OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

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MEMORANDUM

SUBJECT: **Update to "EFED Support Documentation for the Proposed Revisions to the Atrazine Interim Registration Review Decision Regarding Risks to Aquatic Plant Communities"**
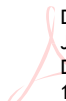
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1 Background

In 2022, EPA published for public comment the Proposed Revisions to the Atrazine Interim Registration Review Decision and the EFED support documentation (USEPA, 2022a; 2022b). That proposal reconfirmed the 3.4 µg/L atrazine concentration (60-day average) equivalent level of concern (CE-LOC) to protect aquatic plant communities and the aquatic ecosystem (waterbodies), as described in the 2016 Refined Ecological Risk Assessment (USEPA, 2016). In 2022, EPA also proposed potential mitigations to protect aquatic plant communities in aquatic ecosystems where atrazine concentrations were predicted to be above the CE-LOC.

The purpose of this document is to provide an update on EPA's recalculation of the CE-LOC, which reflects the results of EPA's reevaluation of the microcosm and mesocosm (cosm) studies[1] that were the focus of the August 2023 Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) meeting and a reevaluation of two additional cosm studies as requested by public commenters. This update also includes the results of EPA's work to correct errors EPA made in the 3.4 µg/L CE-LOC calculation and in the Watershed Regressions for Pesticides for Multiple Pesticides (WARP-MP) analysis. Finally, updated maps are provided that show where the CE-LOC is exceeded based on the revisions to the CE-LOC and WARP-MP modeling and therefore, where EPA expects to propose mitigation measures to protect aquatic plant communities. A high resolution map has been published along with this update. This document addresses CE-LOC and WARP related public comments received on the Proposed Revisions to the Interim Decision. EPA will respond to the public comments on other topics at a later date.

2 Updates to the CE-LOC

2.1 Changes to the Cosm Database

2.1.1 Reevaluation of a Subset of the Cosm Studies

In the comments received on the Proposed Revisions to the Interim Decision, there were requests for EPA to meet with the FIFRA SAP to reconsider a subset of the cosm studies used in the calculation of the 3.4 µg/L CE-LOC. In August 2023, EPA held a FIFRA SAP meeting to present and receive feedback on its reevaluation of 11 cosm studies identified by the 2012 SAP as warranting further review because of concerns about study design or performance flaws, as well as EPA's interpretation of the results (USEPA, 2012b). For the 2023 SAP, the 11 cosm studies identified by the 2012 SAP and nine other studies that focused on the same experiments were divided into seven study groups and reevaluated in the White Paper for the 2023 SAP (USEPA, 2023a). The 2023 SAP's final report (USEPA, 2023b) and EPA's response to the report, which indicated concurrence with the SAP's overall recommendations and presented final conclusions (USEPA, 2024), have since been made public. A summary of the changes that were made to the cosm database for the seven study groups can be found in **Table 2-1**. For more in-depth information on the reevaluations and conclusions, please refer to the White Paper, SAP final report, and EPA's response located in the 2023 SAP docket[2] (USEPA, 2023a; 2023b; 2024).

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[1] EPA defines a cosm "study" as a single publication, while a cosm "study group" is defined as a group of studies (i.e., publications) that all discuss a single unique cosm experiment. Cosm experiments can be defined as complex experiments used to examine aquatic plant communities under semi-controlled conditions that simulate natural environments.

[2] The docket can be found at https://www.regulations.gov/docket/EPA-HQ-OPP-2023-0154

In addition to the studies brought to the 2023 SAP, the 2022 public comments identified several other studies where there was disagreement with EPA, which included: Baxter *et al.* (2011), Downing *et al.* (2004), Pannard *et al.* (2009), and a group of studies that focused on the same microcosm experiment, collectively referred herein as the Bérard study group (Bérard *et al.*, 1999a; Bérard *et al.,* 1999b; Bérard and Benninghoff, 2001; Leboulanger *et al.,* 2001; Seguin *et al.*, 2001b[3]).

Downing *et al.* (2004) and the Bérard study group were in the cosm database at the time of the 2012 SAP (USEPA, 2012a) and were not identified by the panel members as needing reevaluation (USEPA, 2012b). As for the other two studies, EPA reviewed Baxter *et al.* (2011) and Pannard *et al.* (2009) after the convening of the 2012 SAP and presented those reviews in the 2016 Refined Ecological Risk Assessment (USEPA, 2016). EPA decided to reevaluate these two studies given the public comments in 2022. The reevaluation of these two studies is presented in **Appendix I** and the resulting database changes are summarized in **Table 2-1**.

2.1.2 Additional Database Changes

While updating the database, EPA discovered two errors that likely originated during the preparation of the 2016 cosm database (USEPA, 2016). First, endpoint[4] #46 (Moorhead and Kosinski, 1986) in the 2016 cosm database had an initial test concentration of 100 µg/L, but the associated chemograph showed an initial concentration of 1000 µg/L. In pre-2016 databases, endpoint #46 was assigned to the treatment with the initial test concentration of 1000 µg/L instead (removed because it was >500 µg/L), while the 100 µg/L initial test concentration was associated with endpoint #45. Thus, it seems that the endpoints were switched in the cosm database at some point. To correct this error, the 100 µg/L treatment was reassigned to #45 and is properly linked to the chemograph associated with this treatment. In the CE-LOC recalculation, this had minimal impact (-0.3 µg/L). Second, endpoints #69 and #70 (Gustavson and Wängberg, 1995) had initial test concentrations in the 2016 cosm database of 10 and 20 µg/L, respectively. However, the initial concentrations in the chemographs were 20 and 10 µg/L, respectively. To correct this error, EPA switched the chemographs back, which had no impact on the revised CE-LOC because both were "No Effect."[5]

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[3] This study includes microcosm and mesocosm experiments. The mesocosm experiment was part of the 2023 SAP.

[4] Endpoint numbers were assigned to each concentration (i.e., unique test level) within an experiment and were logged as an endpoint.

[5] EPA made effect/no-effect conclusions for each endpoint based on 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. These terms are not to be confused with language associated with the Endangered Species Act (ESA) species-specific effects determinations.

Table 2-1. Summary of Cosm Database Changes due to Reevaluations

Study Group	Associated Citations	Endpoint Number[A]	Nominal Concentration (µg/L)	USEPA, 2016 Conclusion[B]	USEPA, 2024 Conclusion[B]
Studies Brought to the 2023 SAP					
Lampert	Fleckner (1988)[C]; Lampert et al. (1989)	58b	0.1	Effect	**EXCLUDED**
		58	1	Effect	
University of Kansas: 1979 Experiment	deNoyelles and Kettle (1980)[C]; deNoyelles et al. (1982; 1989); Kettle (1982)[C]; Kettle et al. (1987); Larsen et al. (1986)[C]	52	20	Effect	**EXCLUDED**
		3	500	Effect	
University of Kansas: 1981-1983 Experiment[D]	Carney (1983); deNoyelles and Kettle (1983)[C]; deNoyelles et al. (1989; 1994[C]); Dewey (1986); Huggins (1990)[C]; Huggins et al. (1994)[C]; Larsen et al. (1986)[C]	2 (YR 1)	20	Effect	**EXCLUDED**
		4 (YR 1)	100	Effect	
		41 (YR 1)	100	Effect	
		42 (YR 1)	200	Effect	
		5 (YR 2)	200	Effect	
		1 (YR 2)	500	Effect	
Detenbeck	Detenbeck et al. (1996)	22	15	Effect	**EXCLUDED**
		23	25	Effect	
		24	50	Effect	
		25	74	Effect	
Kosinski	Kosinski (1984); Kosinski and Merkle (1984)[C]	28	10	Effect	**EXCLUDED**
		44	100	Effect	
Seguin: 2001 Studies[E]	Seguin et al. (2001a)	84 (PERI)	2	Effect	**No Effect**
		83 (PERI)	30	Effect	**Effect**
	Seguin et al. (2001b)	86 (PHYTO)	2	Effect	**Combined with #84**
		85 (PHYTO)	30	Effect	**Combined with #83**
Seguin: 2002 Study	Seguin et al. (2002)	87	30	Effect	**Effect**
Additional Reevaluations Since the 2023 SAP					
Baxter	Baxter et al. (2011)	108	1	Effect	**No Effect**
		109	10	Effect	**No Effect**
		110	30	Effect	**No Effect**
		111	100	Effect	**Effect**
Pannard	Pannard et al. (2009)	102	0.1	Effect	**Effect**
		103	1	Effect	**Effect**
		104	10	Effect	**Effect**

[A] Endpoint numbers were assigned to each concentration within the experiment and were logged as an endpoint.
[B] EPA made effect/no-effect conclusions for each endpoint based on 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. These terms are not to be confused with language associated with the Endangered Species Act (ESA) species-specific effects determinations.
[C] Studies associated with the original 11 identified by the 2012 SAP.
[D] YR 1 and YR 2 = From year 1 or year 2 of the study, respectively.
[E] PERI and PHYTO = Endpoints were originally based on the periphyton results from Seguin et al. (2001a) or the phytoplankton results from Seguin et al. (2001b; later determined to represent the same study and combined).

2.2 Recalculation of the CE-LOC

After concurring with the 2023 SAP overall recommendations, reevaluating the Baxter *et al*. (2011) and Pannard *et al*. (2009) cosm studies, and correcting two errors in the cosm database, EPA made all necessary changes noted (see **Appendix II** for the updated cosm database) and recalculated the CE-LOC following the protocol described in **Appendix III**.

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The updated CE-LOC is 9.7 µg/L

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This concentration is based on the median[6] of the CE-LOC estimates (see **Table 2-2** for more descriptive statistics).

Table 2-2. Description of the population of CE-LOC results (n=1000)

Descriptive Statistic	Updated Value (µg/L)
5th Percentile	5.6
25th Percentile	7.9
Median (50th percentile)	9.7
75th Percentile	12
95th Percentile	17
Range	2.5-27

The files used to calculate the updated CE-LOC can be found in **Appendix IV**. This appendix also contains a folder with only the starting files, which can be used in combination with the protocol in **Appendix III** to reproduce the CE-LOC.

