

Appendix B: Supporting Information for the Aquatic Community-Level Threshold Concentrations

The Agency has selected an atrazine level of concern (LOC) for aquatic community effects in the 2003 IRED (EPA, 2003a and b) that is consistent with the approach described in the Office of Water's (OW) draft atrazine aquatic life criteria (EPA, 2003c). Based on these previously published analyses (EPA, 2003a, b, and c), aqueous atrazine concentrations, obtained from monitoring studies or model predictions, can be interpreted to determine if a water body within the action area is likely to be significantly altered via effects on primary productivity and resultant impacts on the aquatic community. The LOC provides the means to assess the potential for a measured or predicted (modeled) atrazine exposure regime to cause indirect effects to the Alabama sturgeon due to via changes in the aquatic community.

As described in subsequent sections of this appendix, responses in microcosms and mesocosms exposed to atrazine were evaluated to differentiate no or slight, recoverable effects from significant, generally non-recoverable effects (EPA, 2003d). Because effects varied with exposure duration and magnitude, there was a need for methods to predict relative differences in effects for different types of exposures. The Comprehensive Aquatic Systems Model (CASM) (Bartell et al., 2000; Bartell et al., 1999; DeAngelis et al., 1989) was selected as an appropriate tool to predict these relative effects, and was configured to provide a simulation for the entire growing season of a 2nd and 3rd order Midwestern stream as a function of atrazine exposure. CASM simulations conducted for the concentration/duration exposure profiles of the micro- and mesocosm data showed that CASM seasonal output, represented as an aquatic plant community similarity index, correlated with the micro- and mesocosm effect scores, and that a 5% change in this index reasonably discriminated micro- and mesocosm responses with slight versus significant effects. The CASM-based index was assumed to be applicable to more diverse exposure conditions beyond those present in the micro- and mesocosm studies.

To avoid having to routinely run the CASM model, simulations were conducted for a variety of actual and synthetic atrazine chemographs to determine 14-, 30-, 60-, and 90-day average concentrations that discriminated among exposures that were unlikely to exceed the CASM-based index value (i.e., 5% change in the index). The chosen method defines time integrated exposure doses that correlate with the CASM simulation results for a series of original atrazine monitoring chemographs, as described below in Section B.7. The threshold concentrations for aquatic community effects developed in EPA (2003d), and used in this endangered species assessment are as follows:

- 14-day average = 38 ppb
- 30-day average = 27 ppb
- 60-day average = 18 ppb
- 90-day average = 12 ppb

A step-wise evaluation scheme for interpreting measured or monitored atrazine concentrations over these averaging periods is provided in Figure B.1

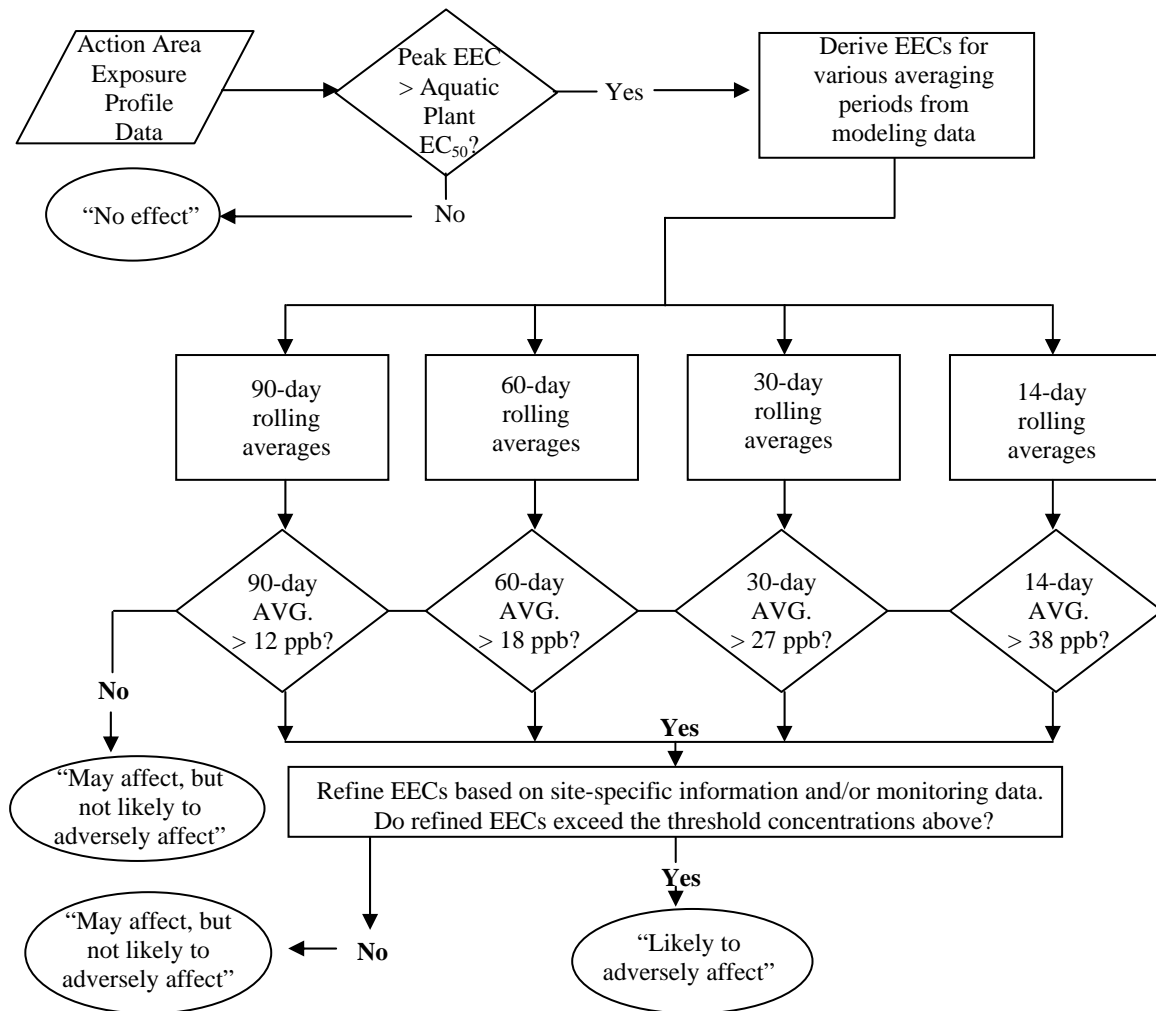


Figure B.1. Use of Threshold Concentrations in Endangered Species Assessment

B.1 Endpoints

Based on the reported results from 77 micro- and mesocosm studies for which atrazine was tested, change in aquatic community structure and function of aquatic plants (i.e., primary producers) was chosen as the endpoint of concern. The effect of atrazine on aquatic plants, whether direct or indirect, appeared to be more sensitive than effects on other organisms in aquatic ecosystems, including aquatic invertebrates and fish. Therefore, focus on the protection of aquatic plant community structural changes is intended to also be protective against adverse effects for the rest of the aquatic community.

B.2 Community Level Studies

Ecological responses of aquatic communities to atrazine exposures were assessed using community level responses observed in micro- and mesocosm studies. Twenty-five different studies with 77 reported effects/no effects were reviewed (see Attachment I). Twenty-four results were from tests on ponds or lakes; 20 on artificial streams; and, 33 were microcosm tests. Eight results were on macrophytes, 29 on periphyton, and 40 on phytoplankton. A limited number of exposure profiles were tested in these studies. Typically, one to three concentrations of atrazine were tested, each with a single application to the test system at initiation. Atrazine concentrations were often kept constant for a variable duration period before the concentrations began to slowly decrease with time. Unfortunately, the variable quality of these studies and the many different study designs did not always allow a reliable association of exposure magnitude and duration to a certain community level effect. In addition, the studies were not of sufficient duration to document community recovery in many cases.

To better understand the impact of exposure duration and magnitude on aquatic communities, the effects reported in the micro- and mesocosm studies were related to specific exposure durations and magnitudes. The 77 study results also had to be quantified as to severity of effects of atrazine on the aquatic plant community. Brock et al. (2000) analyzed a majority of the study results and quantified them as follows:

Effect Scores (Brock et al., 2000)

1 = no effect

2 = slight effect

3 = significant effect followed by return to control levels within 56 days

4 = significant effect without return to control levels during an observation period of less than 56 days

5 = significant effect without return to control levels for more than 56 days

Studies not analyzed by Brock, but considered in this analysis, were scored with the same methods. The distribution of the scores for the 77 study results were as follows (also see Attachment 1):

Distribution of Effect Scores:

15 were ranked as 1
12 were ranked as 2
12 were ranked as 3
23 were ranked as 4
15 were ranked as 5

Next, the 77 effect scores representing the results from the 25 micro- and mesocosm studies for atrazine were plotted against the study specific test concentrations and exposure durations, as shown in Figure B.2. As expected, based on atrazine's mode of action to inhibit primary production by reversibly blocking photosynthesis, the effects observed in the micro- and mesocosm studies generally became more severe with increasing exposure and time.

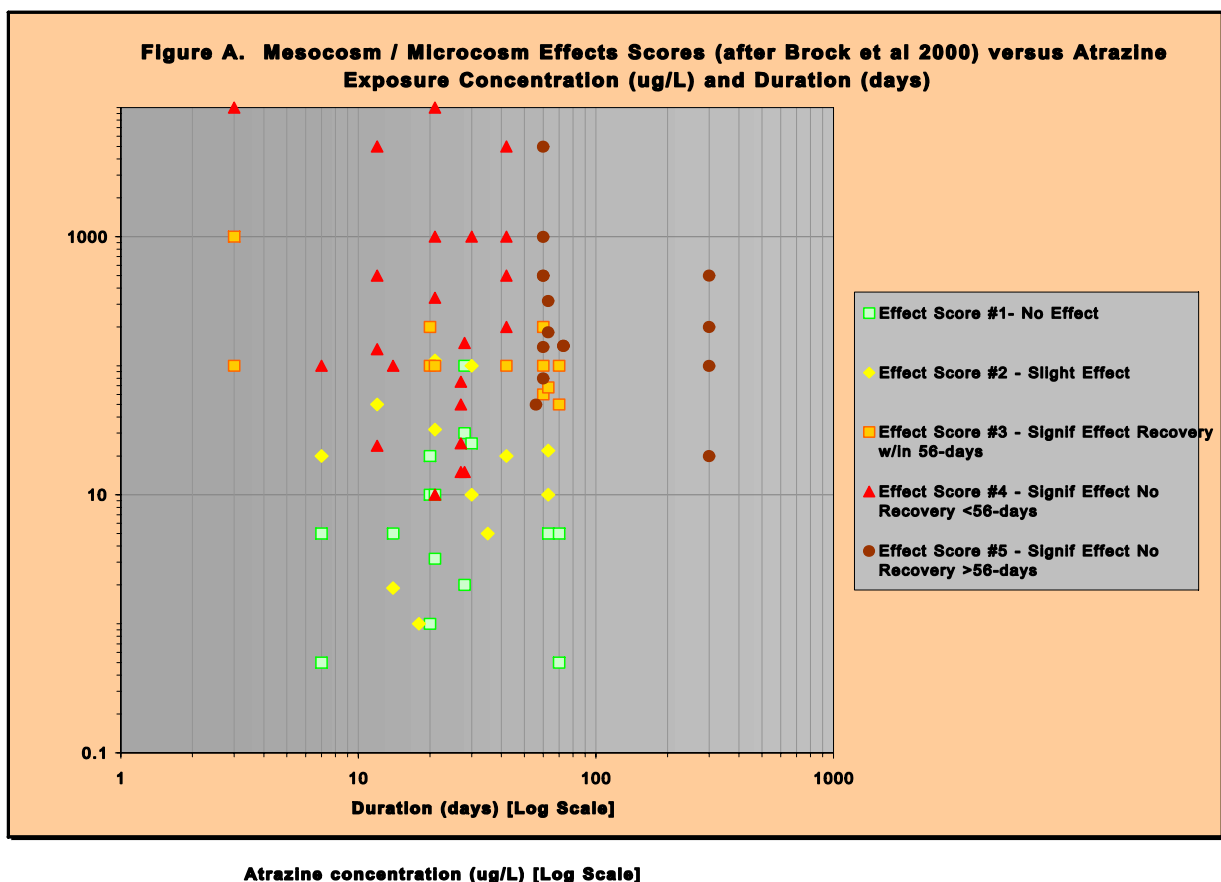


Figure B.2. Micro- and mesocosm study effect concentrations scored according to Brock et al. (2000) and plotted against the study specific exposure duration

To identify the appropriate exposure concentration and duration relationship that defines specific LOCs, ecological modeling was used to simulate a large number of exposure durations and magnitudes based on monitoring data obtained from 2nd to 3rd order Midwestern streams. Two ecological models were initially considered: (1) CASM (Bartell et al., 2000; Bartell et al., 1999; DeAngelis et al., 1989), and (2) AQUATOX (<http://www.epa.gov/waterscience/models/aquatox/about.html> and

<http://www.myweb.cableone.net/dickpark/AQTXFacts.htm>). The decision to use CASM was made after a preliminary comparison revealed that CASM could accommodate a larger number of species in the community structure, which appeared to better support the chosen endpoint.

B.3 Model Parameterization

Single-species laboratory toxicity test results on atrazine toxicity to aquatic organisms (see Giddings et al., 2000), including aquatic plants (macrophytes, periphyton, and phytoplankton), were used to parameterize the model. A subset of the Giddings et al. (2000) data (EC₅₀ geometric means) were selected and used to drive the toxicity of atrazine to aquatic organisms in the CASM simulation model (see Attachment 2). The modeled toxicity profile included 26 producer species (10 plankton, 10 periphyton, and 6 macrophytes) and 17 consumer species. Three toxicity scenarios were modeled: 10th centile, geometric mean, and 90th centile for species with more than one toxicity study. The geometric mean scenario (toxicity scenario 1) was chosen for the reported model results. Figure B.3 shows the plant species sensitivity distribution (SSD) for EC₁₀, EC₅₀, and EC₉₀ values overlaid with the plant SSD (EC₅₀ geometric mean) used to parameterize CASM.

