguin *et al*. (2001b).

EPA solely based the effect call for 2 µg/L on the increased Chrysophyceae abundance on Day 40 (study termination; **Figure 7.3C**). Although the effect was acknowledged by others, there has not been a specific rebuttal of which EPA is aware to not consider the effect on Day 40 as being

treatment related. Perhaps this is because one of the review criteria used by Giddings (Syngenta, 2012a) was "*Did statistically significant differences from controls occur on at least two consecutive sampling dates?*". This criterion would automatically consider the effect on Day 40 to not be treatment related. While there is merit to considering the temporal pattern of observed significant differences, the specific approach by Giddings would exclude potential effects that are first observed at study termination (*note*: this approach also excludes potential effects for endpoints that are only measured at study termination). Therefore, EPA has considered potential effects observed at study termination to be potentially treatment related, with the acknowledgement and caveat that had the study been extended, that effect may have been revealed to be transient.

EPA took a closer look at other lines of evidence to determine in a weight-of-evidence approach the likelihood that the increased abundance of Chrysophyceae may have been transient or slight (*i.e.*, no effect) or potentially was treatment related or more substantial in magnitude or if there was no additional information either way. This approach was taken because the lack of additional time points precludes a direct determination of whether the increased abundance was transient, and the magnitude of effect is unknown. Based on the totality of the lines of evidence below, EPA considers that the author-reported successional dynamics occuring during the experiment combined with similar responses in a second tested chemical (nicosulfuron), the lack of magnitude data, and lack of additional time points to judge the difference between the atrazine treatments and controls, collectively raise enough uncertainty to question if increased Chrysophyceae abundance on Day 40 was an effect due to atrazine exposure:

- The increase in Chrysophyceae density appears only on Day 40 and occurs in all treatment groups (2 and 30 µg/L for both atrazine and nicosulfuron) (**Figure 7.3C**).
- There were successional shifts that occurred throughout the study, which can complicate the ability to discern treatment related effects.
    - There is a sudden increase in Chrysophyceae abundance on Day 40 as shown in **Figure 7.3A**.
    - Although potentially representing control data, unfortunately it is not explicitly stated what is presented in **Figure 7.3A** (*e.g.*, controls only or all experimental tanks), but the text says, "*There was a species succession during the experiment*

in all tanks (Figure 5)." and that "*the succession in all the tanks was similar to that of the controls, with some differences in time lags and proportions*".

- Successional shifts are a potentially confounding factor in any mesocosm study; however, they can be considered in the lens of magnitude of effect and repeated differences between the treatments and controls over time. In this case, the successional dynamics seem to have an overweighted impact as there is enough information to raise considerable uncertainty that the observation at Day 40 was treatment related. Specifically, it would be highly coincidental that two test substances (atrazine and nicosulfuron) with different modes of action would cause the same treatment-related effect on the same class of algae at two test concentrations at the same time point, especially 40 days after the initial exposure. A more reasonable interpretation is that reported successional shifts in the community may have been a major contributing factor for the results on Day 40. Given a concurrent increased abundance of the control and all the treatment groups, the control could have randomly not shown the same rate of succession as the treatment groups. The lack of magnitude of effect and additional time points after Day 40 precludes the ability to exclude the aforementioned successional shift mentioned by the study authors and are confounding factors in distinguishing treatment-related effects from successional changes (for all test concentrations and both test chemicals) for Chrysophyceae abundance on Day 40.

- Although there was a statistically significant increase in Chrysophyceae abundance, there was not a corresponding statistically significant difference in the Bray-Curtis Index on Day 40 (**Figure 7.3B**) between any of the treatment groups (atrazine or nicosulfuron) and the control, suggesting that the magnitude of the change in abundance may not have been large, and least in the context of the entire community. Although the level of taxonomic resolution used for the Bray-Curtis Index was not reported, it is most likely the same or greater resolution (down to species) as the Class level used for the other comparisons. Although the lack of a statistically significant difference does not preclude the potential importance of a shift in abundance of a single taxonomic class, a statistically significant difference in the Bray-Curtis Index would lend support to the possibility that increased abundance of Chrysophyceae was more than slight.

- All other statistically significant effects observed at 2 µg/L prior to Day 40 were considered transient (including for nicosulfuron, **Figure 7.3B** and **C**); therefore, there is a precedence prior to Day 40 for transient effects to randomly appear in the endpoints measured.

After reconsideration, EPA considers that successional dynamics confounds interpretation of the effect in Chrysophyceae at Day 40. EPA previously considered there to be no effect on periphyton at 2 µg/L, as stated in all EPA documentation discussing this endpoint to date. Therefore, for the combined results of Seguin *et al*. (2001a) and Seguin *et al*. (2001b) leads to a "**No Effect**" call for endpoint #84.

7.5.4 Solvent

As indicated above, the 2012 SAP panel pointed out that acetonitrile was used as solvent, it is rarely used in ecotoxicological testing, and that the amount used was not reported. This comment was specific to Seguin *et al*. (2001b) because Seguin *et al*. (2001a) was silent on how atrazine was dissolved. However, this discussion applies to both publications because EPA considers that the results from Seguin *et al*. (2001a) and Seguin *et al*. (2001b) to be from the same experiment (*see* **Section 7.5.1**). Although acetonitrile is not a standard solvent used in ecotoxicity testing and the concentration used was not reported, EPA considers it reasonable to retain the results from this study in the cosm database based on what is known about acetonitrile and the use of a solvent control in the experimental design.

EPA agrees that acetonitrile is not a typical solvent used for ecotoxicity testing (at least in EPA's experience for testing of pesticides). Although EPA has identified preferred solvents for aquatic ecotoxicity testing (*e.g*., dimethylformamide, triethylene glycol, methanol, acetone, and ethanol; see OCSPP 850.1000), the use of other solvents is not exclusionary for study acceptance, including for the atrazine mesocosm database. The preferred solvent in algal tests is dimethylformamide (see OCSPP 850.4500 and 850.4550). That said, the solvent used should be low toxicity to the organisms being tested. EPA is not aware of a large body of experimental studies on the toxicity of acetonitrile to aquatic plants. However, the literature review conducted

by the European Chemicals Agency (ECHA)[38] indicates that acetonitrile has very low toxicity to tested freshwater and marine algae:

> "*Reported toxicity values for acetonitrile in freshwater algae range from 520 mg/L (8-day TT in the blue-green algae Microcystis aeruginosa) to 7,943 mg/L (48-hr EC_{50} in the green algae Raphidocelis subcapitata). A 72-hr NOEC of 400 mg/L was reported in a Guideline study of the marine algae Phaeodactylum tricornutum.*
>
> *The toxicity of acetonitrile has been studied in freshwater and marine algae. A summary of the available data is provided in the table below. Toxicity threshold values based on the first detectable inhibition of cell multiplication for the green algae (Scenedesmus quadricauda) and for the blue-green algae (Microcystis aeruginosa) has been reported by Bringmann and Kühn as 7300 and 520 mg/L respectively*."
>
> *Chen et al. (2005) reported 48 -hour, static EC_{50} values of 5926 mg/L (based on dissolved oxygen production) and 7943 mg/L (based on growth rate) for acetonitrile in Green algae, (Raphidocelis subcapitata). The methods used were designed to eliminate head space, and thus avoid loss of test substance by volatilization. This method is reported by the investigators to be more sensitive than the conventional (open) algal batch tests.*
>
> *In a guideline (OECD 201) GLP study, MCSI of Japan (1996) reported the 72 -hr NOEC values (biomass, growth rate) for acetonitrile in alga (Selenastrum capricornutum) to be >1000 mg/L.*
>
> *In a Guideline GLP study (CEMR, 2010) the effect of acetonitrile on the unicellular marine algae (Phaeodactylum tricornutum) was assessed over 72 hours using a static, sealed test system with no headspace to avoid loss of the test substance by volatilisation from the test system. After 72 hours the following effect concentrations were calculated:*

[38] https://echa.europa.eu/registration-dossier/-/registered-dossier/15440/6/2/6
Website accessed 2-24-23

ErC50(growth rate) = 9696 (95% CI: 7484 – 17,597) mg/L

EyC50(yield) = 3560 (95% CI: 1604 – 7017) mg/L

The "no observed effect concentration" (NOEC) and "lowest observed effect concentration" (LOEC) for both growth rate and yield where statistically derived as 400 and 1600 mg/L respectively."