3 Updates to WARP-MP modeling

3.1 WARP-MP Inputs

In addition to updating the CE-LOC, EPA corrected the WARP-MP modeling and updated the mapping of watersheds. A commenter on the proposed revisions to the atrazine interim decision suggested that one of the WARP-MP modeling parameters (i.e., precipitation in May and June) was incorrectly calculated. EPA acknowledges that the precipitation parameter was calculated incorrectly as the average precipitation in May and June rather than the total precipitation in May and June. EPA updated the calculation for this parameter and updated the WARP-MP estimated atrazine concentrations accordingly.

To model atrazine concentrations with WARP-MP using the updated precipitation parameter, several other input files required regeneration or updating in ArcGIS, to ensure consistency across datasets. Updates included specifying the same projection/coordinate system, cell size, and snap raster across all rasters, because these data were missing in some input raster files. Detailed information regarding ArcGIS and Python preparation for WARP-MP are available in **Appendix V.**

Updating the WARP-MP modeling generally increased the predicted atrazine concentrations. This result was anticipated because the correction to the WARP-MP modeling doubled the precipitation input (i.e.,

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[6] The median of the data is used because the data is not normally distributed (p-value = 2.2 X 10^{-64}; Jarque-Bera Test).

from an average of two months of precipitation to a total of two months of precipitation), which would lead to higher predicted atrazine concentrations.

3.2 Mapping Updates

EPA identified an error in the 12-digit hydrologic unit code (HUC 12) boundaries used in ArcGIS to produce the map released in the 2022 proposed revisions to the interim decision. WARP-MP is run using watershed boundaries from NHDPlusV2 and calculates atrazine concentrations for each associated watershed. The 2022 map proposing HUC 12 watersheds for mitigation was mistakenly generated using a different dataset of watershed boundaries than WARP-MP is run with, causing some watersheds to be misidentified or excluded from the map as a result of changes to watershed names and/or watershed boundaries. The updated maps are based on the NHDPlusV2 watershed definitions used by WARP-MP so that the maps are consistent with the WARP-MP modeling.

4   Watersheds Proposed for Mitigation

Watersheds with higher predicted atrazine concentration are expected to have waterbodies with greater vulnerability to atrazine runoff due to landscape features (e.g., soil restrictive layers), precipitation, and atrazine usage. To identify which HUC 12 watersheds may need mitigation to reduce atrazine runoff, EPA compared predicted atrazine concentrations from WARP-MP and monitoring data to the updated CE-LOC. **Figure 4-1** shows these watersheds exceeding the updated CE-LOC in green. A high-resolution map with county boundaries has been published along with this update.
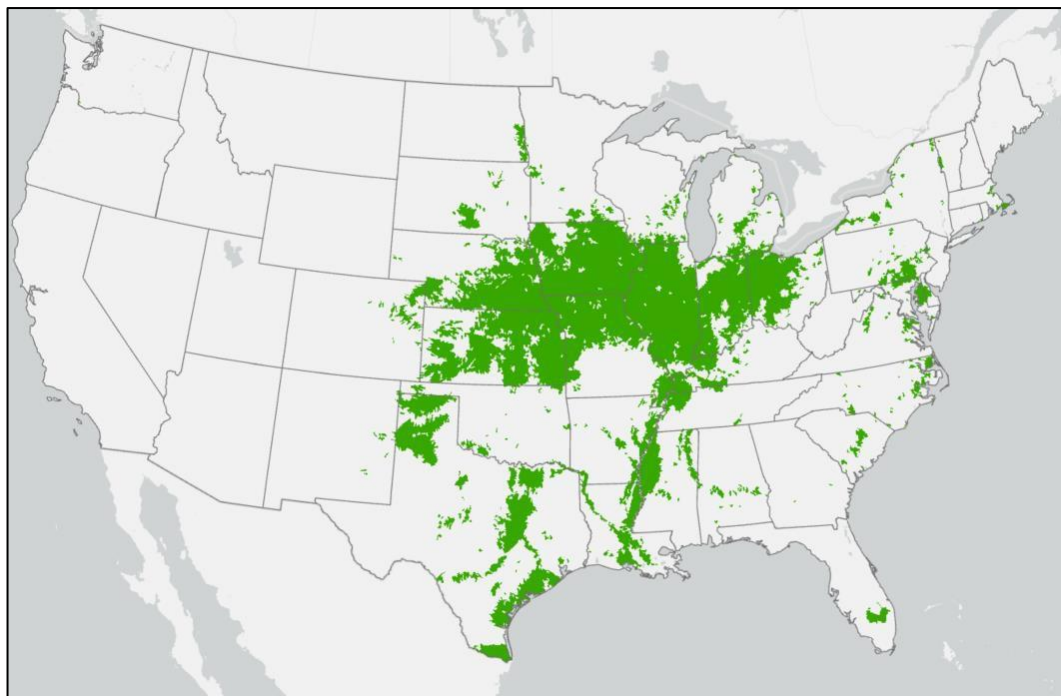

Figure 4-1. Updated map depicting HUC 12 watersheds that exceed the updated CE-LOC.

The update to the CE-LOC and WARP-MP modeling resulted in many acres of cultivated land that are no longer predicted to exceed the CE-LOC. These watersheds are depicted in **Figure 4-2**.
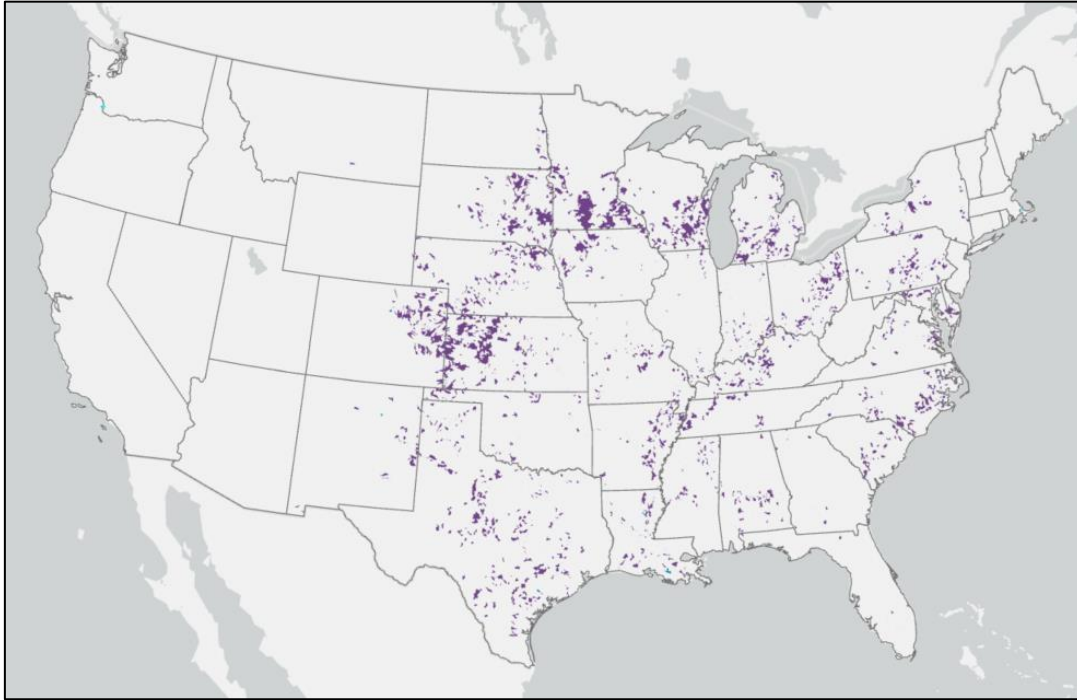
Figure 4-2. HUC 12 watersheds that no longer exceed the CE-LOC. Coloring reflects the map provided in the 2022 mitigation proposal: purple-colored watersheds had predicted atrazine concentrations between 3.4-9.8 µg/L in 2022, teal-colored watersheds had predicted atrazine concentrations above 9.8 µg/L in 2022.

Conversely, as a result of the update to the WARP-MP modeling and mapping, there are watersheds now proposed for mitigation that were not in the 2022 proposed revisions to the atrazine interim decision. That is, estimated atrazine concentrations in these watersheds increased due to the adjustment to the WARP-MP modeling, primarily from the correction to the precipitation input, or were previously identified and not displayed properly on the map. These watersheds are primarily in Mississippi, but also include at least one watershed in numerous other states: Oregon, Nebraska, South Dakota, Missouri, Kansas, Illinois, Indiana, Texas, Louisiana, Arkansas, Alabama, North Carolina, Virginia, Maryland, Kentucky, Michigan, Ohio, Pennsylvania, New York, Rhode Island, Massachusetts, and Vermont. These watersheds are depicted in **Figure 4-3** in orange for easier identification.

Figure 4-3. Watersheds that are proposed for mitigation that were not previously proposed for mitigation.

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[7] Master Record Identifier is used to track and manage information submitted to the Office of Pesticide Programs.

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Appendix I.   Additional Reevaluations (Baxter and Pannard)

Baxter *et al.* (2011)

Experimental Design

This 73-day study was conducted at the University of Guelph (Guelph, ON, Canada) in 12,000 L cosms (3.9 m diameter, 1.2 m deep, filled with water approximately 1 m deep) lined with a black PVC pond liner. Before the start of the study, spring water, sediment trays (covering 44% of bottom), substrate to collect periphyton, and pots of *Myriophyllum spicatum* and *Elodea canadensis* were added. To begin the experiment, cosms were divided into five treatments: 0, 1, 10, 30, and 100 µg/L of atrazine (nominal; 3 cosms/treatment). Each atrazine-treated cosm received one dose of technical-grade atrazine (96% active ingredient) dissolved in acetone (10 mL total). Control cosms (0 mg/L) received acetone only (there was no negative control). Throughout the experiment, water chemistry, periphyton, phytoplankton, filamentous algae, and snails (not discussed here) samples were taken. Water chemistry included atrazine concentrations, total nitrogen, total phosphorus, total hardness, alkalinity, pH, conductivity, temperature, dissolved oxygen (DO), and photosynthetically active radiation (PAR). For periphyton and phytoplankton, ash-free dry weight (AFDW) and chlorophyll *a* were measured on days 1, 4, 7, 14, 21, 28, 49, and 70. Filamentous algae was assessed weekly with a visual scoring system. At the end of the experiment, macrophyte wet and dry mass were measured for above-sediment shoot growth (both species) and below-sediment root growth (*M. spicatum* only). For statistical analysis, parameters were assessed with repeated-measures analysis of variance (ANOVA), followed by one-way ANOVA if there was a time effect. Dunnett's test was used for all post-hoc analyses. Nominal concentrations of atrazine and a significance level of 0.05 were used in all statistical analyses.