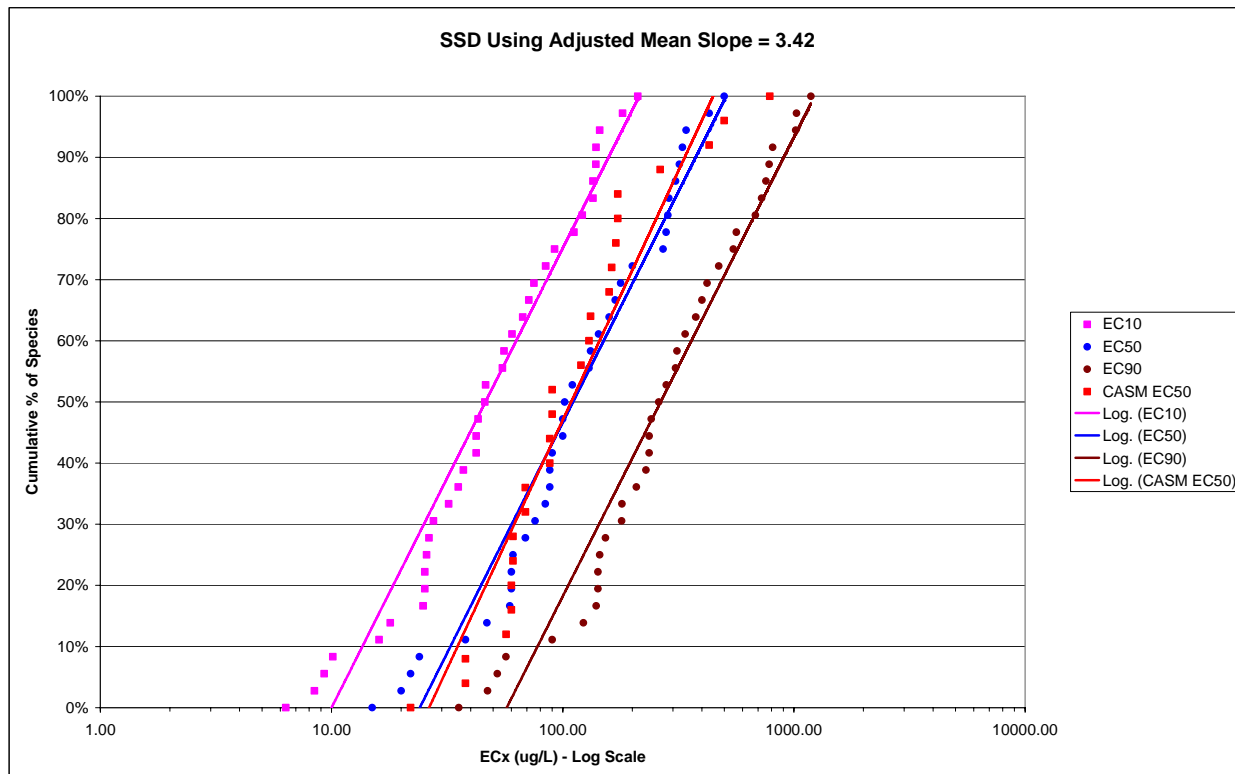


Figure B.3. Plant Species Sensitivity Distribution for EC₁₀, EC₅₀, and EC₉₀ values overlaid with the Plant Species Sensitivity Distribution (EC₅₀ geometric mean) used to parameterize CASM

B.4 CASM Model Simulations

CASM is an ecological food chain model. It was set up to run simulations for exposure durations from 1 to 260 days, and concentrations from 20 to 220 ppb atrazine. The scenarios were designed to simulate a generic 2nd or 3rd order Midwestern stream, typical for the majority of atrazine use on corn and sorghum. The CASM model provides the following results: production – modeled as biomass production (g Carbon m⁻²) for 1 m² surface area (Attachment 3a), and community structure (similarity) – modeled as species population size derived from species daily biomass (Attachment 3b). Therefore, the model integrates direct and indirect effects to indicate changes in community structure. The endpoint selected for the model results is percent (%) change in aquatic community structure (as determined by the Steinhaus Similarity coefficient) of primary producers (phytoplankton, periphyton, and macrophytes).

B.5 CASM Steinhaus Similarity Analysis

Coefficients of similarity are used to determine whether the composition of two communities is similar. The Steinhaus coefficient or similarity index is based on the species abundance (in this case indicated by the species specific daily biomass) common to two communities. The index is described in the following equation:

$$S = \frac{2 * \sum_{k=1}^n \text{Min}(a_{1,k}, a_{2,k})}{\sum_{k=1}^n a_{1,k} + \sum_{k=1}^n a_{2,k}}$$

Where $a_{1,k}$ = abundances of species k in sample 1.

The similarity indices for each possible pair of samples per day were calculated, resulting in a matrix of between (different treatments) similarities, shown in Figure B.4.

	1d1	1d2	1d3	etc.	Xd260
1d1		B	B	B	B
1d2			B	B	B
1d3				B	B
etc.				B	B
Xd260					B

Figure B.4. Example of a matrix similarities resulting from Similarity Index calculations

Similarity indices were calculated for primary producers, consumers, and fish over exposure periods from 1 to 20 days (see Attachment 3b). The results show that the changes in percent change in aquatic community structure of primary producers is a more sensitive (conservative) measurement than the same for consumers or fish.

B.6 Determining the LOC – CASM Steinhaus Similarity vs. the Effects of Atrazine Exposure in Micro- and Mesocosm Studies

A wide range of single pulses of different duration and magnitude were simulated and used to calculate community structure changes. Community structure changes were expressed as percent (%) change in the Steinhaus similarity index that was calculated based on the simulated daily biomass for each individual species and plotted over time.

The maximum daily percent (Table 1A), year-end percent (i.e., at day 260 post application; Table 1B), and average percent change in community structure in the primary producer community (Table 1C) were calculated. Maximum daily deviations represent the short-term (temporary) maximum changes in community structures. The average community structure change integrates short-term changes and long-term recovery of the communities. A comparison of short- and long-term %-impact shows that for concentrations >20 ppb, short-term changes are always between 1- to 2-fold the average response. For example, an average 5% community structure change may cause a less than or equal to 10% short-term (temporary) change in the primary producer community structure. The average percent change in community structure was chosen for the reported results because it captures the short-term changes as well as recovery.

The modeling results reported in Table 1C were used to help define duration-specific levels of concern. Two approaches were used. First, the simulated response (or effect) was set in context to the micro- and mesocosm data. A similarity index value was estimated for each micro- and mesocosm test result by finding the average model similarity deviations (%) of a simulated exposure profile closest to the conditions in each study (test concentration and exposure duration; see Attachment 1 for assigned index values for each of the 77 test results). Next, the index values were plotted against the Brock scores for each micro- and mesocosm test results for comparison (Figure B.5).

As shown in Figure B.5, there is much scatter reflective of the diversity of this data; however, there is a clear, strong correlation between the Brock scores and the index. An index value of 5 (vertical red line on Figure B.5) conservatively separates the 3/4/5 from the 1/2 scores. Therefore, a 5% change in community structure (Steinhaus similarity) of the CASM simulations compares to a large majority of the micro- and mesocosm studies with no to slight effects (leaving only 8% potential false negatives and false positives, i.e., false negatives – 6 out of 77 studies above the effects score 3 line and to the left of the 5% line; false positives – 6 out of 77 studies below the effects score 3 line and to the right of the 5% line).

Table 1: A) Maximum daily percent change^a in community structure (Steinhaus similarity) of primary producers for a modeled generic 2nd-3rd order Midwestern stream.

Atrazine conc. [µg/L]	Pulse duration [d] ^b							
	1	3	5	10	20	60	130	260
20	0.1 ^c	0.2	0.7	0.9	1	1.2	1.2	2.3
25	0.8	1.9	2.9	5	7.8	11.7	13	15.5
30	0.8	1.9	2.9	5	7.8	11.7	13	15.8
40	1.1	2.3	3.2	5.2	8	11.7	13.1	16.6
50	1.1	2.3	3.1	5.2	7.9	11.6	13.1	17.5
70	3.7	8	10.7	13.8	16.1	17.3	18.1	22.5
90	4.4	9.4	12.6	15.9	18.2	18.2	18.3	23.5
130	4.5	9.6	12.7	15.8	17.8	17.8	17.8	20.1
170	5.6	13.1	18.1	24.1	29.7	56.3	67.1	72.4
220	5.7	13.2	18.2	24	29.7	56.3	67.1	72.3

B) Year end percent change^a in community structure (Steinhaus similarity) of primary producers for a modeled generic 2nd-3rd order Midwestern stream.

Atrazine conc. [µg/L]	Pulse duration [d] ^b							
	1	3	5	10	20	60	130	260
20	0 ^c	0	0	0.2	0.2	0.2	0.2	2.3
25	0.7	1.7	2.7	4.7	7.3	10.9	12.1	15.5
30	0.7	1.7	2.7	4.6	7.2	10.8	12.1	15.8
40	0.7	1.9	3	4.9	7.5	11	12.4	16.6
50	0.7	1.9	2.9	4.9	7.5	10.9	12.9	17.5
70	1.5	3.7	5.2	7.9	10.9	14.6	17.6	22.5
90	1.7	4.1	5.7	8.5	11.6	15.5	18.3	23.5
130	1.7	4	5.7	8.4	11.5	15.3	16.4	20.1
170	2	5.4	8.1	15.5	27.9	51.7	61.2	71.5
220	2	5.3	8.1	15.4	27.8	51.6	61.1	71.1

C) Average percent change^a in community structure (Steinhaus similarity) of primary producers for a modeled generic 2nd-3rd order Midwestern stream.

Atrazine conc. [µg/L]	Pulse duration [d] ^b							
	1	3	5	10	20	60	130	260
20	0 ^c	0	0.1	0.4	0.4	0.5	0.5	0.7
25	0.5	1.2	1.9	3.4	5.1	7.4	8.2	8.5
30	0.4	1.2	2	3.5	5.2	7.6	8.4	8.7
40	0.8	1.8	2.6	4.1	5.8	8.3	9.3	9.7
50	0.8	1.8	2.6	4.2	6	8.9	10.1	10.7
70	2.2	4.8	6.4	9.1	11.6	14.9	16.9	17.5
90	2.6	5.6	7.4	10.2	12.8	15.8	17.5	18
130	2.6	5.6	7.4	10.2	12.7	15.4	16.3	16.4
170	2.9	6.8	9.8	16.3	25.5	40.6	46.3	48.4
220	2.9	6.8	9.8	16.4	25.5	40.6	46.3	48.4

^aBased on the mean values of 100 Monte Carlo simulations using the Comprehensive Aquatic Systems Model (CASIM)

^bConsecutive days of constant exposure beginning on model day 105 (April 15)

^cResults using the geometric mean values of EC₅₀ assigned to modeled populations (Toxicity Scenario 1)

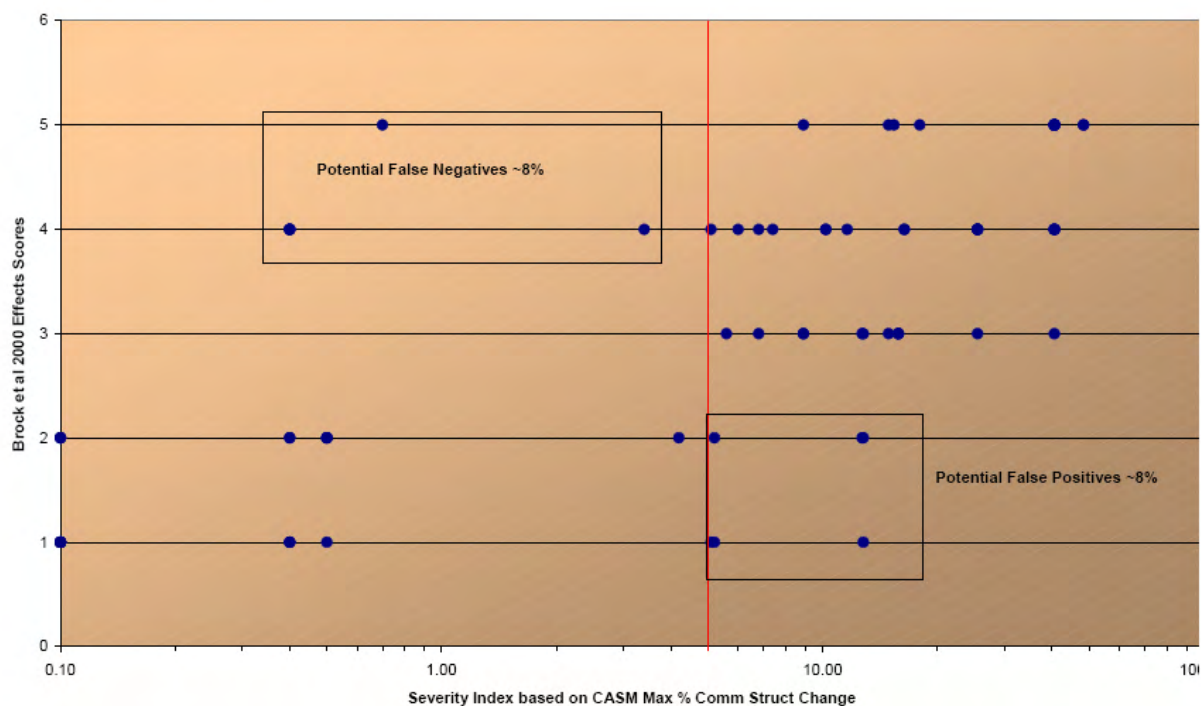


Figure B.5. Correlation Between the Similarity Index (CASM average % change in community structure for 77 atrazine micro- and mesocosm studies) and the Brock Effect Scores

For the second approach, the CASM simulation results in Table 1C were interpolated to develop a set of concentration/duration pairs equivalent to 5% effect from CASM. The interpolated results are as follows:

<u>Time (days)</u>	<u>Concentration (ppb)</u>
1.1	220
1.6	130
3	75
5	63
10	53
20	24.8
60	23.3
130	22.9
260	22.7

For times greater than 3 days, a linear interpolation was performed across the different concentrations at each time. For times from 60 to 260 days, the abrupt shift in response between 20 and 25 ppb made interpolation tenuous; however, the best estimate appears to be in the mid-part of the range. For times less than 3 days, the response did not reach 5%, but the additional points seem to be points needed at high concentrations. Thus, interpolations were performed across times at a fixed concentration rather than across concentrations at a fixed time.