Toxicity of acetonitrile to algae

SPECIES	TEST TYPE	Duration	TOXICITY END POINT (mg/l)	REFERENCE
Raphidocelis subcapitata(green algae)	Static, nominal concentration, no head space	48 hr	EC_{50}= 5926 Disolved oxygen production	Chen et al (2005)
Raphidocelis subcapitata(green algae)	Static, nominal concentration, no head space	48 hr	EC_{50}= 7943 Growth rate	Chen et al (2005)
Microcystis aeruginosa (blue-green algae)	Static, nominal concentration	8 days	TT = 520	Bringmann and Kühn (1978)
Scenedesmus quadricauda (green algae)	Static, nominal concentration	8 days	TT = 7300	Bringmann and Kühn (1978)
Selenastrum capricunutum (green algae)	Static, nominal concentration	72 hr	NOEC > 1000	MCSI, Japan (1996)
Phaeodactylum tricornutum (marine algae)	Static, nominal concentration, no head space	72 hr	NOEC = 400	CEMR (2010)

TT = toxicity threshold for inhibition of cell multiplication.

Therefore, while avoiding the use of organic solvents is preferred, their use is sometimes necessary (though it is not evident that one was necessarily needed in this case). While data suggest that acetonitrile is not of particular toxicological concern, there is uncertainty if it could have effects other than toxicological on the test system. Ideally, a negative and solvent control would be used to show if the solvent had any potential impact on the experiment. In this case, the experiment included a solvent control for comparison. Although it would be ideal to also have a negative control, the use of multiple controls is generally prohibitive in large scale mesocosm experiments, and the inclusion of the solvent control is the more important of the two in accounting for potential impact of the solvent on the biological response. Nonetheless, it remains unknown if the solvent had any effect without the inclusion of a negative control.

The other potential concern regarding solvent use is that the publications do not describe the concentration or amount of acetonitrile in either the stock solution or the mesocosm tanks. The primary concern about the absence of this information is if the concentration of acetonitrile was high enough to potentially confound the interpretation of results. For example, 2.55 L of acetonitrile would be needed to achieve an acetonitrile concentration in the mesocosm (5000 L) that reaches the lowest reported NOAEC for acetonitrile (400 mg acetonitrile/L, keeping in mind that this is for a marine species; NOAEC coverts to 0.51 mL acetontrile/L[39])[40]. Coincidently, this is essentially the same concentration for which the study acceptance criteria require the solvent to be below (<0.5 mL solvent/L in static systems or 2.5 L of acetonitrile in the mesocosms used in this experiment; USEPA, 2011).

Although speculative, the solubility of atrazine in acetonitrile can be used to provide perspective on the amount of acetonitrile in the mesocosm if excessive amounts of acetonitrile were not used to dissolve the atrazine. EPA is not aware of published data on the solubility of atrazine in acetonitrile; however, information is available from commercial atrazine standard products that inform on the minimum solubility of atrazine in acetonitrile. The maximum reported solubility that EPA has located is ≥1000 µg atrazine/mL acetonitrile.[41] At this concentration, ≤0.15 L of acetonitrile would need to be added to the 5000 L mesocosm to achieve the highest concentration tested (30 µg atrazine/L), which is at least 17 times lower than the amount (2.5 L) that would be equivalent to the lowest NOAEC of tested algal species and the screening criterion for maximum solvent concentration (<0.5 mL solvent/L or 0.05% in static systems; USEPA, 2011).[42] The most common atrazine standard concentration sold seems to be 100 µg atrazine/mL acetonitrile (for example, see footnoted citations[43]) and even with that lower concentration, the amount of

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[39] Toxicity calculation
NOAEC in mg: 400 mg acetonitrile/L
Density of acetonitrile: 786 mg/mL (from https://www.chemicalbook.com/ChemicalProductProperty_EN_CB2127174.htm)
NOAEC converted to mL/L: 400 mg acetonitrile/L / (786 mg/mL) = 0.51 mL

[40] Total in cosm calculation: 0.51 mL acetonitrile * 5000 L mesocosm = 2.55 L acetonitrile

[41] https://www.spex.com/getmedia/e2c57ab0-ab5f-4dc2-a63a-28a82edd70d9/Product-Guide-to-Pesticide-Solubility.pdf?ext=.pdf

[42] 30 µg atrazine/L * 5000 L mesocosm = 150,000 µg atrazine per mesocosm. Assumed stock concentration = 1000 µg atrazine/mL acetonitrile. 150,000 µg atrazine / 1000 µg atrazine/mL acetonitrile = 150 mL acetonitrile per mesocosm

[43] https://www.chemservice.com/atrazine-solution-s-11106a1-1ml.html
https://www.lgcstandards.com/US/en/p/DRE-XA10330000AL
https://www.delta-sci.com/products/dre-xa10330000al-atrazine-100-ug-ml-in-acetonitrile

acetonitrile added to the mesocosms would be 1.5 L (same calculations as presented in footnote for ≥1000 µg atrazine/mL acetonitrile), which again is less than 2.5 L acetonitrile. Although the concentration of solvent used is unknown and uncertainty remains, the apparent typical atrazine analytical standard concentration and the highest analytical standard concentration located by EPA (which may not represent the upper limit of solubility of atrazine in acetonitrile) indicate that the amount of acetonitrile used in this experiment would not need to exceed EPA's acceptance criteria for the cosm studies.

Finally, it can be argued, independent of EPA's acceptance criteria for solvent use (<0.5 mL solvent/L; USEPA, 2011), that an untested algal species could be more sensitive than that concentration and could be impacted by use of a solvent; however, this is an uncertainty that is not exclusive to acetonitrile. It applies to the use of any solvent in the absence of testing of all potential species in the plant communities used across all of the mesocosm experiments. Furthermore, as stated above, a solvent control was available in this experiment for comparison. This is in contrast to the Lampert (1989) study, which also did not report the concentration of solvent used but provided no indication that anything other than a negative control was used for comparison. Therefore, assuming conservation of any impact of the solvent among the mesocosms in the Seguin *et al*. study, differences between the treatment groups and controls can be reasonably considered to reflect potential treatment related effects.

7.5.5 EPA's 2023 Conclusions

While the studies have deficiencies, EPA has decided that the studies are sufficient to contribute to our knowledge about the effects of atrazine to aquatic plant communities under the conditions of the experiment. These studies contribute to our understanding of potential effects on natural aquatic plants communities exposed to atrazine when considered within the context of the larger collective body of experimental data from other cosm studies. Therefore, while there is uncertainty in the results, it is reasonable to include these studies in the cosm database. **Based on the changes discussed above, EPA's cosm database now has two endpoints (compared to four) for the Seguin *et al*. (2001a) and Seguin *et al*. (2001b) group with one "Effect" (#83) and one "No Effect" (#84) endpoint** (**Table 7.2**).

Table 7.2. A summary of the cosm endpoints associated with this group that remain after the 2023 reevaluation. These endpoints will be used to evaluate the potential effects of atrazine on aquatic plant communities.

References	Endpoint Number	Duration (days)	Nominal Conc. (µg/L)	Plant Group	Results and Recovery[A]	Effect/No-Effect Conclusion
Seguin *et al*. (2001a); Seguin *et al*. (2001b)	84	40	2	Peri Phyto	*Periphyton*: No effect Recovery: Not applicable *Phytoplankton*: Increased Chrysophyceae abundance at study termination (Day 40; unknown magnitude)[A]. Recovery: Not reported	No Effect
Seguin *et al*. (2001a); Seguin *et al*. (2001b)	83	40	30	Peri Phyto	*Periphyton*: Increase (61%) in periphyton chlorophyll *a* concentration at Day 40[B]. Recovery: Not reported *Phytoplankton*: Change in phytoplankton community structure (change in Bray-Curtis index starting on day 2 and increased Chrysophyceae abundance of unknown magnitude on day 40) [A]. Recovery: Not reported	Effect

[A] Successional dynamics combined with concurrent similar responses in a second chemical and the lack of magnitude data raises uncertainty if this effect was due to atrazine exposure.

[B] Stimulatory effects on biomass are not considered adverse effects. Inconclusive if it represents a shift in the structure of the overall non-macrophyte part of the plant community (periphyton + phytoplankton) at the end of the study (day 40) given that there was no change in biomass of phytoplankton in the atrazine treatment compared to the control.

CHAPTER 8. SEGUIN – 2002 PUBLICATION

8.1 Overview

Seguin *et al*. (2002) was one of the 11 studies flagged by the 2012 SAP as warranting further review (USEPA, 2012b). This publication presents results from freshwater outdoor lentic mesocosms (tanks) located in France. The experiment presented is similar to, but independent from, the experiment presented in Seguin *et al*. (2001a) and Seguin *et al*. (2001b); however, the results presented in Seguin *et al*. (2002) only focus on the phytoplankton community. The Seguin *et al*. (2002) publication also includes details on short-term experiments; however, they are not considered further because they were previously excluded from the cosm database due to the uncertainty surrounding exposure concentrations.