EPA's Use as of 2016

In the 2016 Refined Ecological Risk Assessment (USEPA, 2016), EPA included four endpoints from Baxter *et al*. (2011) based only on the macrophyte results (see **Table AI-1**). Endpoint #108 represented the 1 µg/L treatment and was scored as "**No Effect**" due to the lack of statistically or biologically significant differences in macrophyte biomass after 70 days. EPA stated that "there may have been effects from the exposure, but these had recovered to control levels by day 70." Endpoint #109 represented the 10 µg/L treatment and was scored as an "**Effect**" due to what were determined to be biologically significant decreases in *M. spicatum* shoot weight (46%), despite a lack of statistical significances. Endpoint #110 represented the 30 µg/L treatment and was scored as an "**Effect**" due to what were determined to be biologically significant decreases in *M. spicatum* (19%) and *E. canadensis* (17%) shoot weight, despite a lack of statistical significances. Endpoint #111 represented the 100 µg/L treatment and was scored as an "**Effect**" due to biologically and statistically significant decreases in *M. spicatum* (78%) and *E. canadensis* (81%) shoot weight. In addition, EPA determined that potential biologically significant effects to M. spicatum root dry weight were reported at 1 (-46%), 10 (-54%), 30 (-31%) and 100 (-77%) µg/L, but there was less certainty in the significance of the reductions in root dry weight as compared to the reductions in shoot weight due to overlapping error bars across the doses.

Criticism in the 2022 Public Comments

In comments submitted on the 2022 proposed revisions to the Interim Decision, Syngenta[8] said the study was high quality but that the variance puts the 30 µg/L treatment results within the control range and the results cannot be considered biologically significant (Syngenta, 2022). That, combined with the non-monotonic response led to their scoring of 1, 10, and 30 µg/L as "**No Effect**" and 100 µg/L as an "**Effect**."

The Triazine Network shared these conclusions saying that the "biological significance argument is without scientific merit" due to a lack of dose response and variance that that puts the results "well within the range observed in the control treatment" (Triazine Network, 2022).

EPA's 2024 Reevaluation

In EPA's reevaluation, EPA found no major flaws that would warrant exclusion of the study from the cosm databases and concludes that it is suitable for use in the cosm database.

Effect/No-Effect Conclusion

The study authors reported that there were no statistically significant differences in water chemistry parameters or filamentous algae when atrazine treatments were compared to the control. For periphyton and phytoplankton, there were no consistent differences, and they were not dose responsive (**Figure AI-1**).

Fig. 3. Mean surface-level periphyton (top panel) and phytoplankton (bottom panel) ash-free dry weight (AFDW) during 70-d exposures to atrazine. Bars represent maximum and minimum values for each of the three microcosms per treatment. No significant differences between treatments were found.
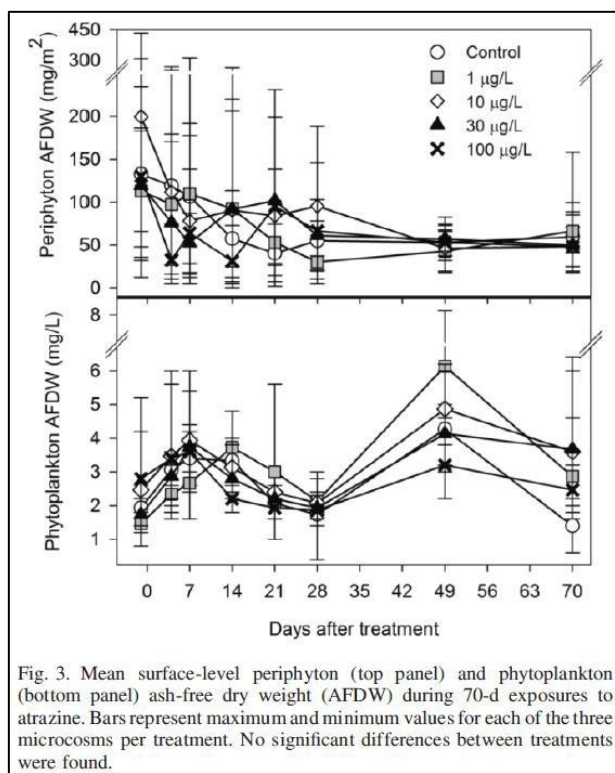
Figure AI-1. Excerpt of Figure 3 from Baxter et al. (2011)

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[8] Syngenta Crop Protection comments include those from Jeffrey Giddings, a Syngenta contractor. The comments from Syngenta Crop Protection and Jeffrey Giddings are collectively referred to as "Syngenta" in this document.

For macrophytes, the study authors reported that the shoot wet and dry weights for *M. spicatum* and *E. canadensis* and the root wet and dry weight for *M. spicatum* were statistically significantly reduced in the 100 µg/L treatment relative to controls (**Figure AI-2**). Macrophytes in the 1, 10, and 30 µg/L treatments were not statistically significantly different from the controls (**Figure AI-2**).
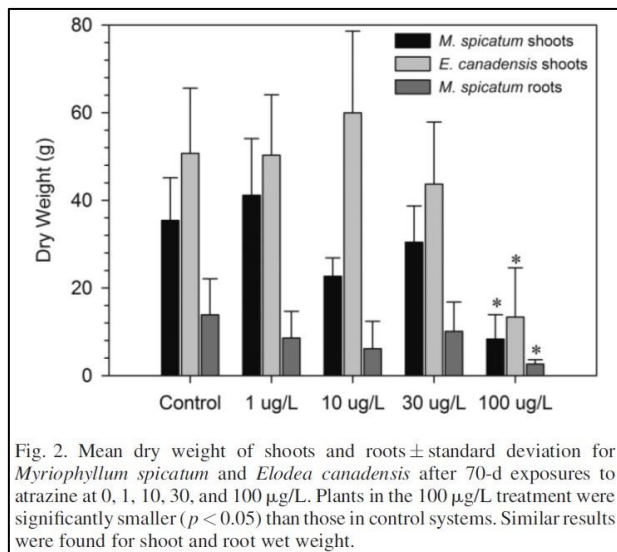

Fig. 2. Mean dry weight of shoots and roots ± standard deviation for *Myriophyllum spicatum* and *Elodea canadensis* after 70-d exposures to atrazine at 0, 1, 10, 30, and 100 µg/L. Plants in the 100 µg/L treatment were significantly smaller ($p < 0.05$) than those in control systems. Similar results were found for shoot and root wet weight.

Figure AI-2. Excerpt of Figure 2 from Baxter et al. (2011)

In 2016, EPA determined that the magnitude of the effect based on the mean dry shoot weight was biologically significant (46% for 10 µg/L and 17-19% for 30 µg/L), despite not being statistically significant. Depending on the endpoint measured, there is a general trend of decreasing dry weight compared to the control, starting as low as 1 µg/L. Although not statistically significant, some of those reductions could be biologically meaningful given the magnitude of the difference. These reductions would not have been detected as statistically significant because of the relatively high variability in one or more of the treatment groups and/or the control. The raw data are not available for review, but control variability, as reported in terms of the standard deviation, is approximately +/-28% for shoot dry weight for both macrophyte species, which is roughly conserved across many of the treatment group responses. Based on the available information (figures only, no raw data), this pattern suggests that there was inherently high variability in the test system as a whole. This leads to reduced confidence in the mean differences being treatment related, as opposed to background variability. Therefore, while the magnitude of the effect based on the means might be biologically significant, there is enough uncertainty due to variability that confounds any conclusion that there was an effect at 10 and 30 µg/L.

Therefore, upon reevaluation of the results, EPA has decided to change the effect/no-effect conclusion for endpoints #109 and #110 from "**Effect**" to "**No Effect**".

EPA's 2024 Conclusions

EPA determined 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 plant 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 will retain the four endpoints associated with Baxter *et al*. (2011), but EPA will change the effect/no-effect conclusion for endpoints #109 and 110 from "Effect" to "No Effect".**

Table AI-1 summarizes Baxter *et al*. (2011) in the 2016 cosm database and the changes that were made after this reevaluation.

Table AI-1. A summary of the cosm endpoints associated with the Baxter *et al*. (2011) study that appeared in the 2016 Refined Ecological Risk Assessment (Appendix G.2) and the adjustments after this 2024 reevaluation (effect/no-effect changes highlighted in gray). These endpoints will continue to be used to evaluate the potential effects of atrazine on aquatic plant communities.

Endpoint Number[A]	Nominal Conc. (µg/L)	2016 Effect/No-Effect Conclusion	Original Results[B]	2024 Effect/No-Effect Conclusion	Updated Results[C]
108	1	No Effect	No statistical or biologically significant differences in macrophyte biomass at 70-days, however earlier effects may have occurred but recovered.	No Effect	No statistically or biologically significant effect on periphyton, phytoplankton, or macrophyte.
109	10	Effect	46%[D] reduction in macrophyte biomass, biologically significant	No Effect	No statistically significant effect on periphyton, phytoplankton, or macrophyte.
110	30	Effect	17-19%[D] reduction in macrophyte biomass, biologically significant	No Effect	No statistically significant effect on periphyton, phytoplankton, or macrophyte.
111	100	Effect	78-81%[D] reduction in macrophyte biomass, Statistically and biologically significant	Effect	No statistically significant effect on periphyton or phytoplankton. Statistically significant reduction (78-81%) in macrophyte shoot and root biomass

[A]All endpoints will still have a duration of 70 days and the Plant Group will be updated to include phytoplankton, periphyton, and macrophytes.

[B]Unable to address macrophyte recovery time because only day 70 results were provided. Based on the mode of action, an assumption is that more significant effects may have occurred to other endpoints earlier in the study, but these are reflected in the chosen biomass endpoints and would include a recovery period.

[C]For macrophytes only, earlier effects may have occurred but recovered in the 1, 10, and 30 µg/L treatments. Unable to address recovery in the 100 µg/L treatment because only day 70 results were provided.

[D]The 2016 Refined Ecological Risk Assessment document and Appendix G.2 report these percents as 48%, 14%, and 83-86%, respectively, based on an earlier version of the Data Evaluation Record (DER). The numbers here represent the updated and signed DER (see Appendix G.3 in USEPA, 2016).