Next, these concentration duration pairs, representing the 5% index points based on interpolation, were plotted with lines connecting each point on Figure B.6.

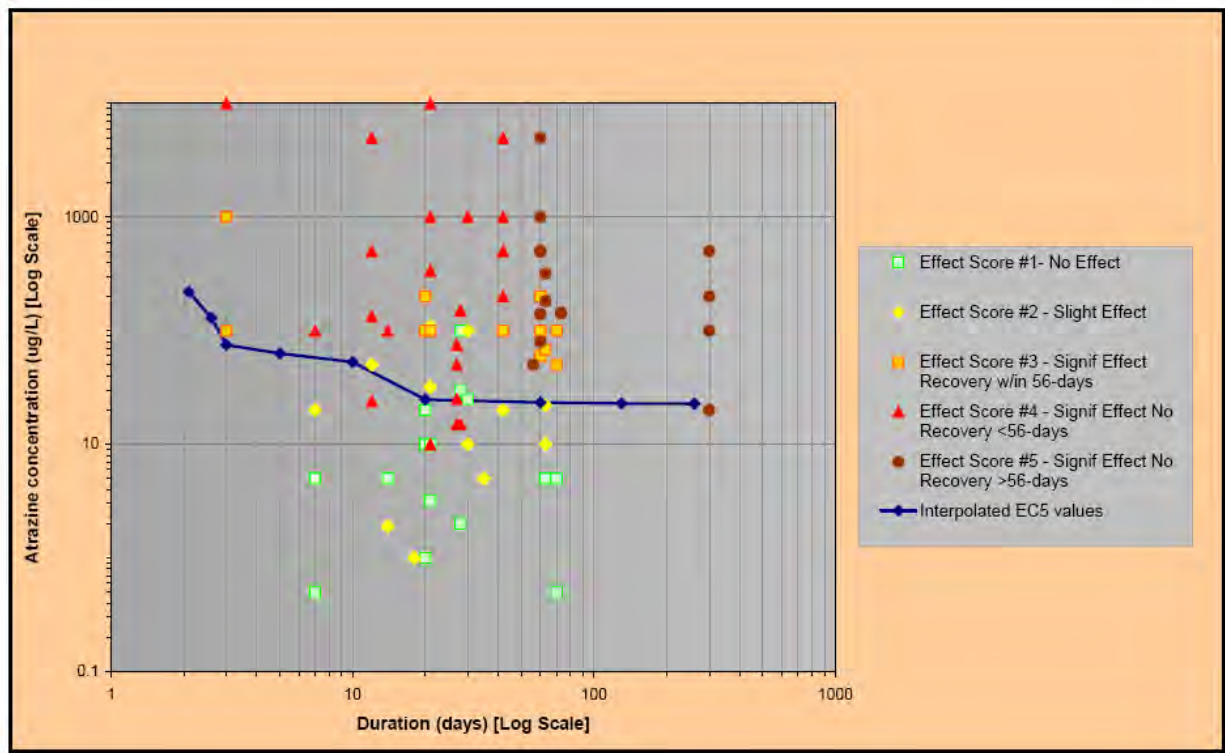


Figure B.6. Micro- and Mesocosm Study Effect Concentrations Scored According to Brock et al. (2000) and Plotted Against the Study Specific Exposure Duration (Interpolated 5% CASM similarity index points plotted)

The plot of interpolated 5% Similarity index points, like Figure B.5, conservatively separates the 3/4/5 from the 1/2 scores. Based on both approaches, an index of 5%, meaning 5% change in the community structure of primary producers, was chosen as a reasonable LOC for atrazine exposures in freshwater environments.

B.7 Use of Threshold Concentrations as LOCs to Determine Indirect Effects to Endangered Species

The 14-, 30-, 60-, and 90-day average concentrations were originally intended to be used as conservative values to trigger the need to run CASM to determine if the LOC (i.e., 5% index delineating no to slight effect in available micro- and mesocosm studies from those with significant effects) was actually exceeded for a specific atrazine chemograph. Therefore, the 14-, 30-, 60-, and 90-day threshold concentrations were developed as an easy method to avoid repeated CASM simulations for exposure profiles that are of no concern. As such, they were set to be conservative, producing a low level (1%) of false negatives (i.e., a CASM simulation would show the LOC to be exceeded even though the threshold concentrations were not exceeded) relative to false positives. The method used to derive the rolling averages provides time integrated exposure doses that correlate with the CASM simulation results for a series of original atrazine monitoring chemographs.

The original monitoring chemographs were obtained from monitoring datasets for a range of Ohio rivers and streams in agricultural areas (i.e., Heidelberg College data set; see EPA, 2003d). Time-integrated exposure concentrations from these chemographs were calculated for averaging periods of 14-, 30-, 60-, and 90-days. Selection of four various durations ranging from two weeks to approximately three months is intended to be representative of typical durations of chronic exposure. The time-weighted concentrations were correlated to the CASM results of the corresponding chemographs by seeking the concentrations that would be unlikely to trigger CASM simulations with plant community structure changes > 5%. As shown in Table 1C and Figure B.6, there appears to be a shift in CASM simulations that trigger the > 5% change in plant community at exposure durations ranging from 10 to 20 days and atrazine concentrations ranging from 25 to 50 ppb. Therefore, these averaging periods reasonably capture the duration of atrazine exposure that may be necessary to elicit a change in the plant community structure based on the relationship shown in Figure B.6. The threshold concentrations for aquatic community effects developed in EPA (2003a), and used in this endangered species assessment are as follows:

- 14-day average = 38 ppb
- 30-day average = 27 ppb
- 60-day average = 18 ppb
- 90-day average = 12 ppb

The 14-, 30-, 60-, and 90-day threshold concentrations developed by EPA (2003d) are used to evaluate potential indirect effects to aquatic communities and/or adverse modification to designated critical habitat for the purposes of this endangered species assessment. Use of these threshold concentrations is considered appropriate because: (1) the CASM-based index meets the goals of the defined assessment endpoints for this assessment; (2) the threshold concentrations provide a reasonable surrogate for the CASM index; and (3) the additional conservatism built into the threshold concentration LOCs, relative to the CASM-based index, is appropriate for an endangered species risk assessment. Therefore, these threshold concentrations are used to identify potential indirect effects (via aquatic plant community structural change) to the listed mussel species and their designated critical habitat. If modeled atrazine EECs exceed any one of the 14-, 30-, 60- and 90-day threshold concentrations following refinements with available monitoring data, the CASM model could be employed to further characterize the potential for indirect effects.

B.8 Uncertainties Related to Selection of the LOC and Use of the CASM Model

Uncertainties associated with the reference data for LOC(s), quantification of micro- and mesocosm testing results and extrapolation to different exposure time series, parameterization of the model, selection of model variables relating to micro- and mesocosm results, setting the LOC relative to micro- and mesocosm results, and applying model-based LOCs to action area exposure profile results are discussed in Sections B.8.1 through B.8.8 below.

B.8.1 Reference Data for LOC(s)

Because the potential risk of atrazine to aquatic communities is based on a set of micro- and mesocosm tests, it is critical to provide rationale for their inclusion. The large set of available studies for atrazine included in this analysis (Attachment 1) have various strengths and weaknesses and use many different testing designs and methods. The set of studies chosen to review was inclusive to avoid excluding data for various limited uncertainties or ambiguities. This approach provided a more robust data set for weight-of-evidence and allows for addressing “false-negatives” and “false-positives” in light of the overall frequency/magnitude of the wide range of possible exposure situations. It was concluded that the LOC decisions should not rely on any one or two of these studies.

B.8.2 Quantification of Results of Micro- and Mesocosm Tests

Effect scores in Brock et al. (2000) were used to quantify the results of the micro- and mesocosm tests. It was concluded that the scores assigned to the 77 results (see Attachment 1) were reasonable, and that scores of 2 (‘slight effect’) do not constitute a level of concern, while scores of 3 (‘significant effect followed by return to control levels within 56 days’) do constitute significant effects. Brock et al. (2000) further characterized a score of 2 as *“effects reported in terms of ‘slight’; ‘transient’, and short-term and/or quantitatively restricted response of sensitive endpoints, and effects only observed at individual samplings.”* Scores of 3 were characterized as a *“clear response of sensitive endpoints, but total recovery within 8 weeks after the last application, and effects reported as ‘temporary effects on several sensitive species’; ‘temporary elimination of sensitive species’; ‘temporary effects on less sensitive species / endpoints’, and effects observed at some subsequent samplings.”* The decision to differentiate a level of concern based on the Brock scores is critical, because these scores define the actual level of protection being sought. Therefore, Attachment 1 is arranged by decreasing effects score and shows the range and nature of effects represented by the different scores.

Another aspect of quantification is the relationship of the exposure duration to the effects score and concentration. There is uncertainty associated with the use of the study exposure duration when severe effects are observed early in studies with longer exposure periods. For example, the significant effects (scored as a 5 and described as a decrease in macrophytes coverage in the pond by 95%) in the Kettle et al. (1987) study were related to an exposure duration of 300 days. However, the study also reported that there was ~60% decrease in macrophytes coverage after 60 days. The 300-day test duration was used because: (1) the exposures in the study were constant over the whole time period, (2) Brock et al, as well as other authors, reported the test duration as ~1 year, and (3) the most dramatic effect without testing for recovery did occur after the ~year long exposure duration. It could be argued that the 60% decrease in macrophytes coverage is significant and should be scored as a 5 and included. However, the uncertainty resulting from this observation for the calculation of the time-specific LOC(s) is very small because, as shown in Figure B.5, the concentrations causing community structure changes do not further decrease for constant exposure periods longer than 20 to 30 days (i.e., longer

exposure periods do not significantly change the effect threshold). The Kettle et al. (1987) study was conducted at the borderline of this threshold concentration (~ 20 ppb). In the weight-of-evidence approach, the Kettle et al. study constitutes only one of a large number of such studies that also measured less severe impact at the comparable concentrations and exposure durations.

B.8.3 Extrapolation of Micro- and Mesocosm Tests to Different Exposure Time Series

Another critical decision was to use an aquatic ecological community model as the extrapolation tool to interpret atrazine chemographs. It is important to emphasize that the use of a model is not intended to predict the effects in any particular community, but rather to provide a useful means for integrating the kinetics of various processes (toxic effects on photosynthesis, plant growth dynamics, interactions among plant species across a growing season) and describing the RELATIVE effects of different exposure time series on the overall response.

The choice of CASM for these efforts was made based both on logistical grounds and on the large set of species used in it. It is important to point out that the response simulated by this model must always be set in relation to the micro- and mesocosm data identified as the most relevant measure for setting the aquatic community LOC(s).

Another development area is the formulation of CASM with regard to toxic effects (i.e., the general stress syndrome [GSS]). Sublethal effects in CASM are modeled using the GSS. Daily exposure concentrations are used to calculate a 'toxic effects factor' for each modeled population. The 'toxic effects factor' addresses differential growth characteristics of each population and population-specific sensitivity to atrazine. A comparison of GSS with reduction in photosynthesis showed that the latter produced smaller effects, making application of the results less practical. However, concern was expressed regarding model results that rely on a toxic response that differs from reduction in photosynthesis (EPA, 2003a). As such, it was recommended that further evaluation of available data be conducted to better justify use of GSS. In addition, it was also recommended that a comparison of how the overall assessment (not just the immediate model results) would differ using the GSS versus an alternative formulation of sublethal endpoints.

B.8.4 Parameterization of the CASM Model

The critical data used to parameterize the model are the plant laboratory toxicity data assigned to each species in CASM. These data are the key factor in determining the concentration at which CASM predicts significant effects (slightly above 20 ppb) and describing the "step-wise" nature of the effects versus concentration. Given the concerns with effect levels that reflect the more sensitive organisms, Figure B.3 (and the information provided in Attachment 2) shows that the decision to use the geometric mean toxicity values (EC₅₀s) for CASM appears to adequately represent the plant species sensitivity distribution. However, one consequence of the limited number of possible

species in the model is that only a few species represent sensitivities below the 10th centile and above the 90th centile. Additional analyses using the 10th and 90th centile of the EC₅₀ instead of the geometric means was conducted to test for the potential impact of the species sensitivity on the CASM results (Attachment 5). For the majority of simulations, the lower toxicity profiles (scenario 2) did not cause significantly higher responses than the geometric mean scenario. It was also observed that the higher and lower toxicity scenarios did not necessarily bracket the geometric mean scenario. This can partly be explained by the complex nature of the food chain interactions in the ecological model. The impact of slightly different species sensitivity distributions used to parameterize the model is therefore probably low, when compared to the relative importance of the species composition in the food chain model.

Different species have different relative importance in the results of CASM, and this varies seasonally. Even if each CASM species is linked to the most relevant laboratory species, the original selection of CASM species and the assignment of the laboratory data represent a major uncertainty. Therefore, evaluation using model parameterizations representing different generic aquatic communities could influence the relationships observed (EPA, 2003a).

B.8.5 Selection of Model Variables to Relate to Micro- and Mesocosm Results

The selection of the endpoint of concern is a critical decision, even if model results are calibrated to the micro- and mesocosm data, because different endpoints have different time-dependencies. These differences will affect the relative level of concern for different exposure series. While the average similarity index is a reasonable choice, it was recognized that its meaning is somewhat uncertain (EPA, 2003a). The critical point is the time trajectory of the index when the effect on the average community structure is less than that at the end of the year. The recommended average index combines direct toxic effects and consequent shifts in later season plant succession. However, it is important to note that this index can have different time dependence than an endpoint such as overall primary productivity, and thus is a key decision. This uncertainty could be estimated by taking a few different endpoints through the whole process and documenting how much the end results are affected (EPA, 2003a).

B.8.6 Setting the LOC for Model Variables Relative to Micro- and Mesocosm Results

Plotting the Brock scores against a similarity index effects based on direct interpolation of the dense matrix of model results provides a reasonable means of selecting the LOC for the model index (EPA, 2003a). The accepted frequency and nature of false positives and negatives is critical in identifying the LOC. This decision, as well as the decision to use the Brock scoring for the reported results of the micro- and mesocosm tests, is key decision points. The LOC threshold values for the different time-averaging periods given in Figure B.1 are based on the most conservative calculations made at the 99th centile prediction accuracy.