The 2012 SAP comment on Seguin *et al*. (2002): "*While the results indicate that there was a 30% reduction in algal biomass over a 21-day exposure to 30 µg/L atrazine, recovery was not studied. The preponderance of evidence in the literature indicates that recovery would be expected*."

8.2 Experimental Design and Execution

The Seguin *et al*. (2002) experiment took place during spring 2000. Mesocosms consisted of 4 outdoor circular tanks at the INRA (Institut National de la Recherche Agronomique, Rennes, France) experimental platform. The tanks were 3.2 m in diameter, 1.2 m high, held approximately 5000 L, and were sunk about 0.4 m into the ground to keep temperatures more stable. The mesocosms were created by first filling the tanks to 7 cm with natural sediment from a local uncontaminated pond, then filling them to a depth of 0.7 m with natural pond water from a nearby site (not stated whether the site is the same as the sediment collection pond, or whether sediment and/or pond water were sampled for atrazine contamination). The natural pond water was used as the source for phytoplankton. Zooplankton added to the mesocosms were sampled from "several local ponds" (not described further) using a net with a mesh size of 63 µm. The aquatic macrophyte *Glyceria maxima* was also added. Mesocosms had 3 weeks to develop before atrazine treatment. Two mesocosms were treated with 30 µg/L atrazine (initial concentration) and two served as controls. The brand, grade, and application method of atrazine were not described, and it is not stated whether a solvent was used. However, the authors cite Seguin *et al*.

(2001b) for "*method and setup of the experimental units*". Seguin *et al.* (2001b) indicates that atrazine was 98% purity and obtained from Greyhound Service, UK and that acetonitrile was used as a solvent of atrazine (concentration not reported) with the use of solvent controls.

Dissolved oxygen (DO) and pH were monitored in the mesocosms daily (early morning). Water temperature was measured continuously *in situ*, and surface irradiation measurements were taken from a nearby meteorological station (exact location/distance from mesocosms not stated). Phytoplankton was sampled using a homemade sampler consisting of a 70 cm long Plexiglass tube with a total volume of 2.5 L. Samples were pooled from four different points in each experimental unit (mesocosm). Chlorophyll *a* was determined once a week in each mesocosm. One liter water samples from each mesocosm were filtered through a Whatman GF/C filter, then chlorophyll *a* was extracted using 90% acetone. Phytoplankton dry weight was measured after filtering samples through 1 μm Nucleopore filters and drying at 105°C to a constant weight. Cells and filaments were counted in preserved samples at 400x using the Utermöhl method[44]. Phytoplankton analysis was done on Days -1 (the day before atrazine treatment), 2, 4, 9, 11, 16, 22, and 25 after treatment.

Shannon-Weaver diversity (H') was calculated for the mean species densities of the two replicates for the treatment and control communities. Bray-Curtis dissimilarity indices (BCI) for differences between treatment and control communities were calculated for each sampling day. The BCI was calculated from phytoplankton cell densities. The Mann-Whitney test was used to detect significant differences between C-C (within-control) BCIs and A-C (control vs. atrazine treatment) BCIs[45]. The publications did not report what level of taxonomic resolution was used to calculate the BCI. A principal component analysis was conducted on the total densities of dominant species in the treatment and control communities using the ADE-4, a multivariate analysis and graphical display software.

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[44] Utermöhl method uses a chamber, into which a 5–50 mL sub-sample is placed and left to settle onto a coverslip, after which phytoplankton are counted using an inverted microscope. https://doi.org/10.1093/plankt/22.12.2255

[45] The authors state "*Mann Whitney test (90%)*", which presumably means an alpha level was 0.1. There is some potentially confusing wording in the publication about the implementation of the Bray-Curtis dissimilarity index. The methods state that the Bray-Curtis dissimilarity index was used, but its implementation by the study authors suggests that it was presented as a similarity index (1-dissimilarity). Another publication by these authors (Berard *et al.*, 1999) is cited for more information on the approach, and the discussion in that paper is consistent with presentation of the BCI data as a similarity (1-dissimilarity).

8.3 EPA's Use as of 2016

In the 2016 Refined Ecological Risk Assessment (USEPA, 2016), EPA included one endpoint that was based on the outdoor mesocosm studies in the Seguin *et al*. (2002) publication (**Table 8.1**). The 30 µg/L treatment group (endpoint #87) was scored as an "Effect" based on reductions in chlorophyll *a* (22%), dry weight (30%) and DO (ca. 20%) and a change in community structure and the Bray-Curtis similarity index (note that the magnitudes of effect are based on the first day observed).

Table 8.1. A summary of the cosm endpoints associated with this group that appeared in Appendix G.2 of the 2016 Refined Ecological Risk Assessment. These endpoints were used to evaluate the potential effects of atrazine on aquatic plant communities.

References	Endpoint Number	Duration (days)	Nominal Conc. (µg/L)	Plant Group	Results and Recovery	Effect/No-Effect Conclusion
Seguin *et al.* (2002)	87	25	30	Phyto	Decrease in chlorophyll *a* (22%) and dry weight (30%); change in community structure and Bray-Curtis similarity index; decrease in DO (20%). Recovery: No	Effect[A]

[A] Changes in biomass (chlorophyll *a* and dry weight) were evident at day 9, changes in community structure began at day 11, changes in Bray-Curtis began day 9, and changes in DO about day 3. Although DO showed an apparent recovery about day 12, it diverged from the control again starting about day 17 through the end of the experiment.

8.4 Stakeholders' Major Concerns and Criticisms

There have been two primary criticisms about this study, one regarding potential recovery of biomass and the other about apparently conflicting results from those presented by Seguin *et al.,* 2001b. Neither of these comments directly disagreed with EPA's "Effect" classification. There is also potential uncertainty about the use of a solvent through association of this study with Seguin *et al*. (2001a) and Seguin *et al*. (2001b).

8.4.1 Recovery of Biomass

The 2012 SAP (USEPA, 2012b) briefly commented on this study and endpoint, not directly disagreeing with the effects classification, but mentioned that the study did not report recovery. The panel stated that "*while the results indicate that there was a 30% reduction in algal biomass*

over a 21-day exposure to 30 µg/L atrazine, recovery was not studied. The preponderance of evidence in the literature indicates that recovery would be expected."

8.4.2 Contradictory Biomass Results

Syngenta (2012a, 2016) has asserted contradictory biomass results in Seguin *et al*. (2001b) and Seguin *et al*. (2002) citing that phytoplankton biomass decreased relative to the controls in the experiments described in the 2002 publication but not the 2001b publication.

8.4.3 Solvent

Seguin *et al*., 2002 cites Seguin *et al*. (2001b) for "*more information on the method and setup of the experimental units*", which indicates that acetonitrile was used (concentration not reported) to dissolve atrazine prior to introduction into the mesocosm tanks and the control tanks contained solvent. Given this wording, the experiment reported in Seguin *et al*., 2002 presumably used acetonitrile to dissolve atrazine and the control tanks contained the solvent.

8.5 EPA's 2023 Reevaluation

After consideration of all of the study's limitations, the determinative concerns will be discussed here, including those brought up by past SAPs and public commenters.

8.5.1 Recovery of Biomass

As discussed above, the 2012 SAP did not directly disagree with the effects classification but mentioned that the study did not report recovery. The panel stated that "*while the results indicate that there was a 30% reduction in algal biomass over a 21-day exposure to 30 µg/L atrazine, recovery was not studied. The preponderance of evidence in the literature indicates that recovery would be expected.*" Although there is the potential for recovery from effects of atrazine exposure in any of the mesocosm experiments, it was not a feature of many studies nor was it a requirement of inclusion in the cosm database. Therefore, recovery is a possibility for many endpoints scored as "effects" in the cosm database. Furthermore, biomass (measured as chlorophyll *a* and dry weight, **Figure 8.1** and **Figure 8.2A**) was consistently lower over multiple and sequential timepoints relative to the control and represented a monotonic trend over that time

period.[46] There were reductions of up to 57% and 68% for chlorophyll *a* and dry weight, respectively, by the termination of the experiment. Recovery was not reported for any variable measured with the exception of the apparent recovery of DO on Day 12, although it diverged from the control again starting about Day 17 (**Figure 8.3**; *note*: "*contaminated*" = atrazine treatment). Finally, even if biomass had recovered, it could potentially be associated with a change in community structure (*e.g.*, a shift in abundance toward less sensitive species), and in this case, there was a clear shift in community structure as evidenced by changes in density of taxonomic groups (especially Cyanobacteria; **Figure 8.2B**) and as represented by the Bray-Curtis Index (**Figure 8.4**) and the Principal Component Analysis (**Figure 8.5**)[47].