Pannard *et al.* (2009)

Experimental Design

In 2001, plankton were collected from a freshwater wetland in France and zooplankton were removed. The resulting phytoplankton community was maintained in semicontinuous culture with weekly dilutions. The medium consisted of filtered wetland water supplemented with nitrate (350 µg $N-NO_3$/L) and phosphate (52.6 µg $P-PO_4^{3-}$/L). A few weeks before the experiment, the culture was "starved" by reducing nutrient inputs and making it phosphorus deficient. Phosphorus was resupplied the day the experiment started onward (52.6 µg $P-PO_4^{3-}$/L). In the experiment, communities were exposed to atrazine for 7 weeks in 500-mL bottles filled with 240 mL of the culture. The treatments were 0 (control), 0.1, 1.0, and 10 µg atrazine/L (4 replicates each). For the atrazine treatments, "commercial pure" atrazine (pestanal, riedel-de-haën; exact purity unknown) was used with no solvent. During the experiment, semicontinuous culture conditions were maintained with weekly fresh inputs with the same atrazine concentration. Phytoplankton biomass was measured via chlorophyll *a* and the biological activity was measured via carbon dioxide incorporation once a week. The taxonomic composition was determined at the beginning of the experiment, then after 1, 4, and 7 weeks of exposure by counting cells and colonies under a microscope. Dominance within the community was characterized by calculating Simpson's index of diversity. For statistical analyses, Friedman non-parametric test was used for biomass and biological activity. A chi-square analysis was used for community structure. Analysis of variance was used for species densities. Lastly, a Kruskall-Wallis analysis was used for Simpson's index. In all tests, significance was set to *p* = 0.05.

EPA's Use as of 2016

In the 2016 Refined Ecological Risk Assessment (USEPA, 2016), EPA included three endpoints from Pannard *et al.* (2009) (see **Table AI-4**). Endpoint #102 represented the 0.1 µg/L treatment and was scored as an "**Effect**" due to a 18-62% reduction in carbon incorporation over time (difficult to interpret significance), a statistically and/or biologically significant reduction in the final density of six species or groups of phytoplankton (40-79%), a biologically significant reduction in the total phytoplankton community density (62%), and changes in Simpson's index of diversity indicating shifts in composition. Endpoint #103 represented the 1 µg/L treatment and was scored as an "**Effect**" due to a 12-47% reduction in carbon incorporation over time (difficult to interpret significance), a statistically and/or biologically significant reduction in the final density of seven species or groups of phytoplankton (27-69%), a biologically significant reduction in the total phytoplankton community density (50%), and changes in Simpson's index of diversity indicating shifts in composition. Endpoint #104 represented the 10 µg/L treatment and was scored as an "**Effect**" due to a 12-47% reduction in carbon incorporation over time (difficult to interpret significance), a statistically and/or biologically significant reduction in the final density of seven species or groups of phytoplankton (33-78%), a biologically significant reduction in the total phytoplankton community density (67%), and changes in Simpson's index of diversity indicating shifts in composition. Some statistics were provided for the end of the study (60 days), which reflects any recovery that may have occurred during the continuous exposure. EPA further said, "An increase in the Simpsons index as well as a shift in the composition in a dose response manner indicates that the concentrations tested affected the community composition leading to more variable communities than the controls and that the dominance in the community had shifted from few taxa being dominant to more even spread of dominance across species" (USEPA, 2016). There was no effect on chlorophyll *a* at any of the tested concentrations.

Criticism in the 2022 Public Comments

In comments submitted on the 2022 proposed revisions to the Interim Decision, Syngenta said that the Pannard *et al.* (2009) study received the lowest data quality and reliability score, which was due to relevance (venue, size, complexity, exposure), lack of atrazine analysis, the addition of nutrients (considered a confounding factor), inconsistent dose-response, and inconsistency among replicates and over time (Syngenta, 2022). They stated that in the Open Literature Review Summaries (OLRS), the reviewer statements conflict with the study text and that the percent changes are difficult to determine from data only presented in figures. On this point, they concluded with, "These discrepancies may be inconsequential, but they are indicative of the difficulty of interpreting a low-quality study; they also influence the effect scores." Syngenta further pointed out that there was a "lack of clear dose-response relationships for nearly all of the measurement endpoints," which contributed to its quality score and complicated interpretation of the results. Syngenta also discussed the "confounding effect" of the nutrient addition at the time of the atrazine treatment and said that the "Community response to atrazine, if any, was overwhelmed by the response to nutrient enrichment." Finally, Syngenta commented on the Simpson's index, which showed a dose-dependent increase in diversity and said that "it is unclear whether an increase in diversity is considered an adverse effect." With all this, Syngenta concluded that, "Evidence for atrazine effects in this study is weak, with no dose-response except (possibly) an *increase* in diversity... the lack of dose-response and the increase in diversity suggest that there were no adverse atrazine effects at any exposure concentration." They scored all three endpoints as "**No Effect**" but they would be eliminated because of the low scores the study received. It is worth mentioning that Giddings[9] *et al*. (2018) scored 0.1, 1, and 10 µg/L as an "**Effect**" based on the dose-related changes in Simpson's diversity index.

The Triazine Network commented on the lack of dose response, the increase in diversity not being an adverse effect, the simplified communities, continuous and constant atrazine concentrations, and the addition of phosphorus (Triazine Network, 2022).

EPA's 2024 Reevaluation

Study Relevance, Analytical Verification, and Added Nutrients

Syngenta and the Triazine Network identified several issues with the Pannard *et al*. (2009) study. Some are common characteristics of cosm studies or were not requirements under EPA's screening criteria (USEPA, 2011), while others could be considered confounding factors or could produce uncertainty in the conclusions.

First, the relevance of the study, which includes the chosen venue (bottles), the size of the venue (500 mL), the simplicity of the community, and the exposure regime. Given that these were microcosm studies, which are also part of the cosm database, the venue (500-mL bottles) is not unusual. Cosm studies range widely in size, including down to this lower end. Being a smaller venue, it also makes since that the plant community was simpler (e.g., no macrophytes). Much like mesocosms (larger venues), microcosms have many positives and negatives associated with them, but they ultimately increase the scientific community's understanding beyond single-organism toxicity studies. In addition, exposure regimes vary across cosm studies and are meant to represent a variety of different scenarios in the

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[9] Syngenta's comments include those submitted by Syngenta but authored by Jeffrey Giddings, a Syngenta contractor. These comments are referred to as "Syngenta" in this document. Giddings *et al*. (2018) is an open literature study authored by Giddings; therefore, it is treated differently.

environment. While a continuous exposure to atrazine might seem unrealistic, the low concentrations tested in Pannard *et al*. (2009) could represent runoff in areas with high atrazine use where atrazine builds up on the field and then slowly runs off into nearby waterways. Overall, variability in venues, communities, and exposures strengthen the cosm database and contributes to the goal of representing aquatic plant communities across the United States.

Next, the lack of analytically verifying atrazine concentration. EPA's screening criteria states, "The amount of test material applied and the exposure concentration in the water column should be determined analytically at the start of exposure (t=0 days). Minimally, nominal exposure concentrations of atrazine must be provided." Therefore, analytically measured concentrations were not a requirement under EPA's screening criteria and where not a feature that would solely exclude a study from the database.

Finally, there was mention of the addition of nutrients being a confounding factor. Similar to the exposure regime, the cosm database includes a variety of nutrient scenarios. Whether this is due to the source water naturally having low or high nutrients or the study authors experimentally manipulating nutrients at some point. In the Pannard *et al*. (2009), nutrients were added to the culture and then the culture was starved before the experiment. This was all done at the whole-culture level (i.e., before the division of the culture into the individual experimental units). Therefore, the starting community would have been approximately the same for each replicate in the experiment. As discussed next, there were a variety of effects observed in this study despite this nutrient addition. Therefore, the manipulation of nutrients in this study strengthens the cosm database and contributes to the goal of representing aquatic plant communities across the United States.