B.8.7 Applying Model-based LOC(s) to Monitored Atrazine Profiles

The evaluation of a rolling average comparison based on CASM results employed actual atrazine chemographs obtained from original and amplified monitoring datasets for a range of rivers and streams from different watershed sizes in agricultural areas in Ohio (Heidelberg data set) (EPA, 2003a). The atrazine concentrations in the data set were increased by a factor of 10 because the actual data were well below the levels associated with the LOCs. Because the threshold values are used as an initial step, an approach to minimize false negatives and include all four averaging periods for a more reliable check was recommended. Even with this conservative screen, there would be only several false positives. An important point to reiterate is that this initial step is NOT a risk decision point. Further evaluation of the appropriate values would be required, if the triggers were exceeded. For the purposes of the endangered species assessment, the threshold concentrations and CASM modeling are used as tools to predict potential indirect effects (via aquatic plant community structural change) to the listed species being evaluated in this assessment. As such, it represents a conservative estimate of potential indirect habitat effects.

B.8 References

- Bartell, S.M., K.R. Campbell, C.M. Lovelock, S.K. Nair, and J.L. Shaw. 2000. Characterizing aquatic ecological risk from pesticides using a diquat dibromide case study III. Ecological Process Models. Environ. Toxicol. Chem. 19(5):1441-1453.
- Bartell, S.M., G. Lefebvre, G. Aminski, M. Carreau, and K.R. Campbell. 1999. An ecosystem model for assessing ecological risks in Quebec rivers, lakes, and reservoirs. Ecol. Model. 124:43-67.
- Berard, A., T. Pelte and J. Druart. 1999. Seasonal variations in the sensitivity of Lake Geneva phytoplankton community structure to atrazine. Arch. Hydrobiol. 145(3):277-295.
- Brock, T.C.M., J. Lahr, P.J. van den Brink, 2000. Ecological risks of pesticides in freshwater ecosystems. Part 1: Herbicides. Wageningen, Alterra, Green World Research. Alterra-Rapport 088. 124 pp.
- Brockway, D.L., P.D. Smith and F.E. Stancil. 1984. Fate and effects of atrazine on small aquatic microcosms. Bull. Environ. Contam. Toxicol. 32:345-353.

- Carder, J.P. and K.D. Hoagland. 1998. Combined effects of alachlor and atrazine on benthic algal communities in artificial streams. *Environ. Toxicol. Chem.* 17(7):1415-1420.
- Carney, E.C. 1983. The effects of atrazine and grass carp on freshwater communities. Thesis. University of Kansas, Lawrence, Kansas.
- DeAngelis, D.L., S.M. Bartell, and A.L. Brenkert. 1989. Effects of nutrient recycling and food-chain length on resilience. *Amer. Nat.* 134(5):778-805.
- deNoyelles, F., Jr. and W.D. Kettle. 1985. Experimental ponds for evaluating bioassay predictions. In: *Validation and predictability of laboratory methods for assessing the fate and effects of contaminants in aquatic ecosystems*. Boyle, T.P. (Ed.). ASTM STP 865, American Society for Testing and Materials, Philadelphia, PA. pp. 91-103.
- deNoyelles, F., and W.D. Kettle. 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.
- deNoyelles, F., and W.D. Kettle. 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.
- deNoyelles, F., Jr., W.D. Kettle and D.E. Sinn. 1982. The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. *Ecol.* 63:1285-1293.
- deNoyelles, F., Jr., W.D. Kettle, C.H. Fromm, M.F. Moffett and S.L. Dewey. 1989. Use of experimental ponds to assess the effects of a pesticide on the aquatic environment. In: *Using mesocosms to assess the aquatic ecological risk of pesticides: Theory and practice*. Voshell, J.R. (Ed.). Misc. Publ. No. 75. Entomological Society of America, Lanham, MD.
- deNoyelles, F., Jr., S.L. Dewey, D.G. Huggins and W.D. Kettle. 1994. Aquatic mesocosms in ecological effects testing: Detecting direct and indirect effects of

- pesticides. In: Aquatic mesocosm studies in ecological risk assessment. Graney, R.L., J.H. Kennedy and J.H. Rodgers (Eds.). Lewis Publ., Boca Raton, FL. pp. 577-603.
- Detenbeck, N.E., R. Hermanutz, K. Allen and M.C. Swift. 1996. Fate and effects of the herbicide atrazine in flow-through wetland mesocosms. *Environ. Toxicol. Chem.* 15:937-946.
- Dewey, S.L. 1986. Effects of the herbicide atrazine on aquatic insect community structure and emergence. *Ecol.* 67:148-162.
- Fairchild, J.F., T.W. LaPoint and T.R. Schwarz. 1994a. Effects of a herbicide and insecticide mixture in aquatic mesocosms. *Arch. Environ. Contam. Toxicol.* 27:527-533.
- Fairchild, J.F., S.D. Ruessler, M.K. Nelson and A.R. Carlson. 1994b. An aquatic risk assessment for four herbicides using twelve species of macrophytes and algae. Abstract No. HF05, 15th Annual Meeting. Society of Environmental Toxicology and Chemistry, Denver, CO.
- Giddings, J.M., T.A. Anderson, L.W. Hall, Jr., R. J. Kendall, R.P. Richards, K.R. Solomon, W.M. Williams. 2000. Aquatic Ecological Risk Assessment of Atrazine: A Tiered, Probabilistic Approach. Prepared by the Atrazine Ecological Risk Assessment Panel, ECORISK, Inc. Novartis Crop Protection, Inc. Project Monitor, Alan Hosmer. Novartis Number 709-00.
- Gruessner, B. and M.C. Watzin. 1996. Response of aquatic communities from a Vermont stream to environmentally realistic atrazine exposure in laboratory microcosms. *Environ. Toxicol. Chem.* 15:410-419.
- Gustavson, K. and S.A. Wangberg. 1995. Tolerance induction and succession in microalgae communities exposed to copper and atrazine. *Aquat. Toxicol.* 32:283-302.
- Hamala, J.A. and H.P. Kollig. 1985. The effects of atrazine on periphyton communities in controlled laboratory ecosystems. *Chemosphere* 14:1391-1408.

- Hamilton, P.B., G.S. Jackson, N.K. Kaushik and K.R. Solomon. 1987. The impact of atrazine on lake periphyton communities, including carbon uptake dynamics using track autoradiography. *Environ. Poll.* 46:83-103.
- Hamilton, P.B., G.S. Jackson, N.K. Kaushik, K.R. Solomon and G.L. Stephenson. 1988. The impact of two applications of atrazine on the plankton communities of *in situ* enclosures. *Aquat. Toxicol.* 13:123-140.
- Hamilton, P.B., D.R.S. Lean, G.S. Jackson, N.K. Kaushik and K.R. Solomon. 1989. The effect of two applications of atrazine on the water quality of freshwater enclosures. *Environ. Pollut.* 60:291-304.
- Herman, D., N.K. Kaushik and K.R. Solomon. 1986. Impact of atrazine on periphyton in freshwater enclosures and some ecological consequences. *Can. J. Fish. Aquat. Sci.* 43:1917-1925.
- Johnson, B.T. 1986. Potential impact of selected agricultural chemical contaminants on a northern prairie wetland: A microcosm evaluation. *Environ. Toxicol. Chem.* 5:473-485.
- Jurgensen, T. A. and K. D. Hoagland. 1990. Effects of short-term pulses of atrazine on attached algal communities in a small stream. *Arch. Environ. Contam. Toxicol.* 19:617-623.
- Juttner, I., A. Peither, J.P. Lay, A. Kettrup and S.J. Ormerod. 1995. An outdoor mesocosm study to assess ecotoxicological effects of atrazine on a natural plankton community. *Arch. Environ. Contam. Toxicol.* 29:435-441.
- Kettle, W.D., F. deNoyelles, B.D. Heacock and A.M. Kadoum. 1987. Diet and reproductive success of bluegill recovered from experimental ponds treated with atrazine. *Bull. Environ. Contam. Toxicol.* 38:47-52.
- Kettle, W.D. 1982. Description and analysis of toxicant-induced responses of aquatic communities in replicated experimental ponds. Ph.D. Thesis. University of Kansas, Lawrence, KS.

- Kosinski, R.J. 1984. The effect of terrestrial herbicides on the community structure of stream periphyton. *Environ. Pollut. (Series A)* 36:165-189.
- Kosinski, R.J. and M.G. Merkle. 1984. The effect of four terrestrial herbicides on the productivity of artificial stream algal communities. *J. Environ. Qual.* 13:75-82.
- Krieger, K.A., D.B. Baker and J.W. Kramer. 1988. Effects of herbicides on stream aufwuchs productivity and nutrient uptake. *Arch. Environ. Contam. Toxicol.* 17:299-306.
- Lakshminarayana, J.S.S., H.J. O'Neill, S.D. Jonnavithula, D.A. Leger and P.H. Milburn. 1992. Impact of atrazine-bearing agricultural tile drainage discharge on planktonic drift of a natural stream. *Environ. Pollut.* 76:201-210.
- Lampert, W., W. Fleckner, E. Pott, U. Schober and K.U. Storkel. 1989. Herbicide effects on planktonic systems of different complexity. *Hydrobiologia* 188/189:415-424.
- Lynch, T.R., H.E. Johnson and W.J. Adams. 1985. Impact of atrazine and hexachlorobiphenyl on the structure and function of model stream ecosystems. *Environ. Toxicol. Chem.* 4:399-413.
- Moorhead, D.L. and R.J. Kosinski. 1986. Effect of atrazine on the productivity of artificial stream algal communities. *Bull. Environ. Contam. Toxicol.* 37:330-336.
- Pratt, J.R., N.J. Bowers, B.R. Niederlehrer and J. Cairns, Jr. 1988. Effects of atrazine on freshwater microbial communities. *Arch. Environ. Contam. Toxicol.* 17:449-457.
- Stay, E.F., A. Katko, C.M. Rohm, M.A. Fix and D.P. Larsen. 1989. The effects of atrazine on microcosms developed from four natural plankton communities. *Arch. Environ. Contam. Toxicol.* 18:866-875.
- Stay, F.S., D.P. Larsen, A. Katko and C.M. Rohm. 1985. Effects of atrazine on community level responses in Taub microcosms. In: *Validation and predictability of laboratory methods for assessing the fate and effects of contaminants in aquatic ecosystems*. Boyle, T.P. (Ed.). ASTM STP 865. American Society for Testing and Materials, Philadelphia, PA. pp. 75-90.

- Van den Brink, P.J., E. van Donk, R. Gylstra, S.J.H. Crum and T.C.M. Brock. 1995. Effects of chronic low concentrations of the pesticides chlorpyrifos and atrazine in indoor freshwater microcosms. *Chemosphere* 31:3181-3200.
- U.S. EPA. 2003a. Interim Reregistration Eligibility Decision (IRED) for Atrazine. Office of Pesticide Programs. Environmental Fate and Effects Division. January 31, 2003. <http://www.epa.gov/oppsrrd1/REDs/0001.pdf>
- U.S. EPA. 2003b. Revised Atrazine Interim Reregistration (IRED). Office of Pesticide Programs. Environmental Fate and Effects Division. October 31, 2003. <http://www.epa.gov/oppsrrd1/REDs/0001.pdf>
- U.S. EPA. 2003c. Ambient Aquatic Life Water Quality Criteria for Atrazine – Revised Draft. Office of Water, Office of Science and Technology, Health and Ecological Criteria Division, Washington, D.C. EPA-822-R-03-023. October 2003.
- U.S. EPA. 2003d. Atrazine MOA Ecological Subgroup: Recommendations for aquatic community Level of Concern (LOC) and method to apply LOC(s) to monitoring data. Subgroup members: Juan Gonzalez-Valero (Syngenta), Douglas Urban (OPP/EPA), Russell Erickson (ORD/EPA), Alan Hosmer (Syngenta). Final Report Issued on October 22, 2003.

Attachment 1: Micro- and mesocosm studies table with Brock scores and estimated average % change in community structure (Steinhaus similarity) of primary producers

#	Duration (d)	Test Conc (µg/L)	Single / Constant / Multiple	Brock et al 2000 Effect Score	Reference(s)	Ecosystem	Results	Measurement Endpoint	Plant Group	Recovery	Comments	> or <	AVG % change in community structure (Steinhaus similarity) of primary producers
1	300	500	single	5	Carney 1983; Kettle et al. 1987; deNoyelles et al. 1989; deNoyelles et al. 1994	mesocosms, experimental ponds	<i>Decrease</i>	cover by emerged, floating and submerged aquatic plants	Macro	> 1 yr		>	48.4
2	300	20	single	5	Carney 1983; Kettle et al. 1987; deNoyelles et al. 1989; deNoyelles et al. 1994, deNoyelles & Kettle 1983, deNoyelles & Kettle 1980, Dewey 1986	mesocosms, experimental ponds	<i>Decrease</i>	cover by floating and submerged aquatic plants	Macro	> 1 yr			0.7
3	60	500	single	5	deNoyelles et al. 1982; Kettle 1982; deNoyelles et al. 1989	mesocosms, experimental ponds	<i>Decrease / Change</i>	¹⁴ C-uptake phytoplankton and biomass phytoplankton; all important phytoplankton species / species composition	Phyto	60 - > 63 d		>	40.6

								phytoplankton					
4	300	100	single	5	deNoyelles et al. 1989 Carney 1983	mesocoms, experimental ponds	<i>Decrease</i>	cover by emerged and submerged aquatic plants	Macro	> 1 yr			18.0
5	300	200	single	5	deNoyelles et al. 1989 Carney 1983	mesocoms, experimental ponds	<i>Decrease</i>	cover by emerged and submerged aquatic plants	Macro	> 1 yr			48.4
6	56	50	single	5	Fairchild et al. 1994	mesocoms, experimental ponds	<i>Change / No Effect</i>	<i>Chara</i> sp. replaces <i>Naja</i> sp. / total biomass aquatic plants	Macro	> 15 wks	esfenvalerate added		8.9
7	60	80	multiple	5	Hamilton et al 1987	lake enclosure	<i>Decrease</i>	number. biomass, composition	Peri	49 d			14.9
8	60	140	multiple	5	Hamilton et al 1987	lake enclosure	<i>No Effect / Change</i>	numbers, biomass, Chl-a and C14 uptake / species composition	Peri	>56 d			15.4
9	73	143	multiple	5	Herman et al. 1986; Hamilton et al. 1988; Hamilton et al. 1989	lake enclosure	<i>Change</i>	species composition	Phyto	>294 d			40.6
10	73	143	multiple	5	Herman et al. 1986; Hamilton et al. 1988; Hamilton et al. 1989	lake enclosure	<i>Decrease / Change</i>	POC (slight), c14 uptake / species composition	Peri	90 d; 14 d; >294 d; >294 d			40.6