Table 1
Some environmental and phytoplankton characteristics of the mesocosm experiments

Date	T(°C)	NH_4^+ (mgN/L)	NO_2^- (mgN/L)	NO_3^- (mgN/L)	PO_4^{3-} (mgP/L)	Chl *a* (μg/L)	DW (mg/L)	H′ (bits/individual)
Mean controls (replicate mesocosms)								
2/4	9.30	2.14	0.00	7.20	0.02	13.66	4.93	0.93
4/4	8.26	1.30	0.06	4.20	0.01	25.42	5.90	0.89
6/4	7.30			8.60	0.00			0.93
9/4	10.08			7.60	0.03			0.91
11/4	7.20	0.78	0.15	5.60	0.02	24.42	6.32	0.91
13/4	8.84			7.40	0.02			
16/4	7.62						7.66	0.88
18/4	9.50						8.76	
20/4	10.38	0.09	0.17	9.60	0.00	30.11	8.80	
25/4	12.26						9.40	0.81
27/4	12.40	0.02	0.15	7.00	0.00	28.63	9.04	0.67
Mean atrazine (replicate mesocosms)								
2/4	9.30	2.18	0.00	7.00	0.01	13.93	4.89	0.91
4/4	8.26	1.35	0.06	4.25	0.01	23.09	5.35	0.91
6/4	7.30			10.75	0.01			0.93
9/4	10.08			6.25	0.03			0.86
11/4	7.20	1.20	0.12	5.50	0.02	19.07	4.40	0.92
13/4	8.84			7.50	0.01			
16/4	7.62						5.70	0.9
18/4	9.50						5.70	
20/4	10.38	0.18	0.14	7.75	0.00	20.86	5.93	
25/4	12.26						4.05	0.87
27/4	12.40	0.49	0.16	6.75	0.00	12.23	2.93	0.76

DW: Dry weight. H′: Shannon diversity index.

Figure 8.1 Excerpt of Table 1 from Seguin *et al.* (2002)

[46] Although tests of statistical significance were not performed on all endpoints, potential treatment related effects were clear based on factors such as trends in the data over time, the magnitude of the differences between treatments and controls, and the amount of variability among replicates.

[47] The PCA analysis is limited in usefulness given that the only component (Axis 2) showing visible changes with treatment explains only 16% of the variance, and the first two components together account for less than half the variance. Nonetheless, it does illustrate shifts in the community over time that are consistent with the other presented measures of community composition (class-level composition and the Bray-Curtis Index).

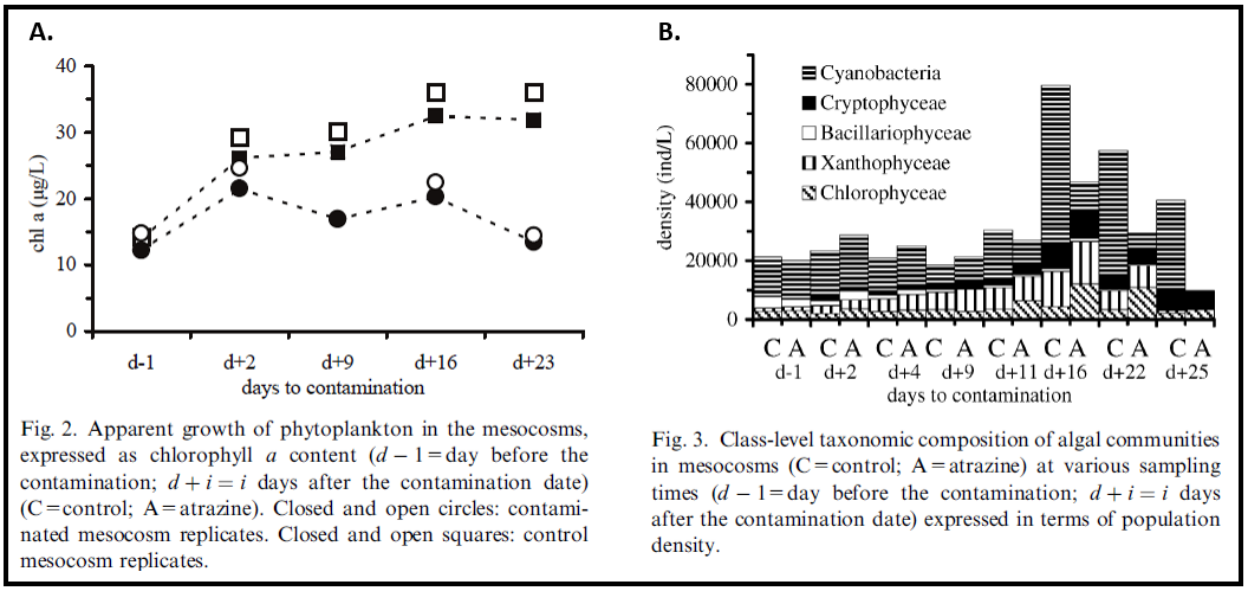

Fig. 2. Apparent growth of phytoplankton in the mesocosms, expressed as chlorophyll *a* content ($d-1$ = day before the contamination; $d+i=i$ days after the contamination date) (C = control; A = atrazine). Closed and open circles: contaminated mesocosm replicates. Closed and open squares: control mesocosm replicates.

Fig. 3. Class-level taxonomic composition of algal communities in mesocosms (C = control; A = atrazine) at various sampling times ($d-1$ = day before the contamination; $d+i=i$ days after the contamination date) expressed in terms of population density.

Figure 8.2. Excerpt of Figure 2 (A) and Figure 3 (B) from Seguin *et al.* (2002)

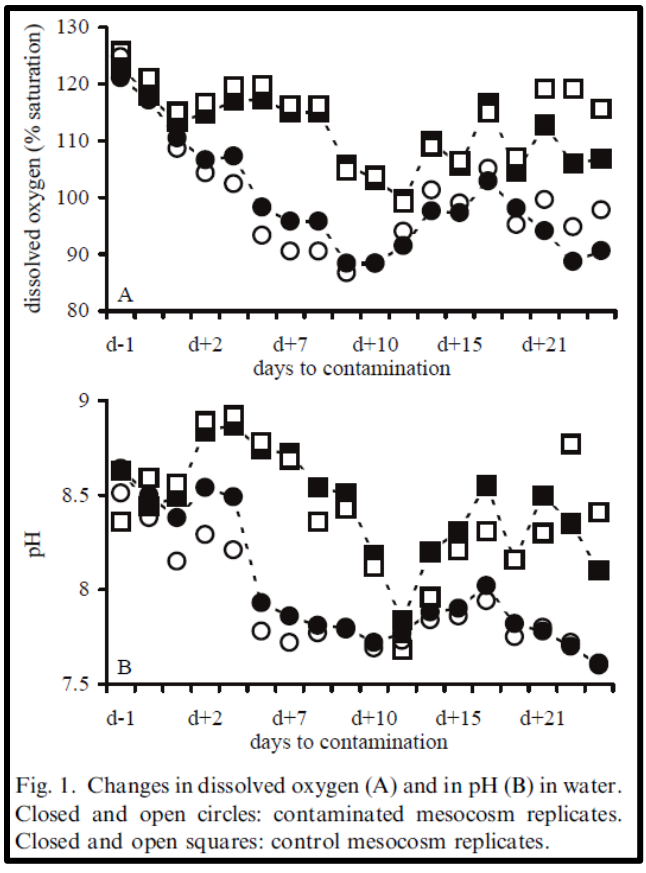

Fig. 1. Changes in dissolved oxygen (A) and in pH (B) in water. Closed and open circles: contaminated mesocosm replicates. Closed and open squares: control mesocosm replicates.