Effect/No-Effect Conclusion

The issues pointed out regarding the results included the difficult to interpret results, inconsistent dose-response, inconsistency among replicates over time, and the consideration of increases as adverse effects. EPA agrees that some results are difficult to interpret, namely carbon incorporation (**Figure AI-3**), which was noted in EPA's review of the study in 2016 and was not included in the results column in the 2016 cosm database. However, even with putting this result aside, there are still several other effects on algal density, which are discussed next.
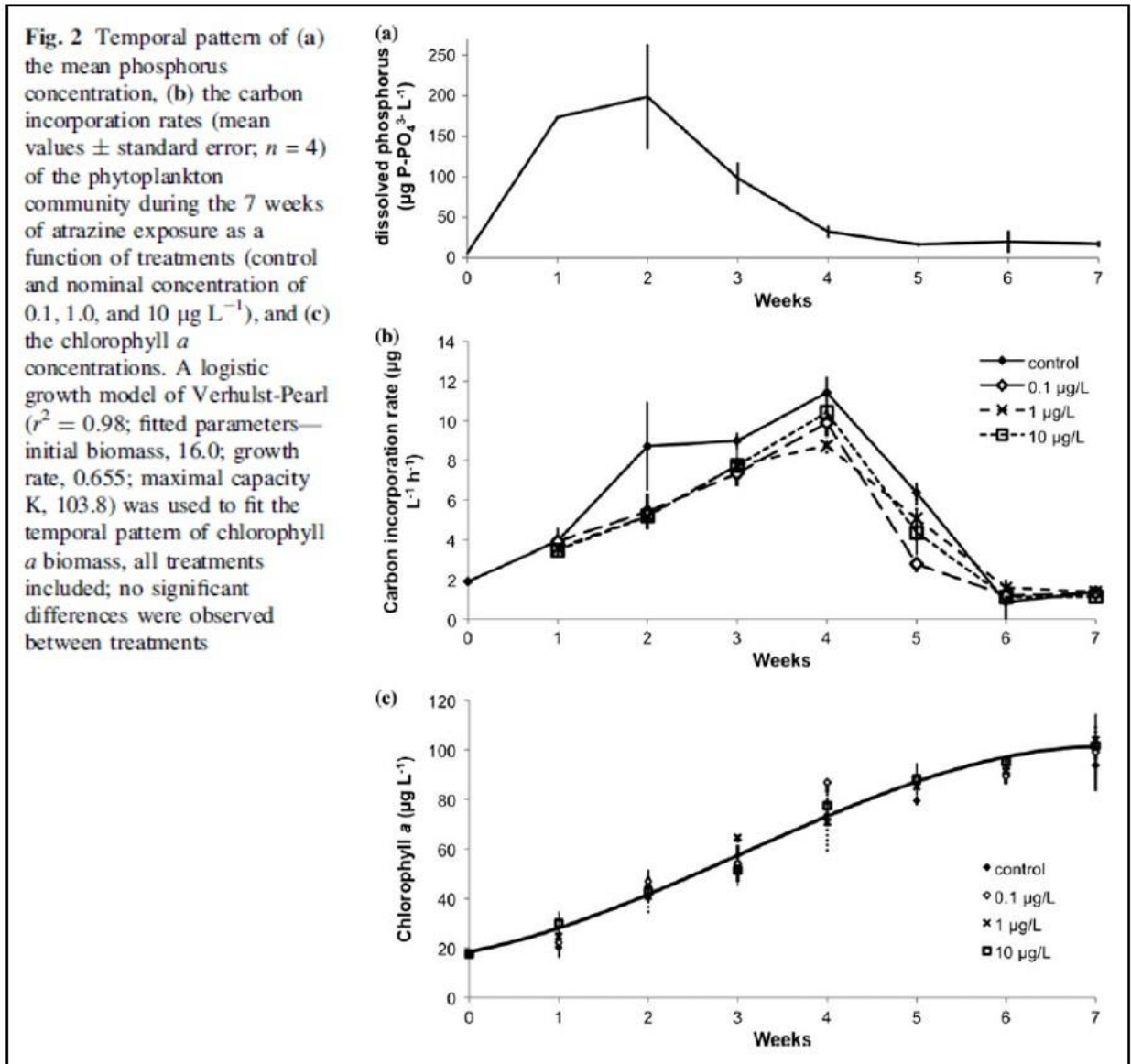
Fig. 2 Temporal pattern of (a) the mean phosphorus concentration, (b) the carbon incorporation rates (mean values ± standard error; $n = 4$) of the phytoplankton community during the 7 weeks of atrazine exposure as a function of treatments (control and nominal concentration of 0.1, 1.0, and 10 μg L^{-1}), and (c) the chlorophyll a concentrations. A logistic growth model of Verhulst-Pearl ($r^2 = 0.98$; fitted parameters— initial biomass, 16.0; growth rate, 0.655; maximal capacity K, 103.8) was used to fit the temporal pattern of chlorophyll a biomass, all treatments included; no significant differences were observed between treatments

Figure AI-3. Excerpt of Figure 2 from Pannard *et al*. (2009). The study authors indicated that carbon incorporation after 3 weeks decreased significantly in atrazine treatments when compared to the control, even with the 0.1 μg/L treatment (p < 0.01, b above). For chlorophyll *a*, there were no significant differences between the control and treated communities (c above).

Algal Species Over Time

Figure AI-4 shows the observed effects on algae species over time. There were treatment-level effects on *Oocystis sp*. (**Figure AI-4a**), *Aphanocapsa sp*. (**Figure AI-4c**), and *Chlorella sp*. (**Figure AI-4d**). The density of *Oocystis sp*. decreased with time and atrazine dose with a clear separation of the control and all other treatments ($p \leq 0.001$; **Figure AI-4a**). *Aphanocapsa sp*. increased in density during the 7-week experiment in all treatments, but abundance was significantly higher in the controls at the end of the experiment ($p < 0.05$; **Figure AI-4c**). *Chlorella sp*. initially decreased in all treatments followed by subsequent increases and decreases (**Figure AI-4d**). The density at the end was significantly higher in the control than in the atrazine treatments ($p < 0.05$). Additionally, while not shown in **Figure AI-4**,

Oscillatoria sp. (colony) initially increased in all treatments, then decreased until the end of the experiment. The density in the controls was higher than in the atrazine treatments ($p < 0.05$).
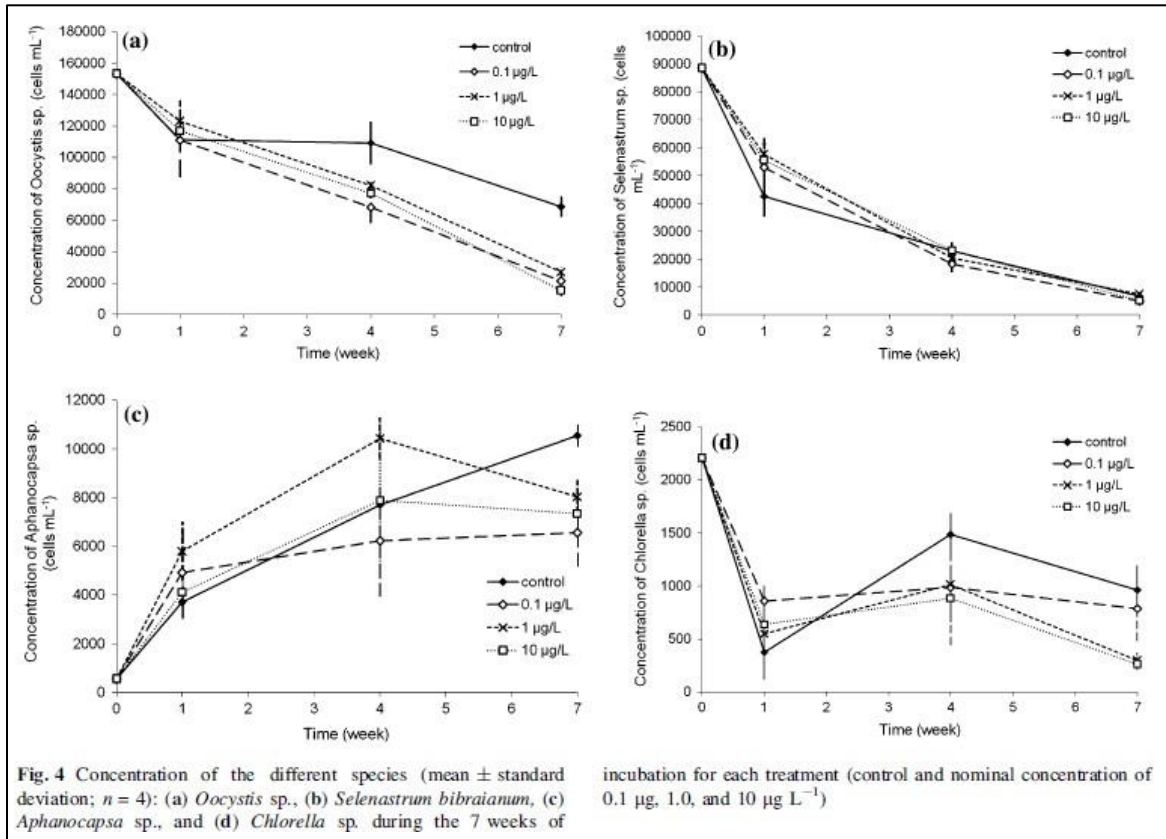

Figure AI-4. Excerpt of Figure 4 from Pannard *et al*. (2009)

Final Algae Densities

Table AI-2 and **Figure AI-5** show the observed effects on the final algal densities. While only two groups followed a dose response, there were observed effects on five other groups plus the total density, meaning seven out of eight quantified algal groups showed statistically and/or biologically significant effects. If only those with statistical significance is considered, there were still four groups affected. Additionally, conclusions about dose responses are based on the mean. The endpoints show variability, meaning the true population means could be somewhere within that range and other endpoints could therefore follow a dose response. Finally, community studies are complex and dynamic. They often involve decreases and increases because of the interactions between species. Therefore, dose responses are not always shown. Overall, one needs to consider if there were effects to species that resulted in a change to the community from the chemical. While the number of species affected can lead to the conclusion that there were changes to the community, the community structure results discussed next also show this.

Table AI-2. Observed effects on the final algal density in the Pannard *et al*. (2009) study that follow a dose response, that do not follow a dose response, or that were insignificant based on Table 1 from the study.

Endpoints with a Dose Response	Mean Effect[A]	
Final *Chlorella sp.* density	0.1 µg/L	-18% (NS)
	1.0 µg/L	-69%*
	10 µg/L	-73%*
Final unidentified chlorophyta density[B]	0.1 µg/L	-43%
	1.0 µg/L	-43%
	10 µg/L	-56%
Endpoints with No Dose Response	**Mean Effect[A]**	
Final *Chlorolobion sp.* density	0.1 µg/L	-73%*
	1.0 µg/L	-39%*
	10 µg/L	-54%*
Final *Ankistrodesmus falcatus* density[B]	0.1 µg/L	-71%
	1.0 µg/L	-37%
	10 µg/L	-66%
Final *Oocystis sp*. density	0.1 µg/L	-69%*
	1.0 µg/L	-60%*
	10 µg/L	-78%*
Final *Aphanocapsa sp.* density	0.1 µg/L	-40%*
	1.0 µg/L	-27%*
	10 µg/L	-33 %*
Final *Oscillatoria limnetica* density[B]	0.1 µg/L	-79%
	1.0 µg/L	-40%
	10 µg/L	-49%
Final community density[BC]	0.1 µg/L	-62%
	1.0 µg/L	-50%
	10 µg/L	-67%
Endpoints with Insignificant Effects	**Mean Effect[A]**	
Final *Selenastrum bibraianum* density	0.1 µg/L	-28% (NS)
	1.0 µg/L	+10% (NS)
	10 µg/L	-22% (NS)

[A] "-" represents a decrease in the mean compared to the control

"+" represents an increase in the mean compared to the control

"*" indicates that the effect was statistically significantly different from the control

"NS" indicates that the effect was determined to be statistically and biologically insignificant

[B] For these groups, the atrazine treatments were not statistically different from the control but EPA determined in 2016 that they were biologically significantly different from the control.