11	63	182	constant	5	Jüttner et al. 1995	pond enclosures	<i>No Effect / Decrease</i>	rotifer / DO, conductivity (slight); algal species [Mallomonas sp (slight); Cryptomonas sp.]	Phyto	>63 d; 50 d; 35 d; 56 d			40.6
12	63	318	constant	5	Jüttner et al. 1995	pond enclosures	<i>Decrease</i>	DO (slight); conductivity (slight); rotifers (slight); algal species [Mallomonas sp (slight); Cryptomonas sp.]	Phyto	> 63 d; 50 d; 25 d; 35 d; >56 d;		>	40.6
13	60	500	single	5	Stay et al. 1985	microcosms, laboratory Taub	<i>Decrease</i>	DO, 14C-uptake, net primary production, respiration, 14C-uptake, Chl-a	Phyto	> 53 d		>	40.6
14	60	1000	single	5	Stay et al. 1985	microcosms, laboratory Taub	<i>Decrease</i>	DO, 14C-uptake, net primary production, respiration, 14C-uptake, Chl-a	Phyto	> 53 d		>	40.6
15	60	5000	single	5	Stay et al. 1985	microcosms, laboratory Taub	<i>Decrease</i>	DO, 14C-uptake, net primary production, respiration,	Phyto	> 53 d		>	40.6

								14C-uptake, Chl-a					
16	21	10	single	4	Berard et al 1999	microcosm, lab stagnant	<i>Change</i>	Change in species composition & density	Phyto	?	not included in brock et al 2000	<	0.4
17	7	100	single	4	Brockway et al. 1984	microcosms, lab stagnant	<i>Decrease</i>	Net O ₂ production	Phyto	> 12 d			7.4
18	12	500	single	4	Brockway et al. 1984	microcosms, lab stagnant	<i>Decrease</i>	Net O ₂ production	Phyto	> 12 d		>	16.4
19	12	5000	single	4	Brockway et al. 1984	microcosms, lab stagnant	<i>Decrease</i>	Net O ₂ production	Phyto	> 12 d		>	16.4
20	28	15	constant	4	Carder and Hoagland 1998	artificial streams, continuous flow	<i>Decrease</i>	Algal community biovolume	Peri	> 28 d	not included in brock et al 2000		0.4
21	28	150	constant	4	Carder and Hoagland 1998	artificial streams, continuous flow	<i>Decrease</i>	Algal community biovolume	Peri	> 28 d	not included in brock et al 2000		25.5
22	27	15	constant	4	Detenback et al 1996	artificial flow-through swamp	<i>Decrease / Increase</i>	DO, metabolism of periphyton in bioassays / nutrients	Peri	?			0.4
23	27	25	constant	4	Detenback et al 1996	artificial flow-through swamp	<i>Decrease / Increase</i>	metabolism of periphyton in bioassays / nutrients	Peri	?			5.1
24	27	50	constant	4	Detenback et al 1996	artificial flow-through swamp	<i>Decrease / Increase</i>	metabolism of periphyton in bioassays / nutrients	Peri	?			6.0
25	27	75	constant	4	Detenback et al 1996	artificial flow-through swamp	<i>Decrease / Increase</i>	metabolism of periphyton in bioassays / nutrients	Peri	?			11.6

26	14	100	constant	4	Hamala and Kollig 1985	microcosm, lab flowing	<i>Decrease / Change</i>	primary production, number of species, Chl-a and biomass of periphyton / species composition	Peri	pp 16-d; > 21 d			10.2
27	30	1000	single	4	Johnson 1986	microcosm, lab stagnant	<i>Decrease</i>	gross primary production; biomass;	Macro	>30 d		>	25.5
28	21	10	constant	4	Kosinski 1984; Kosinski and Merkle 1984	artificial streams, recirculating	<i>Decrease</i>	gross primary productivity; biovolume of periphyton on artificial substrate	Peri	> 21 d		<	0.4
29	21	1000	constant	4	Kosinski 1984; Kosinski and Merkle 1984	artificial streams, recirculating	<i>Decrease</i>	gross primary productivity; biovolume of periphyton on artificial substrate	Peri	> 21 d; 14 d		>	25.5
30	21	10000	constant	4	Kosinski 1984; Kosinski and Merkle 1984	artificial streams, recirculating	<i>Decrease</i>	gross primary productivity; biovolume of periphyton on artificial substrate	Peri	> 21 d		>	25.5
31	12	24	constant	4	Krieger et al. 1988	artificial streams, recirculating	<i>No effect / Decrease</i>	uptake of phosphorous, silicium and nitrogen by periphyton / Chl-a and biomass of periphyton	Peri	?			3.4

32	12	134	constant	4	Krieger et al. 1988	artificial streams, recirculating	<i>No effect / Decrease</i>	silicium uptake by periphyton / uptake of phosphorus and nitrate by periphyton (slight); Chl-a and biomass of periphyton	Peri	?			10.2
33	3	10000	single	4	Moorhead and Kosinski 1986	artificial streams, recirculating	<i>No Effect / Decrease</i>	conductivity, alkalinity, soluble reactive phosphorous, respiration, species composition of periphyton (study probably too short) / pH, net primary production	Peri	7 d		>	6.8
34	21	337	constant	4	Pratt et al. 1988	microcosms, laboratory flowing	<i>Decrease</i>	DO, potassium, magnesium, calcium (slight), number of species, protein biomass and Chl-a protozoa	Peri	> 21 d			25.5

35	42	200	single	4	Stay et al. 1989	microcosms, laboratory Leffler	<i>Decrease</i>	pH and primary production	Phyto	42 - >42 d			40.6
36	42	500	single	4	Stay et al. 1989	microcosms, laboratory Leffler	<i>Decrease</i>	pH and primary production	Phyto	> 42 d		>	40.6
37	42	1000	single	4	Stay et al. 1989	microcosms, laboratory Leffler	<i>Decrease</i>	pH and primary production	Phyto	> 42 d		>	40.6
38	42	5000	single	4	Stay et al. 1989	microcosms, laboratory Leffler	<i>Decrease</i>	pH and primary production	Phyto	> 42 d		>	40.6
39	70	50	constant	3	Brockway et al. 1984	microcosms, lab continuous flow	<i>Decrease / Increase</i>	Net O ₂ -production / Nitrate	Phyto	1 d			8.9
40	70	100	constant	3	Brockway et al. 1984	microcosms, lab continuous flow	<i>Decrease / Increase</i>	Net O ₂ -production / Nitrate	Phyto	1 d; 2 d			15.8
41	20	100	single	3	deNoyelles et al. 1989	mesocosms, experimental ponds	<i>Decrease</i>	¹⁴ C-uptake and biomass phytoplankton	Phyto	20 d			12.8
42	20	200	single	3	deNoyelles et al. 1989	mesocosms, experimental ponds	<i>Decrease</i>	¹⁴ C-uptake and biomass phytoplankton; biomass phytoplankton	Phyto	20 d			25.5
43	63	68	constant	3	Jüttner et al. 1995	pond enclosures	<i>No Effect / Decrease</i>	one algal species, rotifer / DO, conductivity (slight)	Phyto	?			14.9
44	21	100	constant	3	Kosinski 1984; Kosinski and Merkle 1984	artificial streams, recirculating	<i>No effect / Decrease</i>	biovolume of periphyton on artificial substrate; gross primary	Peri	< 3 d			12.7

								productivity					
45	3	100	single	3	Moorhead and Kosinski 1986	artificial streams, recirculating	<i>No Effect / Decrease</i>	conductivity, alkalinity, soluble reactive phosphorous, respiration, species composition of periphyton (study probably too short) / pH, net primary production	Peri	7 d			5.6
46	3	1000	single	3	Moorhead and Kosinski 1986	artificial streams, recirculating	<i>No Effect / Decrease</i>	conductivity, alkalinity, soluble reactive phosphorous, respiration, species composition of periphyton (study probably too short) / pH, net primary production	Peri	7 d		>	6.8
47	60	60	single	3	Stay et al. 1985	microcosms, laboratory Taub	<i>Decrease</i>	DO, ¹⁴ C-uptake, net primary production, respiration /	Phyto	20 - 27 d; 53 d			8.9

								14C-uptake, Chl-a					
48	60	100	single	3	Stay et al. 1985	microcosms, laboratory Taub	<i>Decrease</i>	DO, 14C-uptake, net primary production, respiration / 14C-uptake, Chl-a	Phyto	25 - 32 d; 53 d			15.8
49	60	200	single	3	Stay et al. 1985	microcosms, laboratory Taub	<i>Decrease</i>	DO, 14C-uptake, net primary production, respiration / 14C-uptake, Chl-a	Phyto	25 - 32 d; 53 d			40.6
50	42	100	single	3	Stay et al. 1989	microcosms, laboratory Leffler	<i>Decrease</i>	pH and primary production	Phyto	7 - >42 d			15.8
51	12	50	single	2	Brockway et al. 1984	microcosms, lab stagnant	<i>Decrease</i>	Net O ₂ -production (slight)	Phyto	> 12 d			4.2
52	7	20	single	2	deNoyelles et al. 1982; Kettle 1982; deNoyelles et al. 1989	mesocosms, experimental ponds	<i>Decrease / Change</i>	¹⁴ C-uptake and biomass phytoplankton / composition of phytoplankton species; increase in dinoflagellates	Phyto	7 d			0.1

53	30	10	single	2	Johnson 1986	microcosm, lab stagnant	<i>Decrease</i>	gross primary production (slight)	Macro	7 d			0.4
54	30	100	single	2	Johnson 1986	microcosm, lab stagnant	<i>Decrease</i>	gross primary production (slight)	Macro	7 d			12.8
55	63	22	constant	2	Jüttner et al. 1995	pond enclosures	<i>No Effect / Decrease</i>	one algal species / DO, pH, conductivity (slight)	Phyto	?			0.5
56	63	10	constant	2	Jüttner et al. 1995	pond enclosures	<i>No Effect / Decrease</i>	one algal species / DO, pH, conductivity (slight)	Phyto	?		<	0.5
57	14	1.89		2	Lakshminarayana et al 1992	stream, adjacent to agri tile drainage	<i>Decrease</i>	number of species and cell numbers (during low flow)	phyto	150 d		<	0.1
58	18	1		2	Lampert et al 1989	lake enclosure	<i>Decrease</i>	primary production	phyto	14 d		<	0.1
59	21	32	constant	2	Pratt et al. 1988	microcosms, laboratory flowing	<i>No Effect / Decrease</i>	potassium, protein biomass, Chl-a of Protozoa / DO, magnesium, calcium (slight)	Peri	> 21 d			5.2
60	21	110	constant	2	Pratt et al. 1988	microcosms, laboratory flowing	<i>No Effect / Decrease</i>	potassium, calcium, number of species, protien	Peri	> 21 d			12.7

								biomass, Chl-a of Protozoa / DO, magnesium, (slight)						
61	42	20	single	2	Stay et al. 1989	microcosms, laboratory	Leffler	<i>Decrease</i>	primary production (slight)	Phyto	1-10 d		0.5	
62	35	5	constant	2	van den Brink et al. 1995	microcosms, laboratory		<i>Decrease</i>	photosynthetic activity, as indicated by higher conductivity and alkalinity, and lower Do and pH	Phyto	NA		<	0.4
63	7	0.5	single	1	Brockway et al. 1984	microcosms, lab	stagnant	<i>No effect</i>	Net O ₂ production	Phyto	NA		<	0.1
64	7	5	single	1	Brockway et al. 1984	microcosms, lab	stagnant	<i>No effect</i>	Net O ₂ production	Phyto	NA		<	0.1
65	70	0.5	constant	1	Brockway et al. 1984	microcosms, lab	continuous flow	<i>No effect</i>	Net O ₂ production	Phyto	NA		<	0.1
66	70	5	constant	1	Brockway et al. 1984	microcosms, lab	continuous flow	<i>No effect</i>	Net O ₂ -production / Nitrate	Phyto	NA		<	0.1
67	14	5	constant	1	Gruessner and Watzin 1996	microcosm, lab		<i>No effect</i>	Chl-a of periphyton on artificial substrate	Peri	NA	concentration of atrazine reduced to 1ug/l by day-14	<	0.1
68	20	1	multiple	1	Gustavson and Wängberg 1995	lake enclosure		<i>No effect</i>	community tolerance	Phyto	NA	Copper added; not included in Brock et al 2000	<	0.1

69	20	20	multiple	1	Gustavson and Wängberg 1995	lake enclosure	<i>No effect</i>	community tolerance	Phyto	NA	Copper added; not included in Brock et al 2000		0.4
70	20	10	multiple	1	Gustavson and Wängberg 1995	lake enclosure	<i>No effect</i>	community tolerance	Phyto	NA	Copper added; not included in Brock et al 2000	<	0.4
71	28	2	2 short pulses 24-hours @	1	Jurgensen and Hoagland 1990	stream enclosures	<i>No Effect</i>	cell density, biomass	Peri	NA		<	0.1
72	28	30	2 short pulses 24-hours @	1	Jurgensen and Hoagland 1990	stream enclosures	<i>No Effect</i>	cell density, biomass	Peri	NA			5.2
73	28	100	2 short pulses 24-hours @	1	Jurgensen and Hoagland 1990	stream enclosures	<i>No Effect</i>	cell density, biomass	Peri	NA			12.8
74	63	5	constant	1	Jüttner et al. 1995	pond enclosures	<i>No Effect</i>	one algal species; DO, pH, conductivity	Phyto	NA		<	0.5
75	30	25	?	1	Lynch et al. 1985	artificial streams, laboratory	<i>No Effect</i>	standing biomass, rate of primary production, community respiration	Peri	NA	Use of DMSO as solvent		5.1
76	21	3.2	constant	1	Pratt et al. 1988	microcosms, laboratory flowing	<i>No effect</i>	DO, potassium, magnesium, calcium	Peri	NA		<	0.1