Figure 8.3. Excerpt of Figure 1 from Seguin *et al.* (2002)

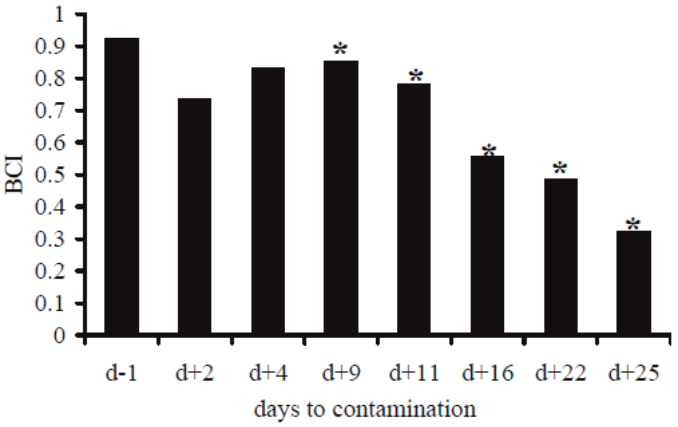

Fig. 5. Bray–Curtis indices (BCI) of the phytoplankton samples in the mesocosms ($d-1$ = day before the contamination; $d+i=i$ days after contamination). The BCI values were calculated for the density of replicate communities, and the significance of the difference between control and treated populations was calculated using a Mann–Whitney test between indices calculated between controls (C–C) and those calculated between control and test values (C–A). *: (C–A/C–C) Mann–Whitney test (90%).

Figure 8.4. Excerpt of Figure 5 from Seguin *et al.* (2002)

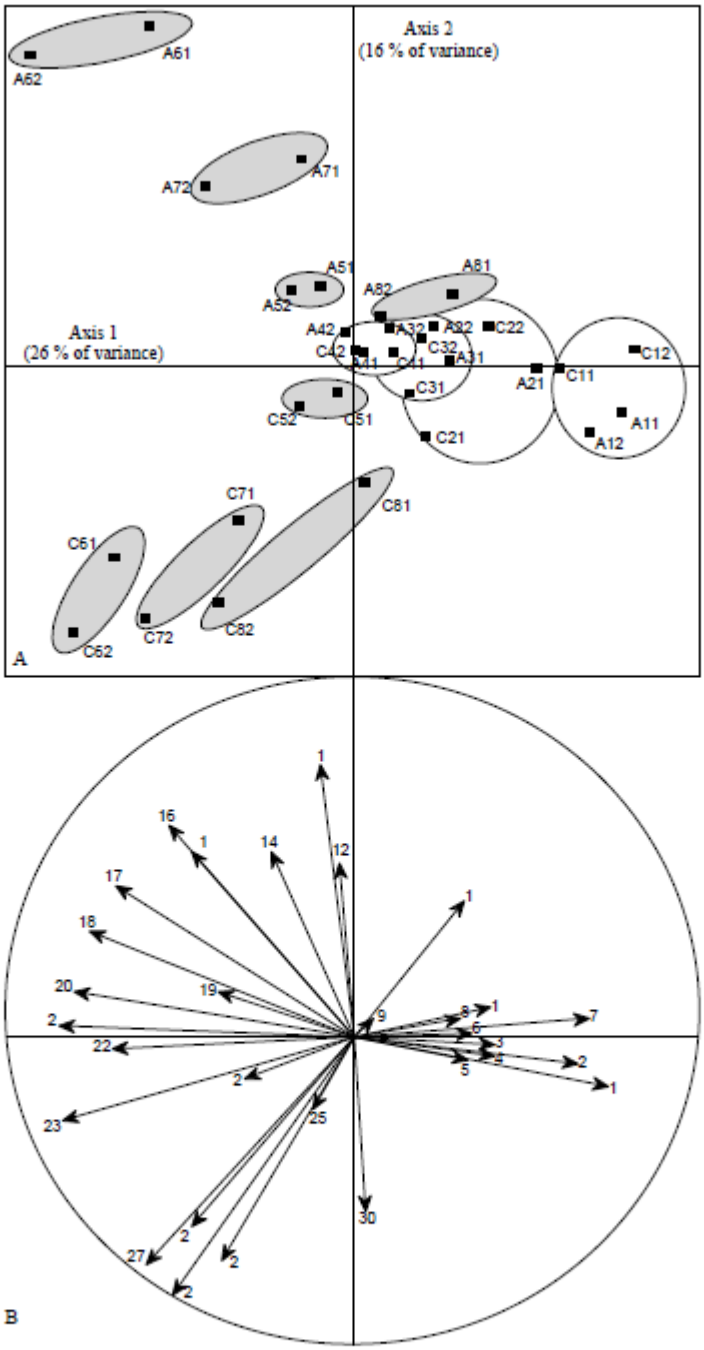

Fig. 4. PCA of phytoplankton communities at various sampling times (projection in the plane defined by axes 1 and 2) (A). X_{ij}: X indicates the nature of the mesocosm (Control or Atrazine), i indicates the sample number (1: 3 April; 2: 5 April; 3: 7 April; 4: 12 April; 5: 14 April; 6: 19 April; 7: 25 April; 8: 28 April), j indicates the replicate. Open areas are for the initial condition and shaded areas are for final conditions. Correlation circle of the explanatory variables (B): 1: *Chlamydomonas* sp.; 2: *Chlorella vulgaris*; 3: *Scenedesmus quadricauda*; 4: *Dictyosphaerium pulchellum*; 5: *Crucigenia apiculata*; 6: *Crucigenia quadrata*; 7: *Synedra acus*; 8: *Nitzschia acicularis*; 9: *Pediastrum duplex*; 10: *Oocystis naegelii*; 11: *Scenedesmus* sp.; 12: *Tetraedron minimum*; 13: *Chodatella quadriseta*; 14: *Scenedesmus acuminatus*; 15: *Monoraphidium minutum*; 16: *Hyaloraphidium contortum*; 17: *Scenedesmus* sp.; 18: Xanthophyceae; 19: *Scenedesmus spinosus*; 20: *Cryptomonas ovata*; 21: *Closterium acutum*; 22: *Nitzschia* sp.; 23: *Scenedesmus* sp.; 24: *Rhodomonas minuta*; 25: *Actinastrum hantzschii*; 26: *Navicula* sp.; 27: *Pseudoanabaena* sp.; 28: *Crucigenia fenestrata*; 29: *Oscillatoria limnetica*; 30: *Pseudoanabaena* sp.

Figure 8.5. Excerpt of Figure 4 from Seguin *et al.* (2002)

EPA acknowledges that some of the measured variables could have potentially recovered; however, there was no direct evidence of it in this study, variables with clear effects followed by recovery are still classified as effects in the cosm database, the possibility of recovery in this experiment is speculative, and several variables were impacted in this experiment, including changes to community structure. Therefore, EPA maintains that there was an effect at 30 µg/L, so endpoint #87 will stay scored as an "**Effect**."

8.5.2 Contradictory Biomass Results

As discussed above, Syngenta has pointed out that phytoplankton biomass decreased relative to the controls in the experiments described in the Seguin *et al*. (2002) publication but not the 2001b publication. Although they do not question the results, they suggest that the results are apparently contradictory. On the surface, these results could seem contradictory, but these were independent experiments and there was a clear difference in the initial composition of the phytoplankton communities tested in the two experiments (**Figure 8.6a** for 2001b and **Figure 8.6b** for 2002). Therefore, there is no reason to expect the exact same response in biomass after exposure. This comment does not change EPA's interpretation of the study.