[C] This group represents the sum of the other groups and was not tested statistically by the study authors. EPA determined that the atrazine treatments were biologically significantly different from the control in 2016.
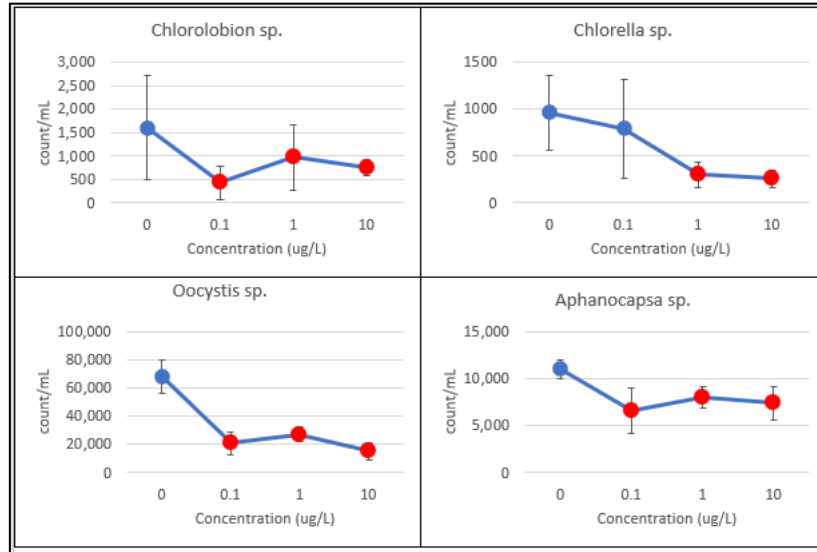
Figure AI-5. Graphical representation of Table 1 from Pannard et al. (2009; statistically significant effects only). Red datapoints are treatments statistically significantly different from the control.

Simpson's Index

There were comments on the community structure results, which show an *increase* in the Simpson's index, or an increase in diversity. While increases in endpoints are often not considered adverse, increases in the Simpson's index can indicate underlying adverse changes in the community composition.

The index calculates diversity using the following equation:

$$D = 1 - \frac{\sum n - (n-1)}{N(N-1)}$$

Where D is diversity, n is the number of individuals of a particular species, and N is the total number of individual organisms. Therefore, changes in either the total number of individuals within each species (numerator) or the total number of individuals overall (denominator) could change the Simpson's index in either direction, or not at all. **Table AI-3** shows a variety of examples where the Simpson's index either stays the same or goes up. In all of these examples, EPA would consider the community to be adversely affected. Diversity goes up in these cases because the Simpson's index determines the probability of randomly choosing different species. If the community shifts to be more even spread, the chance of picking the dominant organism (e.g., Species A below) goes down, while the chance of picking a variety of organisms goes up. Therefore, when combined with the species diversity results discussed above, EPA considers the observed increase in the Simpson's index an adverse effect and it is important to note that it shows a clear dose response with clear separation from the control by week 7 (**Figure AI-6**).

Table AI-3. Examples of how changes in the number of individuals within a species and the total number of individuals impacts the Simpson's index.

Algal Species	Control	Example 1	Example 2	Example 3	Example 4
A	800	400	100	400	40
B	100	50	800	90	50
C	100	50	100	90	50
Sum	1000	500	1000	580	140
Diversity	0.34	0.34	0.34	0.48	0.67
Description of Scenario		All species reduced by 50%	Change in dominance	Most abundant species reduced by 50%, others reduced by 10%	Most abundant species reduced by 90%, others reduced by 50%

Fig. 5 Changes in Simpson's index of diversity, $(1 - D)$ (mean ± standard deviation; $n = 4$) during the 7 weeks of exposure to the different atrazine treatments (control and nominal concentration of 0.1, 1.0, and 10 µg L^{-1})
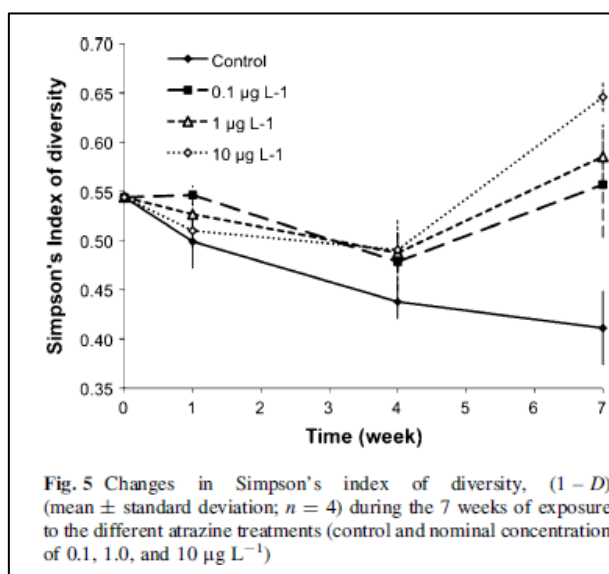
Figure AI-6. Excerpt of Figure 5 from Pannard et al. (2009). Study authors report that after 7 weeks of exposure, diversity was significantly higher in the communities exposed to atrazine than in the controls (p = 0.027) and followed a dose response.

Upon reevaluation of the results, EPA did not change the effect/no-effect conclusion for the endpoints associated with Pannard *et al.* (2009) given the amount of evidence to support these conclusions. So, all endpoints will remain as an "**Effect**".

EPA's 2024 Conclusions

While the study has deficiencies, EPA determined 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 plant 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 will not change for Pannard *et al*. (2009).**

Table AI-4 summarizes Pannard *et al*. (2009) in the 2016 cosm database and any changes that were made after this reevaluation.

Table AI-4. A summary of the cosm endpoints associated with the Pannard *et al*. (2009) study that appeared in the 2016 Refined Ecological Risk Assessment (Appendix G.2) and the adjustments after this 2024 reevaluation. These endpoints will continue to be used to evaluate the potential effects of atrazine on aquatic plant communities.

Endpoint Number[A]	Nominal Conc. (µg/L)	2016 Effect/No-Effect Conclusion	Original Results[B]	2024 Effect/No-Effect Conclusion	Updated Results
102	0.1	Effect	Cell Density: *Chlorolobion sp*. (-73%) *Ankistrodesmus falcatus* (-71%) *Oocystis sp*. (-69%) Unidentified chlorophyta (-43%) *Aphanocapsa sp*. (-40%) *Oscillatoria limnetica* (-79%)	Effect	Only update to the results is the inclusion of: "Community Structure: Dominance and diversity, as demonstrated by changes in Simpson's index of diversity and the provided CCA[C] results"
103	1	Effect	Cell Density: *Chlorolobion sp*. (-39%) *A. falcatus* (-37%) *Chlorella sp*. (-69%) *Oocystis sp*. (-60%) Unidentified chlorophyta (-43%) *Aphanocapsa sp*. (-27%) *O. limnetica* (-40%) Community Structure: Dominance and diversity, as demonstrated by changes in Simpson's index of diversity and the provided CCA[C] results	Effect	No update to the results.
104	10	Effect	Cell Density: *Chlorolobion sp*. (-54%) *A. falcatus* (-66%) *Chlorella sp*. (-73%) *Oocystis sp*. (-78%) Unidentified chlorophyta (-56%) *Aphanocapsa sp*. (-33%) *O. limnetica* (-49%) Community Structure: Dominance and diversity, as demonstrated by changes in Simpson's index of diversity and the provided CCA[C] results	Effect	No update to the results.

[A]All endpoints will still have a duration of 49 days and the Plant Group will remain phytoplankton.
[B]Multiple doses. Effects only reported in terms of final measurement. Concentrations held constant by weekly additions of compound. An increase in the Simpsons index as well as a shift in the composition in a dose response manner indicates that the concentrations tested affected the community structure leading to more variable communities than the controls and that the dominance in the community had shifted.
[C]Canonical Correspondence Analysis

Literature Cited in This Appendix

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Appendix II.   Updated Cosm Endpoint and Chemograph Database (Microsoft Excel)

The updated Cosm Endpoint and Chemograph Database is attached to this document and named "Appendix II. Cosm Endpoint and Chemograph Database 6-24-2024.xlsx". This can be saved or opened as a Microsoft Excel file and contains the detailed cosm database and associated cosm chemographs in Microsoft Excel format. Some of these details also appear in the files associated with **Appendix IV**.

Appendix III.   Step-by-Step Process for Running PATI and Calculating the CE-LOC

Running PATI (Plant Assemblage Toxicity Index)

1. Duplicate the "PATI and CELOC Files_StartingFiles" folder and rename it with your run details[10] (this folder, which contains all the required files, can be found in **Appendix IV**)
    a. E.g., "PATI and CELOC Files_2024UpdatedCELOCRun"
2. In this folder, open "2014UncertaintyAnalysisCosmPATIandLOC.exe" and enter the following:
    a. For "ENTER NAME OF COSM FILE" – Enter the cosm database name:
        i.   "cosmeffects_2016original.dat" for the 2016 database that resulted in 3.4 µg/L
        ii. "cosmeffects_2024update.dat" for the 2024 database that resulted in 10 µg/L
    b. For "ENTER 0 TO NOT INCLUDE TOXICITY UNCERTAINTY, 1 TO INCLUDE" – Enter "1"
    c. For "ENTER 0 TO NOT INCLUDE PATI-LOC UNCERTAINTY, 1 TO INCLUDE" – Enter "1"
    d. For "ENTER DESIRED NUMBER OF SETS TO EVALUATE UNCERTAINTY" – Enter "1000"
    e. For "ENTER DESIRED NUMBER OF SAMPLES TO DEFINE PATI FUNCTION" – Enter "1000"
3. Once it is finished running (could take several minutes), close (it saves in the background)
    a. The following output files will be added to the folder:
        i.   "2014UAPATIFunctionParams.out" – This is the PATI
        ii. "2014UACosmCumulativePATI.out" – This is the cosm cumulative PATI scores
        iii. "2014UACumulativePATI-LOC.out" – This is the LOC_{PATI}
4. Open "2014UncertaintyAnalysisChemPATIandEEF.exe" and enter the following:
    a. For "ENTER NAME OF CHEMOGRAPH FILE" – Enter "AEEMP04to14.txt"
5. Once it is finished running (could take up to 1 hr), close (it saves in the background)
    a. The following additional output files will be added to the folder:
        i.   "2014UAChemographCumulativePATI.out" – This is the site cumulative PATI scores
        ii. "2014UAChemographEEF.out" – This is the Effect Exceedance Factors (EEFs)
        iii. "2014UAChemographMaxRunAvgConc.out" – This is the AEEMP concentrations