77	21	10	constant	1	Pratt et al. 1988	microcosms, laboratory flowing	<i>No effect</i>	DO, potassium, magnesium, calcium	Peri	NA		<	0.4
----	----	----	----------	---	-------------------	--------------------------------	------------------	--	------	----	--	---	-----

Attachment 2: Ecotoxicity profiles for CASM

CASM Population	Description	Assay species	EC50/LC50 ug/L	Duration (d)	NOEC ug/L	Min	centiles				Max	Scenario		
							10	50	90			1	2	3
Phytoplankton														
1	Diatom 1	<i>Navicula pelliculosa</i>	60	5	10							60	60	60
2	Diatom 2	<i>Skeletonema costatum</i>	69	3	14	24	31	69	186	265		69	31	186
3	Chlorophyte 1	<i>Scenedesmus obliquus</i>	38	1	20							38	38	38
4	Chlorophyte 2	<i>Chlamydomonas reinhardtii</i>	57	3	10	19	26	57	224	350		57	26	224
5	Chlorophyte 3	<i>Akistrodesmus braunii</i>	61	3	19	60				61		61	60	61
6	Chlorophyte 4	<i>Chlorella vulgaris</i>	88	4	(33) 500	25	34	88	212	293		88	34	212
7	Cyanophyte 1	<i>Microsystis spp.</i>	90	4	19							90	90	90
8	Cyanophyte 2	<i>Anabaena inaequalis</i>	173	1	100	100	113	173	264	300		173	113	264
9	Cyanophyte 3	<i>Synechococcus leopoliensis</i>	130	3	19							130	130	130
10	Cyanophyte 4	<i>Anabaena flosaquae</i>	264	5	100	58	88	264	576	776		264	88	576
Periphyton														
11	Diatom 1	<i>Navicula pelliculosa</i>	60	5	10							60	60	60
12	Diatom 2	<i>Skeletonema costatum</i>	69	3	14	24	31	69	186	265		69	31	186
13	Diatom 3	<i>Cyclotella spp.</i>	430	2	19							430	430	430
14	Chlorophyte 1	<i>Scenedesmus obliquus</i>	38	1	20							38	38	38
15	Chlorophyte 2	<i>Akistrodesmus braunii</i>	61	3	19	60				61		61	60	61
16	Chlorophyte 3	<i>Selenastrum capricornutum</i>	120	3	8	26	42	120	440	960		120	42	440
17	Cryptophyte 1	<i>Cryptomonas</i>	500	6	19							500	500	500
18	Cyanophyte 1	<i>Microsystis spp.</i>	90	4	19							90	90	90
19	Cyanophyte 2	<i>Anabaena cylindrica</i>	787	2	100	178	277	787	2283	3600		787	277	2,283
20	Euglenoid 1	<i>Chlorella vulgaris</i>	88	4	(19) 500	25	34	88	212	293		88	34	212
Macrophytes														
21	Vallisneria	<i>Vallisneria</i>	163	42	6							163	163	163
22	Myriophyllum	<i>Myriophyllum</i>	132	14	6							132	132	132
23	Ceratophyllum	<i>Ceratophyllum</i>	22	14	6							22	22	22
24	Elodea	<i>Elodea</i>	159	12	10	21	35	159	725	1200		159	35	725
25	Lemna	<i>Lemna</i>	173	7	4	22	41	173	1710	8700		173	41	1,710

26	Potamogeton	<i>Potamogeton</i>	170	8	11	6 G-mean	80	99	170	349	474	170	99	349
Consumers														
27	Copepods	Geo. Mean of 3 SW copepods	272	2	189		121	151	272	422	500	272	151	422
28	Cladocerans	Geo. Mean of 4 FW cladocerans	29,865	2	140		15,599	18,404	29,865	32,060	34,000	29,865	18,404	32,060
29	Common shiner	<i>Notropis atherinoides</i>	15,600	4	189							15,600	15,600	15,600
30	Johnny darter	<i>Pimephales promelas</i>	17,321	4	220							17,321	17,321	17,321
31	Blunt nosed minnow	<i>Pimephales promelas</i>	17,321	4	220							17,321	17,321	17,321
32	Smallmouth bass	<i>Perca sp.</i>	16,000	4	189							16,000	16,000	16,000
		<i>nitrogenase activity in cyanobacteria</i>												
33	Bacterioplankton		100,000	1	189 G-mean							100,000	100,000	100,000
34	Ephemeroptera	Geo. Mean of 5 FW species	12,788	2	132		3300	4904	12788	37561	60000	12,788	4,904	37,561
35	Trichoptera	Geo. Mean of 5 FW species	12,788	2	132		3300	4904	12788	37561	60000	12,788	4,904	37,561
36	Oligochaetes	Geo. Mean of 5 FW species	12,788	2	132		3300	4904	12788	37561	60000	12,788	4,904	37,561
37	Chironomids	Geo. Mean of 2 species	848	2	110		720	747	848	964	1000	848	747	964
38	Bivalves	<i>Anodonta imbecilis</i>	60,000	1	132							60,000	60,000	60,000
39	Creek chub	<i>Lepomis macrochirus</i>	28,033	4	95							28,033	28,033	28,033
40	White sucker	<i>Pimephales promelas</i>	17,321	4	220							17,321	17,321	17,321
41	Central stoneroller	Geo. Mean of 2 FW species	22,737	4 =			21105	21498	22737	24047	24495	22,737	21,498	24,047
42	Grass pickerel	<i>Perca sp.</i>	16,000	4	132							16,000	16,000	16,000
		<i>respiration inhibition in aerobic bacteria</i>												
43	Sediment bacteria		100,000	1	132 G-mean							100,000	100,000	100,000

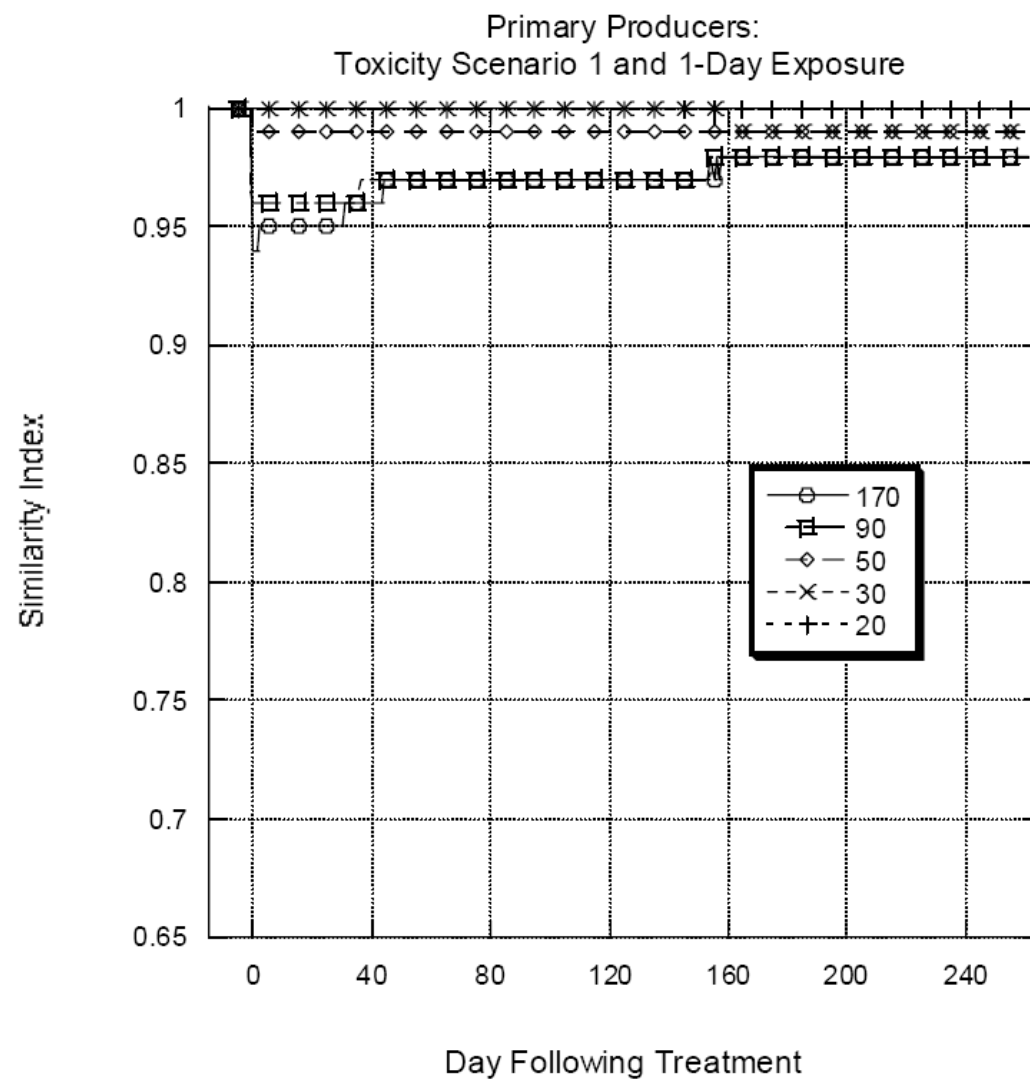
158

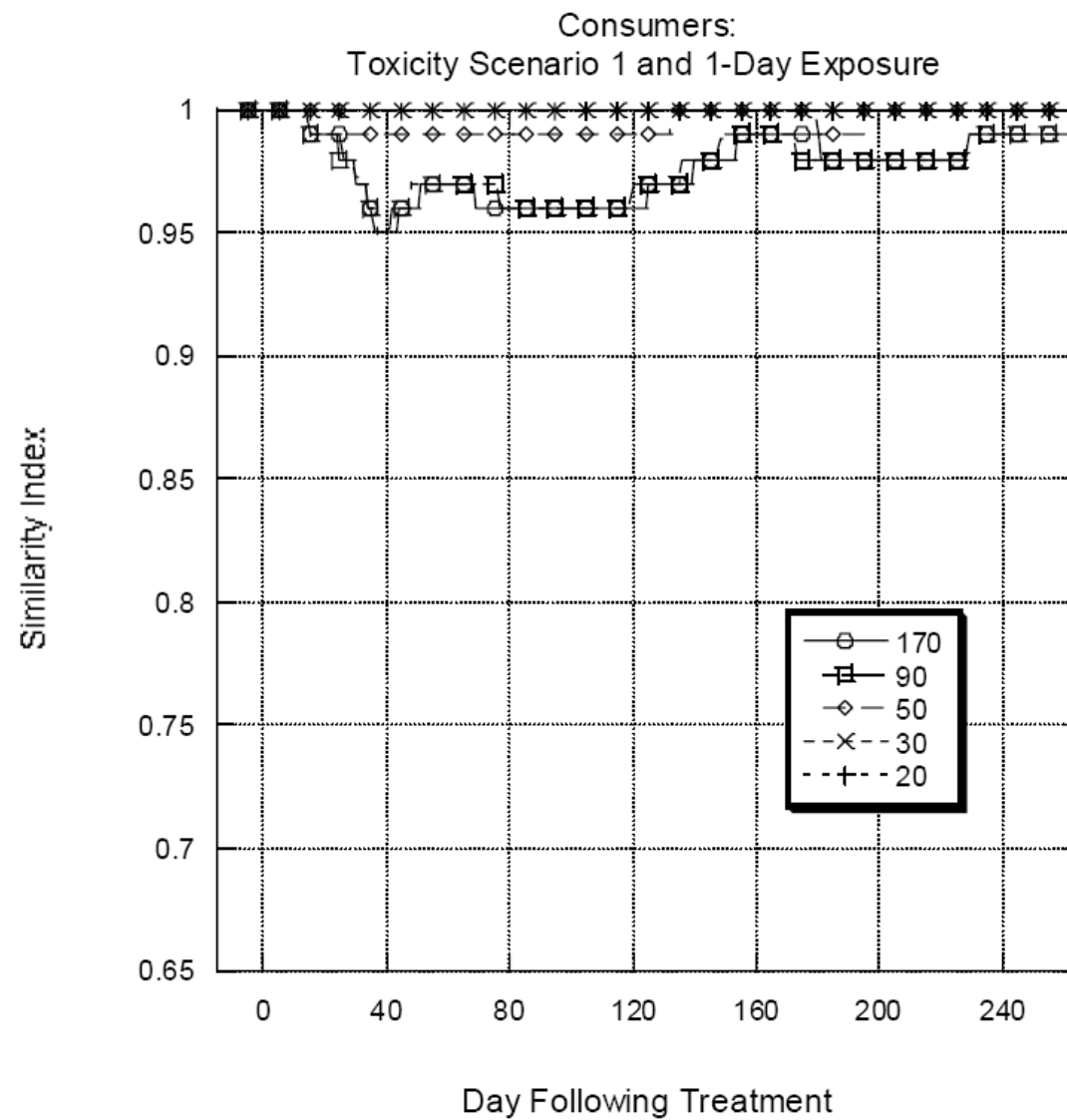
Attachment 3a: CASM Results: Annual Production