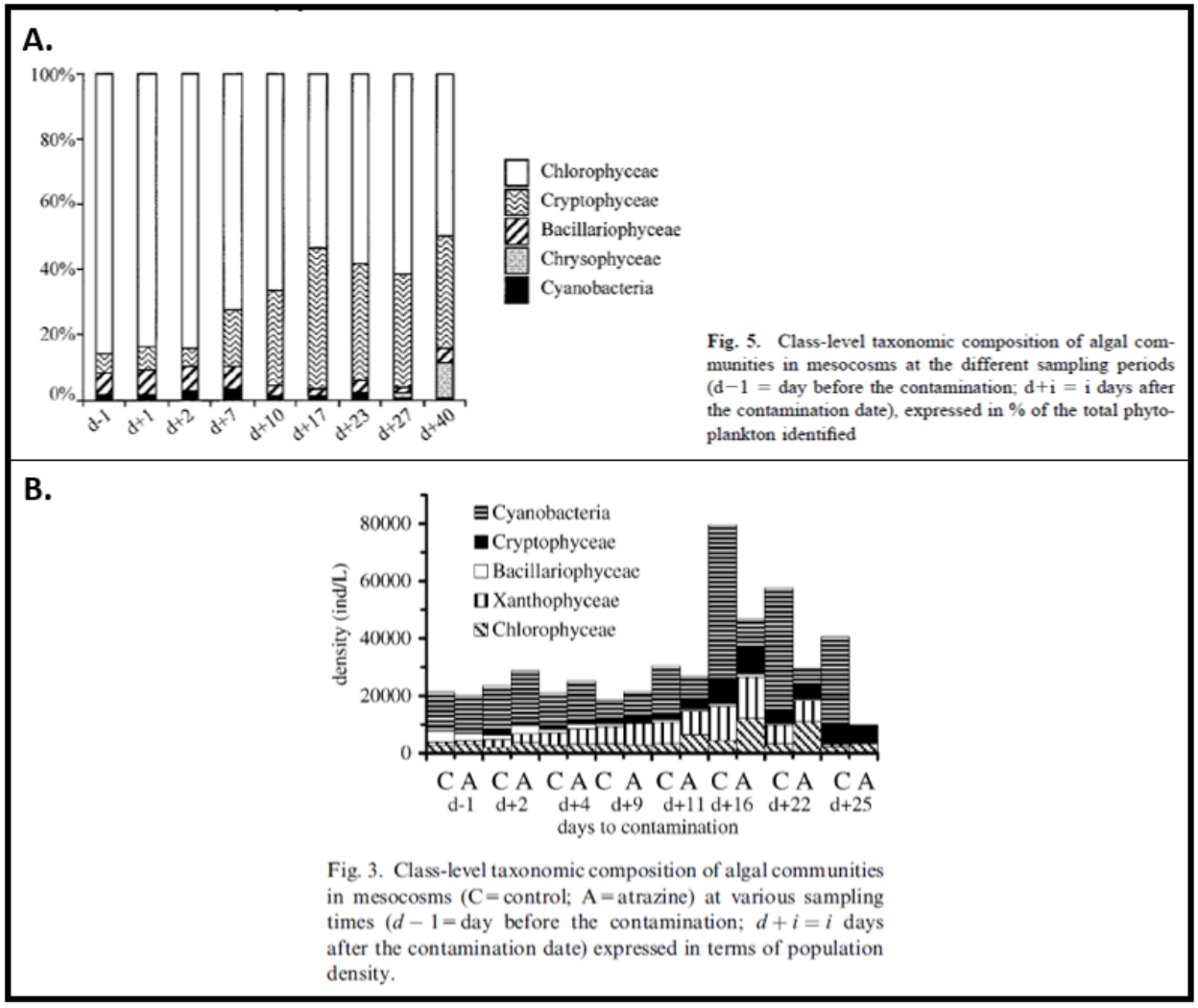

Fig. 5. Class-level taxonomic composition of algal communities in mesocosms at the different sampling periods (d−1 = day before the contamination; d+i = i days after the contamination date), expressed in % of the total phytoplankton identified

Fig. 3. Class-level taxonomic composition of algal communities in mesocosms (C = control; A = atrazine) at various sampling times ($d-1$ = day before the contamination; $d+i=i$ days after the contamination date) expressed in terms of population density.

Figure 8.6. A. Excerpt of Figure 5 from Seguin *et al.* (2001b). B. Excerpt of Figure 3 from Seguin *et al.* (2002)

8.5.3 Solvent

As discussed above, the publication does not report details about atrazine or if a solvent was used, instead citing Seguin *et al.* (2001b) for "*more information on the method and setup of the experimental units*". This is likely because the experimental setup is very similar except the difference in the number of replicates and test concentrations. The Seguin *et al.* (2001b) publication indicates that acetonitrile was used to dissolve atrazine prior to introduction into the mesocosm tanks and the control tanks contained solvent (concentration not reported). Given the presumption that acetonitrile and solvent controls were used, the discussion about the solvent in **Section 7.5.4** applies to Seguin *et al.* (2002) as well.

8.5.4 EPA's 2023 Conclusions

While the study has deficiencies, EPA has decided that the study is sufficient to contribute to our knowledge about the effects of atrazine to aquatic plant communities under the conditions of the experiment. This study contributes to our understanding of potential effects on natural aquatic plants communities exposed to atrazine when considered within the context of the larger collective body of experimental data from other cosm studies. Therefore, while there is uncertainty in the results, it is reasonable to include this study in the cosm database. **Based on the considerations discussed above, EPA's cosm database has not changed for Seguin *et al.* (2002)** (**Table 8.2**).

Table 8.2. A summary of the cosm endpoints associated with this group that remain after the 2023 reevaluation. These endpoints will be used to evaluate the potential effects of atrazine on aquatic plant communities.

References	Endpoint Number	Duration (days)	Nominal Conc. (µg/L)	Plant Group	Results and Recovery[A]	Call
Seguin *et al.* (2002)	87	25	30	Phyto	Decrease in chlorophyll *a* (22%) and dry weight (30%); change in community structure and Bray-Curtis similarity index; decrease in dissolved oxygen (20%). Recovery: No	Effect

[A] Additional results: Changes in biomass (chlorophyll *a* and dry weight) were evident at day 9, changes in community structure began at day 11, changes in Bray-Curtis began day 9, and changes in dissolved oxygen (DO) about day 3. Although DO showed an apparent recovery about day 12, it diverged from the control again starting about day 17 through the end of the experiment.

CHAPTER 9. CONCLUSION

Aquatic plant communities are vital to aquatic and terrestrial food webs and ecosystems; therefore, changes to these communities could alter the functioning of the ecosystem. EPA evaluated the toxicity of atrazine to aquatic plant communities using cosm studies, which are useful tools in understanding complex community interactions under semi-controlled conditions. These cosm studies, while complex, provide a greater understanding of aquatic plant community responses to atrazine than is provided by short duration single-species toxicity studies (*e.g.*, OCSPP 850.4500 and 850.4550 test guideline studies).

The use of cosm studies in the ecological assessment of atrazine has a long, 20-year history involving multiple SAPs and EPA reviews. This White Paper focused on EPA's reevaluation of 11 cosm studies (and associated publications) that the 2012 SAP identified as warranting further review. EPA's 2023 conclusions for these 11 studies (and associated publications) are summarized in **Table 9.1** and presented in full in **Chapters 2** through **8**.

In summary, EPA has decided to exclude the Lampert and Detenbeck studies and the associated endpoints. EPA concluded that the Seguin *et al.* (2002) study and endpoint would remain the same as it was in the cosm database as of 2016. The University of Kansas 1979, University of Kansas 1981-1983, Kosinski, and Seguin *et al.* (2001) study groups continue to be included in the cosm database, however EPA made several changes to the endpoints (**Table 9.1**). Support for these changes is provided in the relevant study group chapters.

The SAP's feedback on EPA's 2023 reevaluation and conclusions regarding these studies will inform how EPA ultimately uses these 11 cosm studies.

Table 9.1 . EPA's 2023 Cosm Review Conclusions. The cosm endpoint numbers, concentrations, and calls (*i.e*., exclude/include or effect/no-effect conclusion) from the new 2023 reevaluation presented in this White Paper (*i.e.,* "EPA 2023") for seven groups that comprise the 11 studies in question and other associated publications.

Chapter: Group	References	Endpoint Number[C]	Nominal Conc. (µg/L)	Call	Summary of Rationale
Chapter 2: Lampert	Fleckner (1988)[A]; Lampert *et al*. (1989)	-	-	Exclude	Uncertainty regarding potential solvent interaction
Chapter 3: University of Kansas – 1979 Experiment	deNoyelles and Kettle (1980)[A]; deNoyelles *et al.* (1982); deNoyelles *et al.* (1989)[B]; Kettle (1982)[A]; Kettle *et al.* (1987); Larsen *et al.* (1986)[AB]	52 3	20 500	No Effect Effect	#1 and #2 not associated with 1979 experiment. #52 rescored due to minimal differences in phytoplankton and no quantification or presentation of macrophyte results.
Chapter 4: University of Kansas – 1981-1983 Experiment	Carney (1983); Dewey (1986); deNoyelles and Kettle (1983)[A]; deNoyelles *et al.* (1994)[A]; deNoyelles *et al.* (1989)[B]; Huggins (1990)[A]; Huggins *et al*. (1994)[A]; Larsen *et al*. (1986)[AB]	2 4	20 100	No Effect Effect	#1 and #5 removed due to duration (>240 days). #41 removed due to duplication. #42 removed due to one replicate. #2 rescored due to no phytoplankton results and no effect on macrophytes.
Chapter 5: Detenbeck	Detenbeck *et al*. (1996)	-	-	Exclude	Excluded based on study design, execution, and results reporting.
Chapter 6: Kosinski	Kosinski (1984); Kosinski and Merkle (1984)[A]	28 44 28a 44a	10 100 10 100	Effect No Effect No Effect No Effect	#28a and #44a added to account for distinct experiments. #44, #28a, and #44a scored as "No Effect" because either no significant effect or slight/transient effect.
Chapter 7: Seguin – 2001 Publications	Seguin *et al*. (2001a); Seguin *et al*. (2001b)	84 83	2 30	No Effect Effect	#86 and #85 removed due to combining. #84 scored as "No Effect" because of potential confounding effects from succession
Chapter 8: Seguin – 2002 Publication	Seguin *et al*. (2002)	87	30	Effect	No change

[A] Publications that were not part of the original 11 identified by the 2012 SAP but are associated with them.
[B] deNoyelles *et al.*, 1989 and Larsen *et al.*, 1986 summarize both the 1979 and the 1981-1983 University of Kansas experiments. Therefore, they are present in both University of Kansas sections and are associated with all endpoints.
[C] Endpoint numbers were assigned to each concentration within the experiment(s) associated with that publication and were logged as a data point number. .