Processing the Data

1. Open "2014UAChemographEEF.out" with Microsoft Word and Save As a .txt file
    a. E.g., Named "2014UAChemographEEF_adjusted.txt"
2. Find and Replace the following in Microsoft Word <u>IN THIS ORDER</u>:
    a. "   " (3 spaces) with a "," (,no space)
    b. " " (2 spaces) with a "," (,no space)
    c. " " (1 space) with a "," (,no space)
    d. "¶****" or "^p****" (paragraph****) with ", " (,1 space)
    e. "¶," or "^p," with "¶" or "^p"
    f. Check beginning of the document for extra "," and remove
3. Open "2014UAChemographEEF_adjusted.txt" in Microsoft Excel and Save As a .xlsx file

---

[10] If you wish to run PATI multiple times, you should duplicate the "StartingFiles" folder each time and complete your new run within a new folder. If you do not create a new run folder for each new run and instead complete multiple runs within one folder, PATI will overwrite the existing output files each time.

      a. Note you may need to change the file type to "All Files (*.*)" in order to find the file

      b. Follow the Text Import Wizard to properly import the text into Microsoft Excel

           i. Change the delimiter from "Tab" to "Comma"

           ii. You should have 1000 rows (ISETS [11]) and 239 columns (ISET label + 238 AEEMP EEFs)

           iii. Note that AEEMP #201 (column GT) is blank (this will be fixed later)

      c. Save As (in .xlsx format)

           i. E.g., Named "2014UAChemographEEF_adjusted_excel.xlsx"

4. Open "2014UAChemographMaxRunAvgConc" in Microsoft Excel and Save As a .xlsx file

      a. Follow the Text Import Wizard to properly import the text into Microsoft Excel

           i. Change delimiter from "Tab" to "Space"

      b. The columns are shifted at AEEMP site 100 (row 100), so select all empty cells in Column A (Rows 1:99) and delete ("Shift cells left") to align rows 1-99 with 100-238

      c. Save As (in .xlsx format)

           i. E.g., Named "2014UAChemographMaxRunAvgConc_excel.xlsx"

5. Change the name of "CELOC Calculation_Template.xlsx" by replacing "Template" with your run details and then open the file

      a. E.g., Named "CELOC calculation_2024UpdatedCELOC.xlsx"

6. Copy over the sheets from "2014UAChemographEEF_adjusted_excel.xlsx" (created in Step 3 above) and "2014UAChemographMaxRunAvgConc_excel.xlsx" (created in Step 4 above) to the "CELOC calculation" workbook (Step 5 above)

7. On the "CELOC Calculation" sheet [12],

      a. Add run details to cells B5-9 (those entered in *Running Pati* Steps 2b-e above)

           i. E.g., B5: cosmeffects_2024update.dat, B6: 1, B7: 1, B8: 1000, B9: 1000

      b. Copy over the data from the "2014UAChemographMaxRunAvgConc" sheet into columns A and B starting at row 18

      c. Transpose the data from the "2014UAChemographEEF_adjusted" sheet so that the ISETs goes across the top (instead of down) starting in cell C18

           i. To avoid copying over the ISET identification number, transpose the data in another sheet and then only copy over the values starting in row 2 of that separate sheet

           ii. Note that AEEMP #201 (row 218) is blank for the ISETs, which prevents the values in rows 1-16 from being calculated and the graphs from being created (fixed next)

8. Duplicate the "CELOC Calculation" sheet and remove row 218 (AEEMP #201)

      a. E.g., Named "CELOC Calculation_201 removed"

      b. All "#VALUE!" should change to numbers and the graphs should be created once #201 (row 218) is removed

      c. The median CE-LOC is used because the data is not normally distributed

---

[11] The number of sets to evaluate uncertainty as defined in Step 2d of the instructions for running PATI.

[12] This sheet is protected to limit accidental changes. Editing is restricted to the cells that need data inputted as identified above. Protection can be removed by going to "Review" in the Microsoft Excel menu bar and then clicking "Unprotect Sheet". There is no password.

Appendix IV.   Accompanying Text for the Zipped Folder Named "PATI and CE-LOC Files"

The files needed to reproduce the CE-LOC and the files EPA used to calculated the CE-LOC in **Section 2** are attached to this document in a zipped folder (.zipx) named "Appendix IV. PATI and CELOC Files". This can be saved and opened as a .zip file. This zipped folder includes two subfolders: "PATI and CELOC Files_StartingFiles" and "PATI and CELOC Files_2024UpdatedCELOCRun". The folder "PATI and CELOC Files_StartingFiles" contains a copy of the program PATI, all input files needed, and a template to calculate the CE-LOC (see **Table IV-1,** "Starting Files" section). The folder "PATI and CELOC Files_2024UpdatedCELOCRun" contains the files EPA used to calculate the CE-LOC in **Section 2**, including the Starting Files (same as the "StartingFiles" folder), the Output Files (see **Table IV-1,** "Output Files" section), and the additional files created following the protocol described in **Appendix III**.

Table IV-1. Files included in compressed file titled "Appendix IV. PATI and CELOC Files"

Starting Files	

Name
 	
2014UncertaintyAnalysisChemPATIandEEF.exe
2014UncertaintyAnalysisCosmPATIandLOC.exe
AEEMP04to14.txt
CELOC Calculation_Template.xlsx
cosmeffects_2016original.dat
cosmeffects_2024update.dat
Exposure#1.txt
Exposure#2.txt
 	
Plus 391 additional cosm and AEEMP chemographs
	PATI Program files
	• "2014UncertaintyAnalysisChemPATIandEEF.exe"
	• "2014UncertaintyAnalysisCosmPATIandLOC.exe"
	
	Databases
	• "AEEMP04to14.txt"
	   o This is the AEEMP input file
	• "cosmeffects_2016original.dat"
	   o This is the 2016 cosm effects input file used to create 3.4 µg/L in 2016
	• "cosmeffects_2024update.dat"
	   o This is the current cosm effects input file used to create 9.7 µg/L (see **Appendix II** for the complete database)
	
	Chemograph files (393 total, only 2 shown to left)
	• Cosm chemographs input files (e.g., Exposure#1.txt)
	• AEEMP chemographs input files (e.g., IA 03 12.txt)
	
	Data processing
	• "CELOC Calculation_Template"

Output Files*	
Name 2014UAChemographCumulativePATI.out 2014UAChemographEEF.out 2014UAChemographMaxRunAvgConc.out 2014UACosmCumulativePATI.out 2014UACumulativePATI-LOC.out 2014UAPATIFunctionParams.out	From "2014UncertaintyAnalysisCosmPATIandLOC.exe" • "2014UACosmCumulativePATI.out" • "2014UACumulativePATI-LOC.out" • "2014UAPATIFunctionParams.out" From "2014UncertaintyAnalysisChemPATIandEEF.exe" • "2014UAChemographCumulativePATI.out" • "2014UAChemographEEF.out" • "2014UAChemographMaxRunAvgConc.out"

*These files appear after the program files are run. The folder "PATI and CELOC Files_2024UpdatedCELOCRun" contains these output files plus additional files described in **Appendix III** that were created to calculate the CE-LOC presented in **Section 2**.

Appendix V.   Detailed Updated to WARP-MP Modeling

Commenters on the 2022 proposed revisions to the atrazine ID noted that the parameter for precipitation in May and June was calculated incorrectly. EPA has now updated the calculation for precipitation in May and June and regenerated atrazine concentration estimates with WARP-MP.

WARP-MP inputs were prepared for the 2016 draft risk assessment using the Python script named generate_warp_inputs.py and the accompanying library named warp_lib.py. Lines 71-79 of the generate_warp_inputs.py script require the user to specify several input datasets (**Table V-1**). Several of these inputs required regeneration due to file loss or corruption since 2016, and others were updated for consistency in GIS processing.

Table V-1. Input variables and description of generate_warp_inputs.py script

Line	Input	Description	Regenerated for this effort?	Directory/file name
71	nhd_dir	Directory with shapefiles for each 2-digit watershed with 12-digit watersheds as objects.	No	NHDPlusV2
72	grid_dir	Directory with rasters for each 2-digit HUC; raster values are the 12-digit watershed OBJECTID from nhd_dir shapefiles.	Yes	objectid_rasters
73	state_dir	Directory with rasters for each US state; raster values are the 5 digit FIPS code (concatenated state and county code).	Yes	state_county_rasters
74	use_grid	Raster with presence/absence of atrazine use areas, as defined by the 2016 DRA.	Yes	atr_cdl
75	climdiv_grid	Raster with climate divisions as values. Used to map precipitation data to geographic area.	Yes	climdiv_30m
76	rfactor_grid	Raster with soil R factor data as values.	Yes	rfactor_30m
77	satof48_grid	Raster with percent of flow due to Dunne's overland flow	Yes	satof48_30m
78	srl25ag_grid	Raster with percent of watershed with a soil restrictive layer as values.	Yes	srl25ag_30m
79	climate_table	Text file with precipitation for each month of the year (columns 1-12) for each climate division (column 0)	No	climdiv-pcpndv-v1.0.0-20150306.txt

a. Preparing inputs for generate_warp_inputs.py

For this effort, all inputs were prepared or updated using ArcGIS Pro 3.2.1. All input rasters were projected with NAD_1983_Albers coordinate system and NAD 1983 geographic coordinate system (WKID 4269). To ensure consistency across raster datasets, all rasters were snapped to match the file 'rfactor_30m' and the cell size was also set to match 'rfactor_30m' (30m x 30m cells). In addition to

ensuring consistency in the projection/coordinate system, raster alignment, and cell size, the inputs grid_dir, state_dir, and srl25ag_grid were regenerated using ArcGIS Pro. Following are descriptions on how these inputs were developed.

i. srl25ag_30m

1. The ssurgo_srlag_grd.zip file from https://www.sciencebase.gov/catalog/item/6314057ad34e36012efa2cb0 was downloaded, and the srl25ag raster was imported into ArcGIS Pro. These files contain estimates of the soil restrictive layer in the upper 25 cm of agricultural land in the conterminous United States.
2. The srl25ag raster was projected with NAD_1983_Albers coordinate system and NAD 1983 geographic coordinate system (WKID 4269), snapped to match 'rfactor_30m', and the cell size was set to match 'rfactor_30m' (30m x 30m cells).