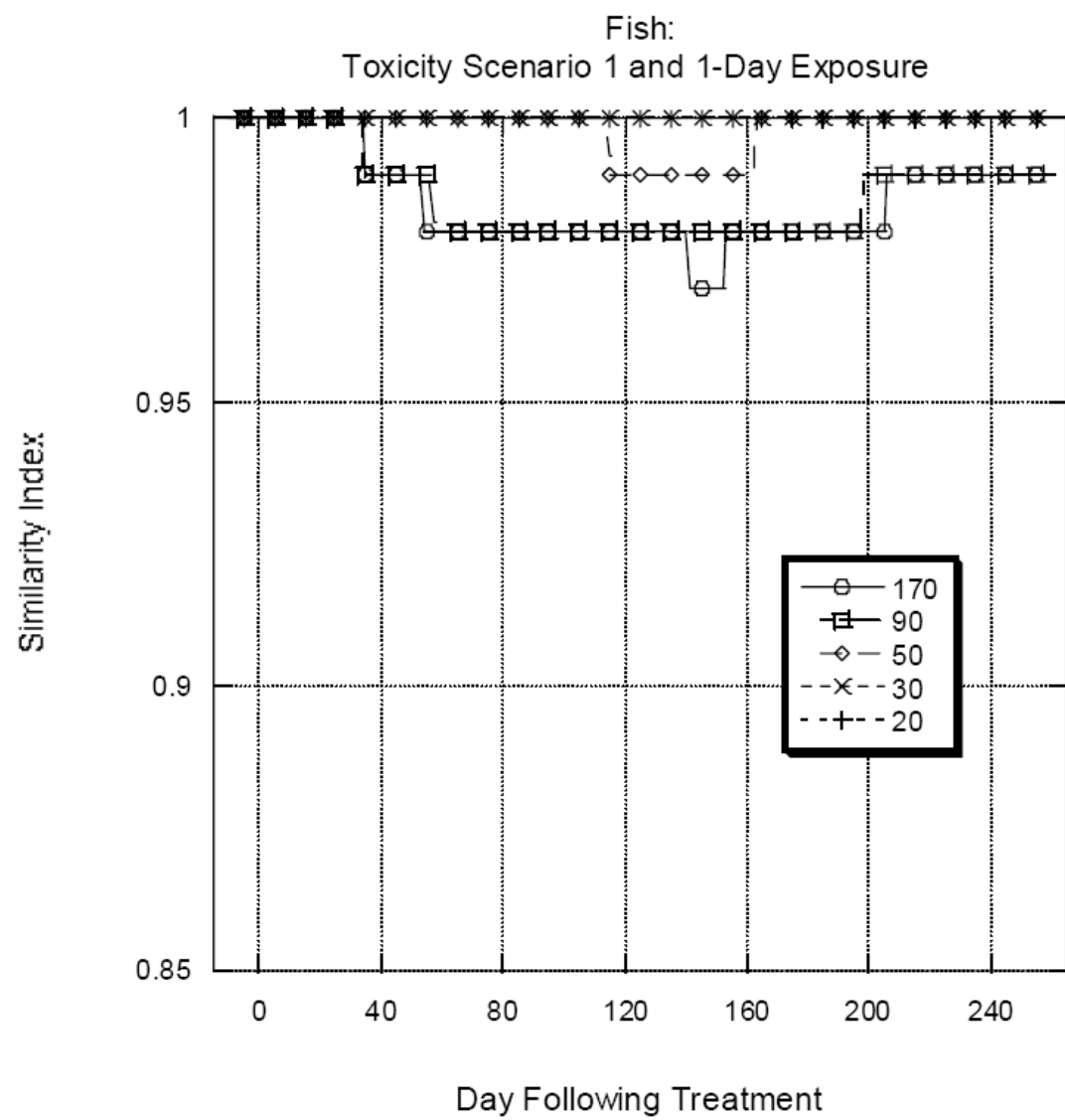
Duration [d]	Test Conc [µg/L]	Decrease in total annual production						fish
		phytoplankton	periphyton	macrophytes	Total primary production	zooplankton	benthic invertebrates	
		264	5,124	8,478	13,866			
1	20	0.3	0.1	0.0	0.0	0.2	0.1	0.1
1	30	0.1	0.1	0.3	0.2	0.1	0.4	0.1
1	50	1.5	0.1	0.2	0.2	2.2	0.3	0.5
1	90	12.5	3.8	1.4	2.5	12.4	1.6	2.0
1	170	13.8	3.8	0.6	2.0	13.2	2.6	2.4
3	20	0.9	0.0	0.0	0.0	0.8	0.0	0.0
3	30	0.4	0.2	1.0	0.7	0.3	0.7	0.3
3	50	1.6	0.5	0.7	0.6	2.9	1.0	0.9
3	90	21.9	7.8	2.4	4.8	23.4	3.5	4.2
3	170	24.2	7.6	2.9	5.0	25.2	6.1	5.1
5	20	2.3	0.1	0.0	0.1	2.1	0.0	0.1
5	30	1.0	0.4	1.5	1.1	0.8	1.1	0.5
5	50	1.5	1.2	1.2	1.2	3.0	1.7	1.3
5	90	25.9	10.5	2.8	6.1	29.3	4.9	5.6
5	170	28.5	10.0	5.3	7.5	31.8	8.9	6.9
10	20	1.2	0.2	0.2	0.2	0.8	0.0	0.1
10	30	1.1	0.6	2.7	1.9	1.4	1.9	0.7
10	50	1.8	2.1	2.4	2.3	0.3	3.1	1.6
10	90	29.3	14.7	2.8	7.7	36.1	7.5	7.5
10	170	32.0	13.5	10.5	12.0	39.4	13.9	9.7
20	20	2.1	0.1	0.2	0.2	2.3	0.1	0.2
20	30	0.2	0.8	4.0	2.7	0.4	2.8	1.0
20	50	2.0	2.8	3.7	3.3	0.5	4.3	2.1
20	90	30.0	16.7	2.0	8.0	39.0	9.3	8.6
20	170	33.0	14.7	17.5	16.8	43.5	19.2	12.1
60	20	2.5	0.1	0.2	0.2	3.3	0.3	0.3
60	30	0.1	1.4	5.5	3.9	0.5	4.3	1.8
60	50	2.8	4.3	5.6	5.1	2.1	6.6	3.1
60	90	24.8	19.2	0.3	7.8	34.3	12.7	9.8
60	170	33.3	14.4	27.7	22.9	43.3	26.2	15.7
130	20	2.5	0.3	0.2	0.3	3.3	0.4	0.4
130	30	0.1	1.7	6	4.3	0.5	4.9	2.1
130	50	7.9	5	6.1	5.7	3.0	7.9	3.8
130	90	22.7	21.7	0.3	8.6	24.2	17.7	11.8
130	170	33.3	12.8	29.9	23.6	43.3	28.1	18.4
260	20	2.5	0.5	0.2	0.4	3.3	0.6	0.5
260	30	0.1	2	6.1	4.5	0.5	5.2	2.3
260	50	9.5	5.5	6.2	6.0	2.8	8.3	4.1
260	90	27.2	22.3	0.4	9.0	24.6	18.5	12.2
260	170	33.3	12.8	27.2	22.0	43.3	31.3	21.2

Attachment 3b: Steinhaus Similarity Exposure: Day 1 to Day 20

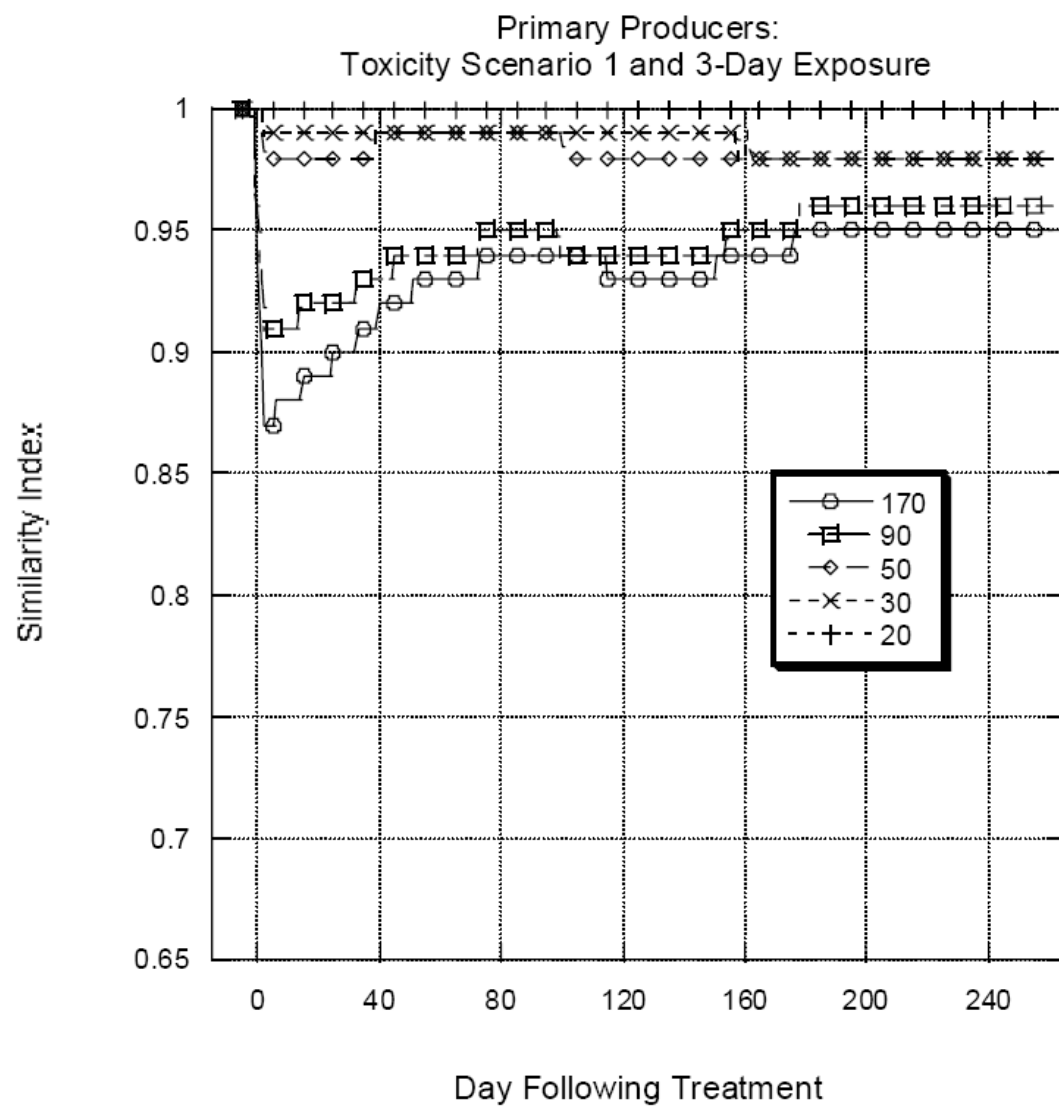
CASM Results: Steinhaus similarity Toxicity Scenario 1 and 1-Day Exposure