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APPENDIX A. RELEVANT DOCKETS AND STUDY REFERENCES

Table A.1. Dockets relevant to the aquatic plant community portion of the ecological risk assessment of atrazine

Category	Docket Title	Docket ID	Items in the Docket
2009 SAP	Notice of FIFRA SAP Meeting; Determination of the Ecological Significance of Atrazine Effects on Primary Producers in Surface Water Streams in the Corn and Sorghum Growing Region of the United States (Part II)	EPA-HQ-OPP-2009-0104	• White Paper • Appendix III-1: Cosm Data Points and Exposure Profiles • Meeting Minutes • Public comments
2012 SAP	Notice of FIFRA SAP Meeting; Problem Formulation for Reassessment of Ecological Risks from Use of Atrazine	EPA-HQ-OPP-2012-0230	• White Paper • Appendix D: Bibliography of Microcosm and Mesocosm Studies and Criteria Used to Screen Studies for Analysis of Atrazine Risks to Aquatic Plant Communities • Meeting Minutes • Public comments
Registration Review	Atrazine Registration Review	EPA-HQ-OPP-2013-0266	• EPA's Data Evaluation Record (DER)/Open Literature Review Summaries (OLRS) for the cosm studies • Addendum to the Problem Formulation • 2016 Refined Ecological Risk Assessment • Appendix G.1. Bibliography of Microcosm and Mesocosm Studies and Criteria • Appendix G.2. COSM Endpoint and Chemograph Database • Regulatory Update • Proposed Interim Decision • Interim Decision • Proposed revisions to the Interim Decision • Public comments

Table A.2. References for the studies associated with Chapter 2 through 8. These studies can be found on the Health and Environment Research Online (HERO) database[A] using the HERO ID number listed below. Data Evaluation Records (DERs)/Open Literature Review Summaries (OLRSs) can be found on docket EPA-HQ-OPP-2013-0266 using the MRID numbers listed below.

Chapter	In-text Citation	Full Reference
2. Lampert	Fleckner (1988)[B]	Fleckner W. 1988. Ökotoxikologische untersuchungen mit herbiziden in eingeschlossenen wasserkörpern in-situ. Direkte und indirekte wirkungen von 2,4-D und atrazin auf planktonbiocoenosen und physikalisch-chemische parameter. Thesis. Christian-Albrechts-University, Kiel, Germany (HERO 11145445; MRID NA)
	Lampert *et al.* (1989)	Lampert W, Fleckner W, Pott E, Schober U, and Storkel KU. (1989). Herbicide effects on planktonic systems of different complexity. Hydrobiologia. 188/189:415-424 (HERO 11145496; MRID 47543511)
3. University of Kansas – 1979 Experiment	deNoyelles and Kettle (1980)[B]	deNoyelles F and Kettle WD. 1980. Herbicides in Kansas waters – evaluations of effects of agricultural runoff and aquatic weed control on aquatic food chains. Contribution Number 219, Kansas Water Resources Research Institute, University of Kansas, Lawrence, Kansas (HERO 11144275; MRID 47543605)
	Kettle (1982)[B]	Kettle WD. 1982. Description and analysis of toxicant-induced responses of aquatic communities in replicated experimental ponds. Thesis. University of Kansas, Lawrence, KS, USA (HERO 11145487; MRID 48273504)
	deNoyelles *et al.* (1982)	deNoyelles F, Kettle WD, and Sinn DE. 1982. The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. Ecology. 63:1285-1293 (HERO 11144279; MRID 47543607)
	Kettle *et al.* (1987)	Kettle WD, deNoyelles F, Heacock BD, and Kadoum AM. 1987. Diet and reproductive success of bluegill recovered from experimental ponds treated with atrazine. Bull. Environ. Contam. Toxicol. 38:47-52 (HERO 11145484; MRID 47543506)
4. University of Kansas – 1981-1983 Experiment	Carney (1983)	Carney CE. 1983. The effects of atrazine and grass carp on freshwater communities. Thesis. University of Kansas, Lawrence, KS, USA (HERO 11144274; MRID 47543604)
	deNoyelles and Kettle (1983)[B]	deNoyelles F and Kettle WD. 1983. Site studies to determine the extent and potential impact of herbicide contamination in Kansas waters. Contribution Number 239, Kansas Water Resources Research Institute, University of Kansas, Lawrence, Kansas (HERO 11144276; MRID 47543606)
	Dewey (1986)	Dewey SL. 1986. Effects of the herbicide atrazine on aquatic insect community structure and emergence. Ecology. 67:148-162 (HERO 11145260; MRID 47543611)
	deNoyelles *et al.* (1994)[B]	deNoyelles F, Dewey SL, Huggins DG, and Kettle WD. 1994. Aquatic mesocosms in ecological effects testing: Detecting direct and indirect effects of pesticides. In: Graney RL, Kennedy J.H., Rodgers J.H. Jr. (Eds.). Aquatic mescocosm studies in ecological risk assessment. Lewis Publishers, Boca Raton, FL. pp. 577-603 (HERO 11144296; MRID 47543609)
	Huggins (1990)[B]	Huggins DG. 1990. The ecotoxic effects of atrazine on aquatic macroinvertebrates and its impact on ecosystem structure. Thesis. University of Kansas, Lawrence, KS, USA (HERO 11145476; MRID NA)
4. University of Kansas –	Huggins *et al.* (1994)[B]	Huggins DG, Johnson ML, deNoyelles F. 1994. The ecotoxic effects of atrazine on aquatic ecosystems: An assessment of direct and indirect effects using structural equation modeling. In: Graney RL, Kennedy J.H., Rodgers J.H. Jr. (Eds.). Aquatic mescocosm studies in ecological risk

Chapter	In-text Citation	Full Reference
1981-1983 Experiment cont.		assessment. Lewis Publishers, Boca Raton, FL. pp. 653-692 (HERO 11145446; MRID NA)
Both 3. and 4.	deNoyelles *et al.* (1989)	deNoyelles F, Kettle WD, Fromm CH, Moffett MF, and Dewey SL. 1989. Use of experimental ponds to assess the effects of a pesticide on the aquatic environment. Department of Systematics and Ecology, University of Kansas. Entomological Society of America. Miscellaneous Publications No. 75:41 – 56 (HERO 11144287; MRID 47543608)
	Larsen *et al.* (1986)	Larsen DP, deNoyelles F, Stay F, and Shiroyama T. 1986. Comparisons of single-species, microcosm, and experimental pond reponses to atrazine exposure. Environmental Toxicology. 5: 179-190 (HERO 11145500; MRID NA)
5. Detenbeck	Detenbeck *et al.*, 1996	Detenbeck NE, Hermanutz R, Allen K, and Swift MC. 1996. Fate and effects of the herbicide atrazine in flow-through wetland mesocosms. Environmental Toxicology and Chemistry. 15:937-946 (HERO 4629908; MRID 47543610)
6. Kosinski	Kosinski (1984)	Kosinski RJ. 1984. The effects of terrestrial herbicides on the community structure of stream periphyton. Environmental Pollution (Series A). 36:165-189 (HERO 10542028; MRID 47543507)
	Kosinski and Merkle (1984)[B]	Kosinski RJ, Merkle MG. 1984. The effects of four terrestrial herbicides on the productivity of artificial stream algal communities. Journal of Environmental Quality. 13:75-82 (HERO 8429851; MRID 47543508)
7. Seguin – 2001 Publications	Seguin *et al.* (2001a)	Seguin F, Druart JC, Le Cohu R. 2001a. Effects of atrazine and nicosulfuron on periphytic diatom communities in freshwater outdoor lentic mesocosms. Annales De Limnologie-International Journal of Limnology 37:3-8 (HERO 11139498; MRID 48273501)
	Seguin *et al.* (2001b)	Seguin F, Leboulanger C, Rimet F, Druart JC, Berard A. 2001b. Effects of atrazine and nicosulfuron on phytoplankton in systems of increasing complexity. Archives of Environmental Contamination and Toxicology 40:198-208 (HERO 11145512; MRID 48261134)
8. Seguin – 2002 Publication	Seguin *et al.* (2002)	Seguin F, Le Bihan F, Leboulanger C, Berard A. 2002. A risk assessment of pollution: induction of atrazine tolerance in phytoplankton communities in freshwater outdoor mesocosms, using chlorophyll fluorescence as an endpoint. Water Research 36:3227-3236 (HERO 11145513; MRID 48261133)

[A] Search the HERO database at the website: https://hero.epa.gov/hero/index.cfm/project/page/project_id/4775

[B] Publications that were not part of the original 11 identified by the 2012 SAP but are associated with them.