ii. grid_dir

1. WBD shapefiles from the nhd_dir were compiled into a single directory (WBD_shapefiles) and renamed according to their 2-digit HUC.
   Ex. NHDPlus01/WBDSnapshot/WBD/WBD_Subwatershed.shp renamed 01.shp
2. Model1 in the 2024 WARP Update.gbd was run
   a. The 'Iterate Feature Classes' iterator was used to iterate through each shapefile in the WBD_shapefiles directory.
   b. For each shapefile, the 'Polygon to Raster' tool was used to rasterize the shapefile with 'OBJECTID' as the raster values, setting the projection, raster snap, and cell size to match the existing raster 'rfactor_30m'.
   c. For each rasterized shapefile, the 'Copy Raster' tool was used to export the raster to an ESRI grid format, named according to the 2-digit HUC region. Pyramids were not built, but statistics were calculated.

iii. state_dir

1. U.S. country boundaries were imported into ArcGIS Pro from the Living Atlas (USA Counties – Generalized).
2. USA Counties – Generalized was exported to a new polygon dataset within ArcGIS using the 'Export Features' tool (USACountiesGeneralized_ExportFeatures).
3. In the attribute table for USACountiesGeneralized_ExportFeatures, a new numerical field 'FIPS_num' was created and populated with the string values in the field 'FIPS' (treated numerically).
4. USACountiesGeneralized_ExportFeatures was rasterized using the 'Polygon to Raster' tool, shapefile with 'FIPS_num' as the raster values; setting the projection, raster snap, and cell size to match the existing raster 'rfactor_30m'. (USACountiesGeneralized_ExportFeatures_PolygonToRaster).
5. Model in the 2024 WARP Update.gbd was run
   a. The 'Iterate Feature Selection' iterator was used to iterate through each feature in USACountiesGeneralized_ExportFeatures_PolygonToRaster, grouping by 'STATE_ABBR'.
   b. For each state, the 'Clip Raster' tool was used to clip the USACountiesGeneralized_ExportFeatures_PolygonToRaster raster.

c. For each clipped raster, the 'Copy Raster' tool was used to export the raster to an ESRI grid format, named according to the state abbreviation. Pyramids were not built, but statistics were calculated.

b. Modifications to generate_warp_inputs.py and warp_lib.py

To prepare input datasets for modeling in WARP-MP, several minor modifications were made to the generate_warp_inputs.py script and the library it calls upon (warp_lib.py), including modification to the precipitation calculation. The precipitation calculation was modified to calculate the total precipitation in May and June, rather than the average precipitation in May and June. These modifications are outlined below. Beneath each line number in a code, two boxes show the previous version of the code as well as the updated code.

i. generate_warp_inputs.py

Line 9

```
all_rasters = {r: os.path.join(grid_dir, "nhdplus{}".format(r)) for r in
overlaps.keys()}
```

```
all_rasters = {r: os.path.join(grid_dir, "{}".format(r)) for r in
overlaps.keys()}
```

Purpose: The naming convention was modified to be consistent with the files exported from ArcGIS Pro.

Line 15

```
satof48_raster = Raster(satof48_grid, no_data=255)
```

```
satof48_raster = Raster(satof48_grid)
```

Purpose: The naming convention was modified to be consistent with the files exported from ArcGIS Pro.

ii. warp_lib.py

Line 46

```
def __init__(self, path, precision=None, no_data=None):
```

```
def __init__(self, path, precision=None):
```

Purpose: The 'no_data' argument was removed from the raster class and will now be specified from the gdal function 'GetNoDataValue'.

Line 50

```
self.cell_size = gt[1]
```

```
self.cell_size = np.int64(gt[1])
```

Purpose: The cell size must be treated as an integer, but was read in as a float value for some inputs.

Line 59

```
self.no_data = no_data
```

```
self.no_data = self.obj.GetRasterBand(1).GetNoDataValue()
```

Purpose: 'no_data' was previously an argument for the Raster class, but this caused an issue if the 'no_data' value was unknown, or varied from the ArcGIS apparent 'no data' value. This modification specifies the 'no_data' value from the raster metadata to ensure the proper value is used for all inputs.

Line 280

```
state_rasters = [Raster(os.path.join(state_dir,
"{}_cty_30m".format(state)), 6) for state in overlaps[region]]
```

```
state_rasters = [Raster(os.path.join(state_dir, "{}_cty".format(state)), 6)
for state in overlaps[region]]
```

Purpose: The naming convention was modified to be consistent with the files exported from ArcGIS Pro.

Line 324

```
climate_dict[year][climdiv] = (float(line[5]) + float(line[6])) / 2.0
```

```
climate_dict[year][climdiv] = (float(line[5]) + float(line[6]))
```

Purpose: Previously the precipitation was incorrectly calculated as the average precipitation in May and June, rather than the total precipitation in May and June. This update fixes the incorrect calculation.

c. Protocol for running WARP-MP

i. Run generate_warp_inputs.py

The generate_warp_inputs.py script was run in a conda environment with a Python 2.7.18 interpreter. The packages and versions installed in the environment are listed in **Error! Reference source not found.V-2**. A .yml file is attached, which can be used to clone the environment (environment.yml).

The user must specify paths to the input and output directories in lines 63-83. Note that new directories must be created by the user prior to running the script. For this work, all years were run (line 65), the compound was 'ATRAZINE' (line 66), and the level was 'low' (line 67).

Table V-2: Packages and versioning in conda environment used to run generate_warp_inputs.py

Name	Version	Build	Channel
boost-cpp	1.70.0	h099cdad_2	conda-forge
ca-certificates	2023.11.17	h56e8100_0	conda-forge
Certifi	2019.11.28	py27h8c360ce_1	conda-forge
console_shortcut	0.1.1	4	
Curl	7.65.3	h2f83ebf_0	conda-forge

Name	Version	Build	Channel
Expat	2.5.0	h63175ca_1	conda-forge
Freexl	1.0.5	haa51a25_1002	conda-forge
Gdal	2.2.2	py27h58389d3_1	
Geos	3.6.2	heec837b_2	
hdf4	4.2.13	ha32470c_1003	conda-forge
hdf5	1.10.1	vc9_2	conda-forge
intel-openmp	2024.0.0	h57928b3_49841	conda-forge
Jpeg	9c	h0c8e037_1001	conda-forge
Kealib	1.4.7	vc9_4	conda-forge
krb5	1.16.4	h7fea353_0	conda-forge
Libblas	3.9.0	8_mkl	conda-forge
Libcblas	3.9.0	8_mkl	conda-forge
Libcurl	7.65.3	h2f83ebf_0	conda-forge
Libexpat	2.5.0	h63175ca_1	conda-forge
Libgdal	2.2.2	h75964b3_1	
Libiconv	1.15	h0c8e037_1006	conda-forge
Libkml	1.3.0	h59e3451_1010	conda-forge
Liblapack	3.9.0	8_mkl	conda-forge
Libnetcdf	4.4.1.1	vc9_10	conda-forge
Libpng	1.6.37	h7a46e7a_0	conda-forge
Libpq	11.5	h5e4800f_1	conda-forge
Libspatialite	4.3.0a	he01c9b4_1023	conda-forge
libssh2	1.8.2	hd5c6ab6_2	conda-forge
Libtiff	4.0.9	he490001_1002	conda-forge
libxml2	2.9.10	h00b1425_0	conda-forge
Mkl	2020.4	hb70f87d_311	conda-forge
Numpy	1.16.5	py27h0d21db5_0	conda-forge
Openjpeg	2.3.1	h9afe63c_0	conda-forge
Openssl	1.1.1e	h0c8e037_0	conda-forge
Pip	19.3.1	py27_0	
proj4	4.9.3	h0c8e037_9	conda-forge
Python	2.7.18	hfb89ab9_0	
python_abi	2.7	1_cp27m	conda-forge
Setuptools	44.0.0	py27_0	
Sqlite	3.30.1	h0c8e037_0	
Tk	8.6.10	h0c8e037_0	conda-forge
Vc	9	h2eaa2aa_6	
vs2008_runtime	9.00.30729.1	haa95532_6	
Wheel	0.37.1	pyhd3eb1b0_0	
wincertstore	0.2	py27hf04cefb_0	

Name	Version	Build	Channel
xerces-c	3.2.2	hc56fc5f_1004	conda-forge
zlib	1.2.11	h3cc03e0_1006	conda-forge

ii. Run batch_atrazine_2.r

Following successful completion of the generate_warp_inputs.py script, the user will use the outputs generated as the inputs to the R script batch_atrazine_2.r. The package 'survival' is the only dependency for batch_atrazine_2.r, and version 3.5-5 was used for this work. In addition to the inputs generated in Run generate_warp_inputs.py, the user will also need to provide 'warp.mp.cal.data.csv'; a .csv file with WARP-MP calibration data (attached).

The user must specify paths to the input and output directories in lines 57 and 58, and the path to the calibration data (warp.mp.cal.data.csv) in line 59. For this work, the cutoffs (line 48) did not need to be updated because they were not used in the final analysis. The years modeled were 2006, 2007, 2008, and 2009 (line 49); consistent with the 2016 DRA.

iii. Process_r_output.py

Following the successful completion of the batch_atrazine_2.r script, the user will use the outputs generated as the inputs to the process_r_output.py python script. This script compiles results from batch_atrazine_2.r into two summary files. One output file contains WARP output broken down by watershed for each year while the other contains average output for each watershed across all available years. The script was run in a conda environment with a Python 3.11.8 interpreter. The packages and versions installed in the environment are listed in **Table V-3**.

The user must specify paths to the input and output directories in lines 92-94.

Table V-3. Packages and versioning in conda environment used to run process_r_output.py

Name	Version	Build	Channel
bzip2	1.0.8	h2bbff1b_5	
ca-certificates	2023.12.12	haa95532_0	
console_shortcut	0.1.1	4	
docutils	0.20.1	pypi_0	pypi
libffi	3.4.4	hd77b12b_0	
nested-dict	1.61	pypi_0	pypi
openssl	3.0.13	h2bbff1b_0	
pip	23.3.1	py311haa95532_0	
python	3.11.8	he1021f5_0	
setuptools	68.2.2	py311haa95532_0	
sqlite	3.41.2	h2bbff1b_0	
statistics	1.0.3.5	pypi_0	pypi
tk	8.6.12	h2bbff1b_0	
tzdata	2024a	h04d1e81_0	
vc	14.2	h21ff451_1	

Name	Version	Build	Channel
vs2015_runtime	14.27.29016	h5e58377_2	
wheel	0.41.2	py311haa95532_0	
xz	5.4.6	h8cc25b3_0	
zlib	1.2.13	h8cc25b3_0	