APPENDIX B. TABLE 1 FROM THE 2012 SAP MEETING MINUTES

Table B.1. Table 1 and associated footnotes taken directly from the 2012 SAP meeting minutes (page 44-45), which was captioned "Summary of the Panel's Evaluation of 11 Cosm Studies". Footnote 3 was added for this White Paper.

Study Author	Study Evaluation
Lampert *et al.* (1989)[1]	This study should be excluded due to solvent bias as noted on p. 173 of Giddings *et al.* 2005; "decreased primary productivity" was actually increased in bacteria growth and respiration (See Appendix D, p. 5). The Panel recommended that this study should be dropped. The Panel was disappointed to see this study still included in the cosm dataset since the 2007 and 2009 SAPs indicated that it be dropped due solvent bias.
DeNoyelles *et al.* (1982)[1,2]	This study showed basically no effects on biomass and C-14 uptake in phytoplankton at an atrazine concentration of 20 µg/L. This is revealed by the overlapping confidence intervals in Fig. 1. In fact, the greatest proportional differences occurred when the 20 µg/L concentration simulated primary productivity compared to the control. Figure 2 shows stimulation of 3 species of algae at 20 µg/L. In addition, this study contained gizzard shad at a total of 7 fish/mesocosm, or 70/acre…in addition to bluegill and channel catfish. There was no accounting of survival of gizzard shad which are very difficult to handle in transfer and stocking. Differential survival would have large indirect effects due to differences in the zooplankton community. Effects noted at 1 µg/L were short-term studies where control pond water was treated with atrazine in lab bioassays. No macrophyte data were cited. These data should not be assigned a 20 µg/L effect.
Carney (1983)[1,3]	Authors cited exclosure experiments in control ponds. Grass carp were stocked at 20/acre, which is an order of magnitude greater than common guidance (2/acre). Macrophytes were totally denuded in control pond. Note: These two ponds may not have been the ponds examined by deNoyelles *et al.* (1982) or Dewey *et al.* (1985); however, this is the exact magnitude of the direct effect on macrophytes that one would expect at this extreme stocking level.
Dewey (1986)[1,2,3]	This study should be excluded because it does not meet the "must not have other stressors present" criterion listed in Appendix D, p. 5. Grass carp, gizzard shad, channel catfish, and bluegill were present with no data on percent survival (especially differential survival of grass carp; however, Kettle *et al.* (1987) mentioned 80% survival of adult bluegill). Grass carp were stocked at 20/acre, which is an order of magnitude greater that common guidance (2/acre). Author indicated that decreased insect emergence was an indirect effect and not primary effect of atrazine. *Macrophyte biomass was decreased by 90 and >95% in the 20 and 500 µg/L treatments, respectively, which makes no ecotoxicological sense based on the large amount of data for atrazine. Visual observations indicated that periphyton was affected at 100 µg/L but no mention of effects at 20 µg/L***.** Note: there was zero grass carp survival in the controls as indicated by DeNoyelles *et al.* 1989, which resulted in high macrophyte biomass and associated insect emergence rates.
Kettle *et al.* (1987)[1,2]	The pertinent reference should be the original 1980 Master's Thesis which led to the 1987 paper. The 1987 paper reports a negative effect of atrazine on bluegill reproductive success. It suffers from the same design flaws as Dewey *et al.* (1985) such as presence of gizzard shad. With no information on differential survival of gizzard shad the results on bluegill reproduction are suspect. Loss of gizzard shad from the controls would lead to increased numbers of zooplankton and higher survival of young bluegill. Note: there was zero grass carp survival in the controls as

Study Author	Study Evaluation
	indicated by DeNoyelles *et al*. (1989), which resulted in high macrophyte biomass that served as refugia for young bluegills and allowed them to avoid predation by channel catfish.
DeNoyelles *et al.* (1989)[1,2]	Ponds were exposed to 20, 100, 200, and 500 µg/L of atrazine. The results indicate that effects occurred at an atrazine concentration of 20 µg/L. However, the paper explicitly states (Fig. 4) that there were no lasting effects on phytoplankton biomass up to 500 µg/L. Species shifts occurred, but they were replaced by tolerant species. Effects were noted in the laboratory in C-14 uptake experiments, but these are short-time bioassays that do not reflect what happens in the mesocosm itself. Four species of fish were present (bluegill, channel catfish, gizzard shad, and grass carp). Biomass of all species was *increased* in the presence of atrazine with two exceptions: one control pond that had zero gizzard shad survival and another control pond that had zero grass carp survival. Fish data and macrophyte data presented in Fig. 7clearly show the effects of grass carp on macrophyte biomass. Macrophyte biomass in the control ponds was high because grass carp were absent. This observation, not mentioned in Dewey *et al*. 1986 and Kettle *et al*. 1987, invalidates these studies as well, as increased insect emergence and bluegill survival were observed in the controls due to decreased predation of young of the year bluegill. In the treated ponds, there was high grass carp survival, macrophyte biomass was decreased due to fish grazing, and emergent insects and larval bluegill were higher due to refugia from predation by bluegills and channel catfish.
Detenbeck *et al*. (1996)	This study design involved steadily increasing does of atrazine at 2-week intervals from 15 to 25 to 50 to 75 µg/L using two controls and two treatments. The authors concluded that there were effects of atrazine on gross primary production at the 15 µg/L level; however, no data were presented for the initial 2-week 15 µg/L exposure other than two reported dissolved oxygen concentrations. Gross photosynthesis is not reliable in this system due to the accumulation of large amounts of sediment and detritus that would result in high respiration rates. Hence the stressor in the early part of the study was not atrazine, but most likely accumulated decaying organic matter unrelated to the dosing. Neither chlorophyll *a* nor ash-free dry weight of periphyton was affected at any concentration. *Elodea* was not affected at concentrations up to 75 µg/L, but *Ceratophyllum* showed effects only at > 75 µg/L. The Panel recommended that this study be excluded from consideration.
Kosinski (1984)[1]	The 10 µg/L atrazine concentration listed for this study in Attachment 3, Appendix D, should be 100 µg/L. Although some streams were colonized (pre-treated at 10 µg/L), there are insufficient data to assign effects at this level. The abstract of Kosinski and Merkle (1984) states succinctly, "There was little evidence that exposure to 0.01 mg/kg herbicide during colonization modified the response of the algae to any of the herbicides."
Seguin *et al*. (2001a)	Exposure to 2 and 30 µg/L of atrazine had a stimulatory effect on periphyton production, but this was incorrectly categorized as a negative effect.
Seguin *et al.* (2001b)	No significant effects on periphyton biomass were observed at 30 µg/L of atrazine. The effects observed appear to be based on shifts in phytoplankton community. For example, as atrazine concentrations increased, the number of Chrysophysceae increased while the numbers of chlorophytes decreased. Acetonitrile was used as solvent, but amount added not listed. Rarely is this solvent used in ecotoxicological dosing.
Seguin *et al.* (2002)	While the results indicate that there was a 30% reduction in algal biomass over a 21-day exposure to 30 µg/L atrazine, recovery was not studied. The preponderance of evidence in the literature indicates that recovery would be expected.

[1]Studies discussed by the 2009 SAP as having flawed methodology, which affected interpretation of the results.

[2] Studies conducted at the University of Kansas from 1979-1991. These studies were considered "ecotoxicological classics" based on the hypotheses tested, study complexity and ecological relevance. However, they were not conducted under any semblance of Good Laboratory Practices (GLPs). Note: The University of Kansas study is accounted for five times in EPA's analysis, which may bias the data in Fig. 16 of the White Paper for atrazine effects at 20 μg/L.

[3] Added for the 2023 White Paper – Carney and DeNoyelles (1986) is not the correct citation and was changed to Carney (1983) above. Dewey *et al.* (1986) is not the correct citation and was changed to Dewey (1986) above.