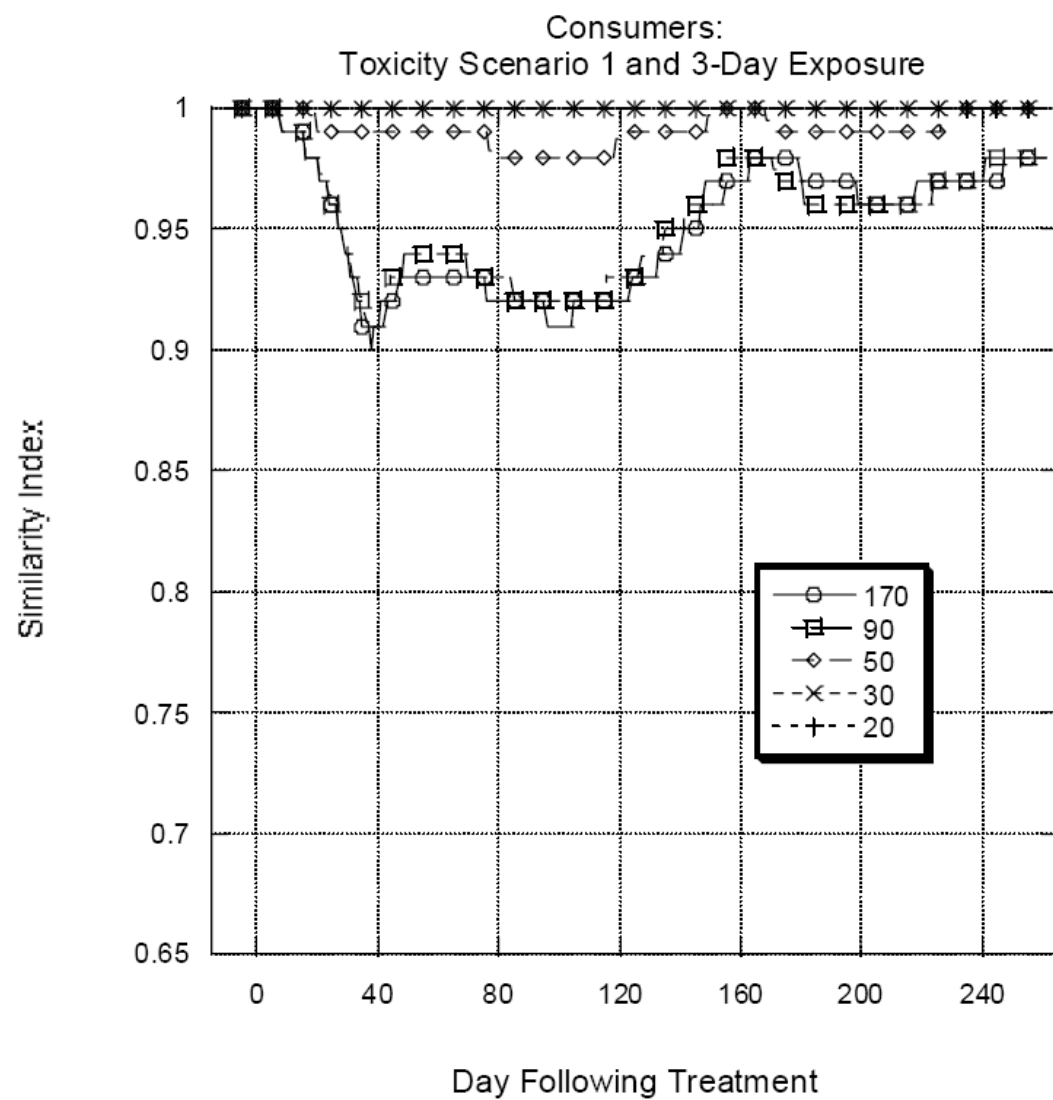


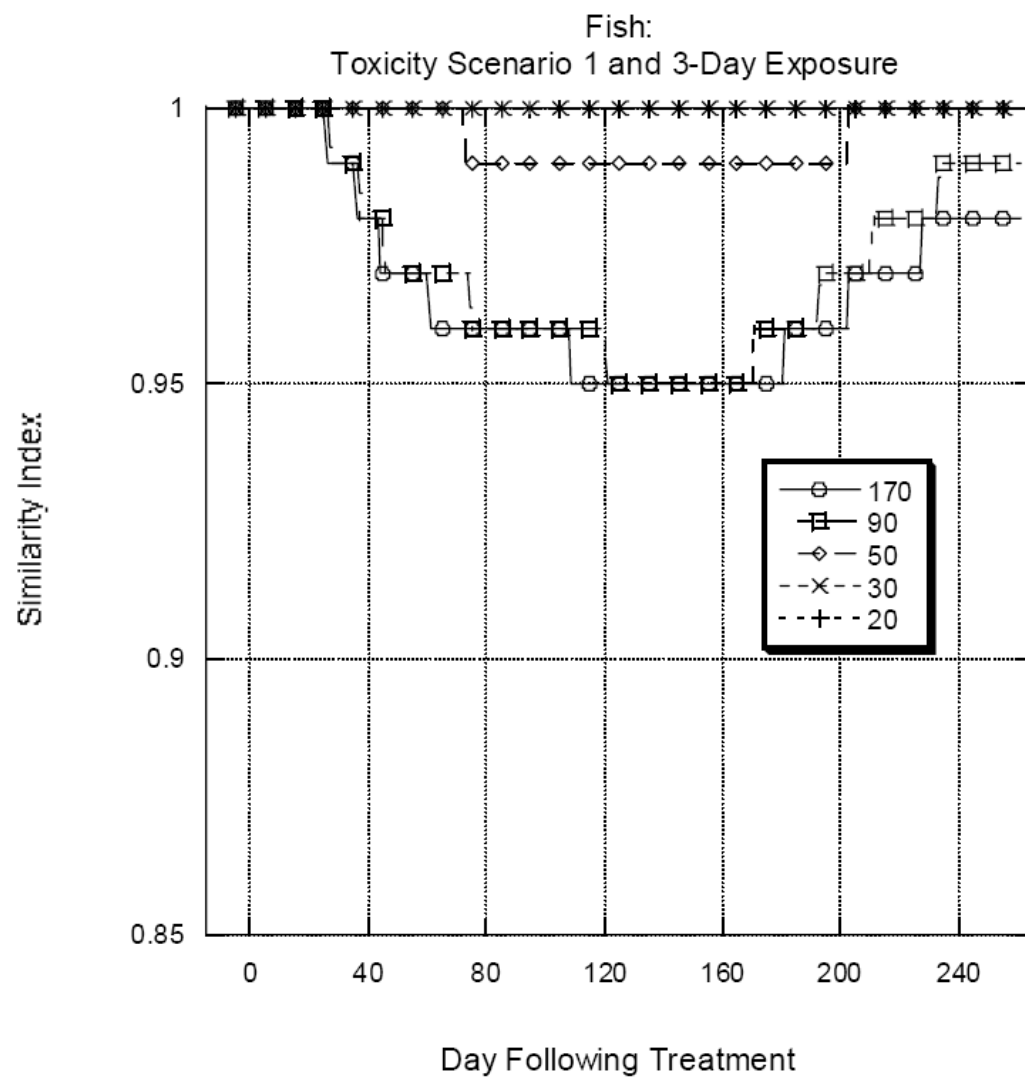




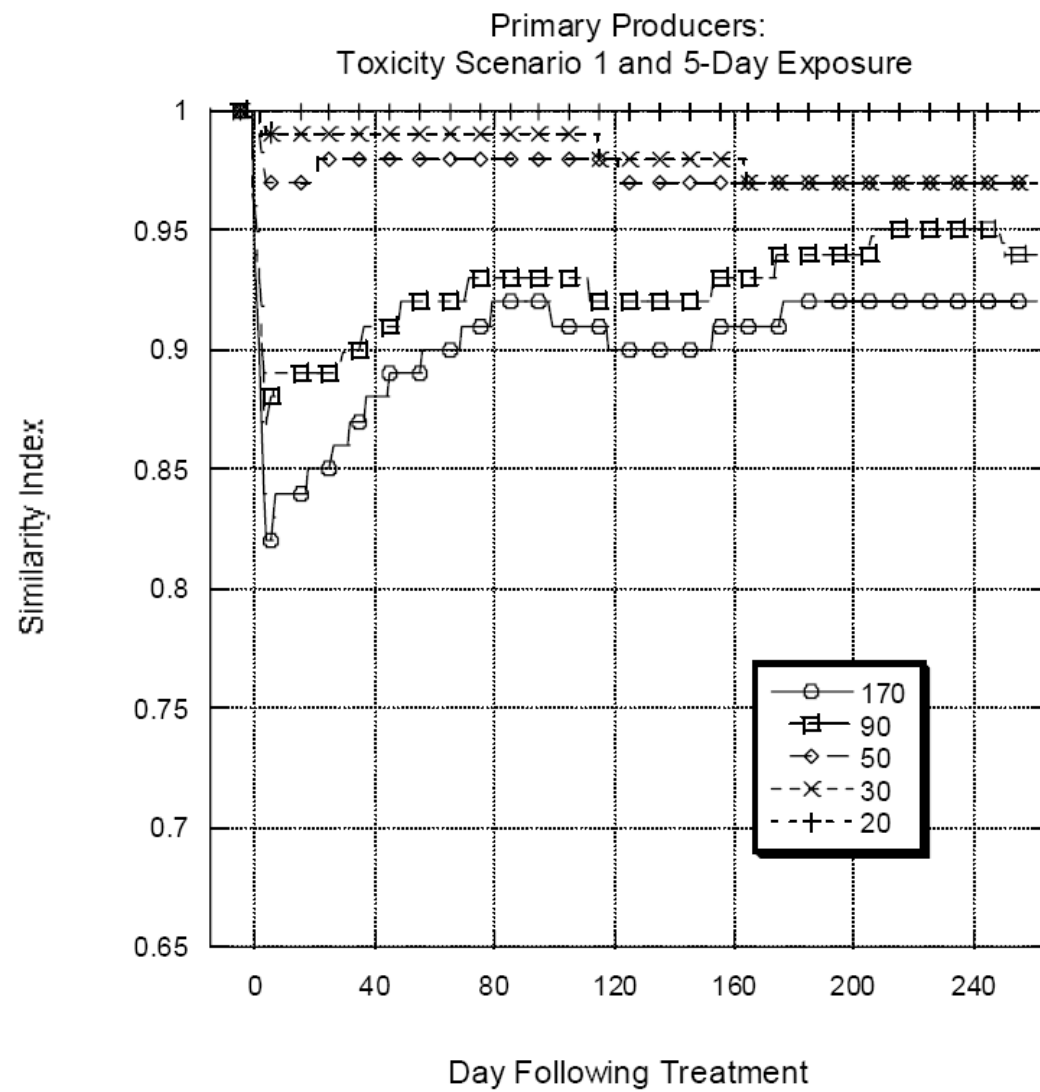
CASM Results: Steinhaus similarity Toxicity Scenario 1 and 3-Day Exposure

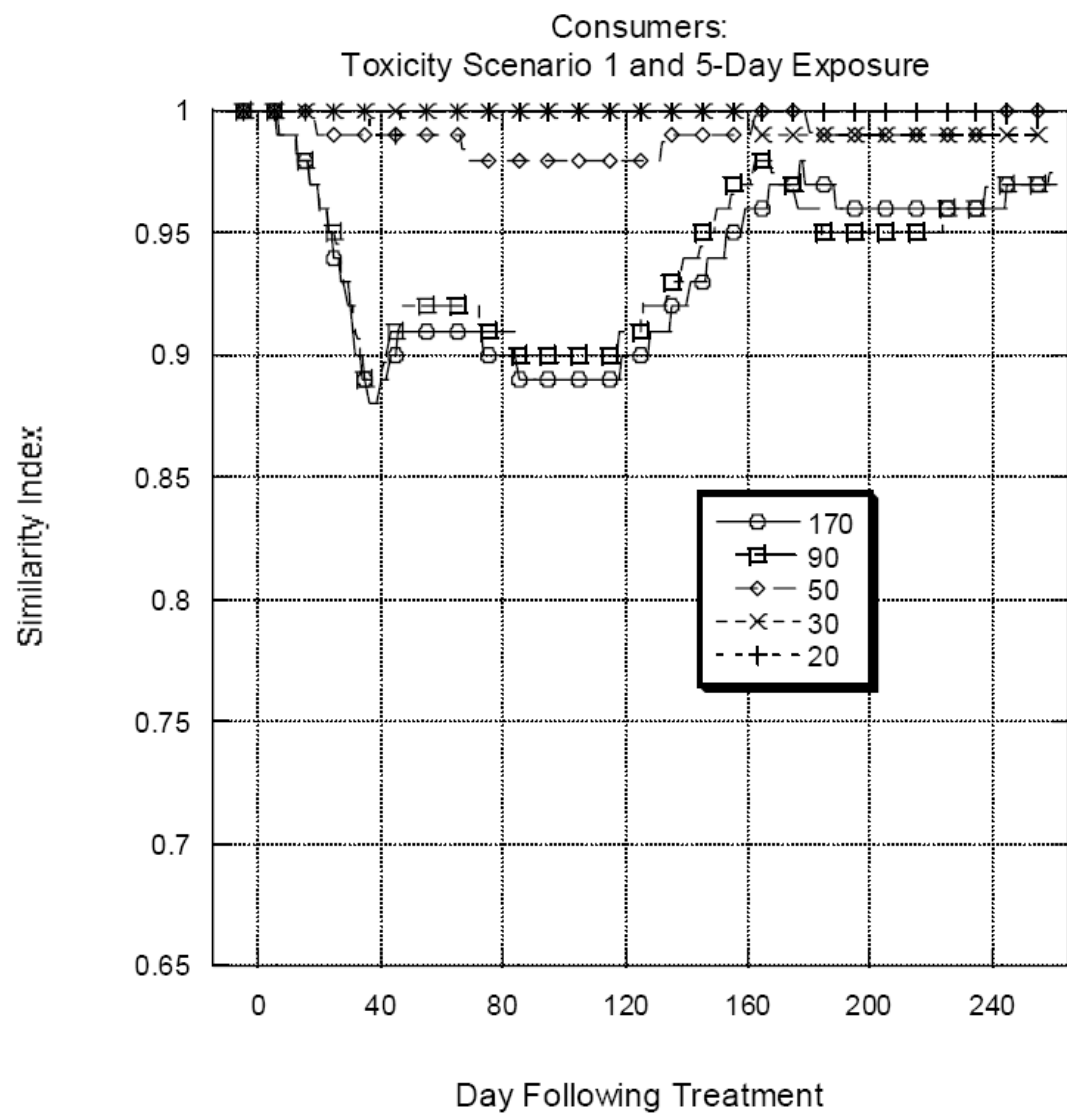


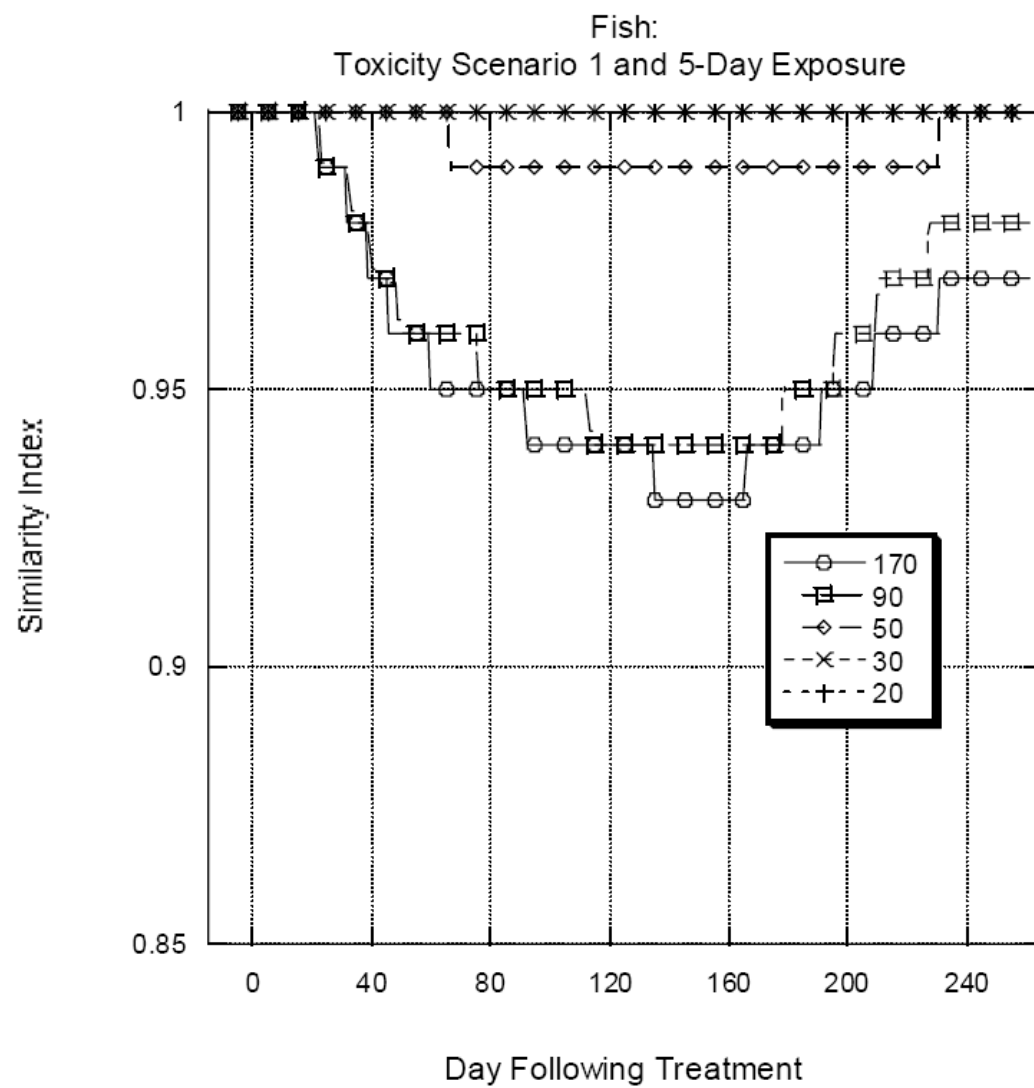




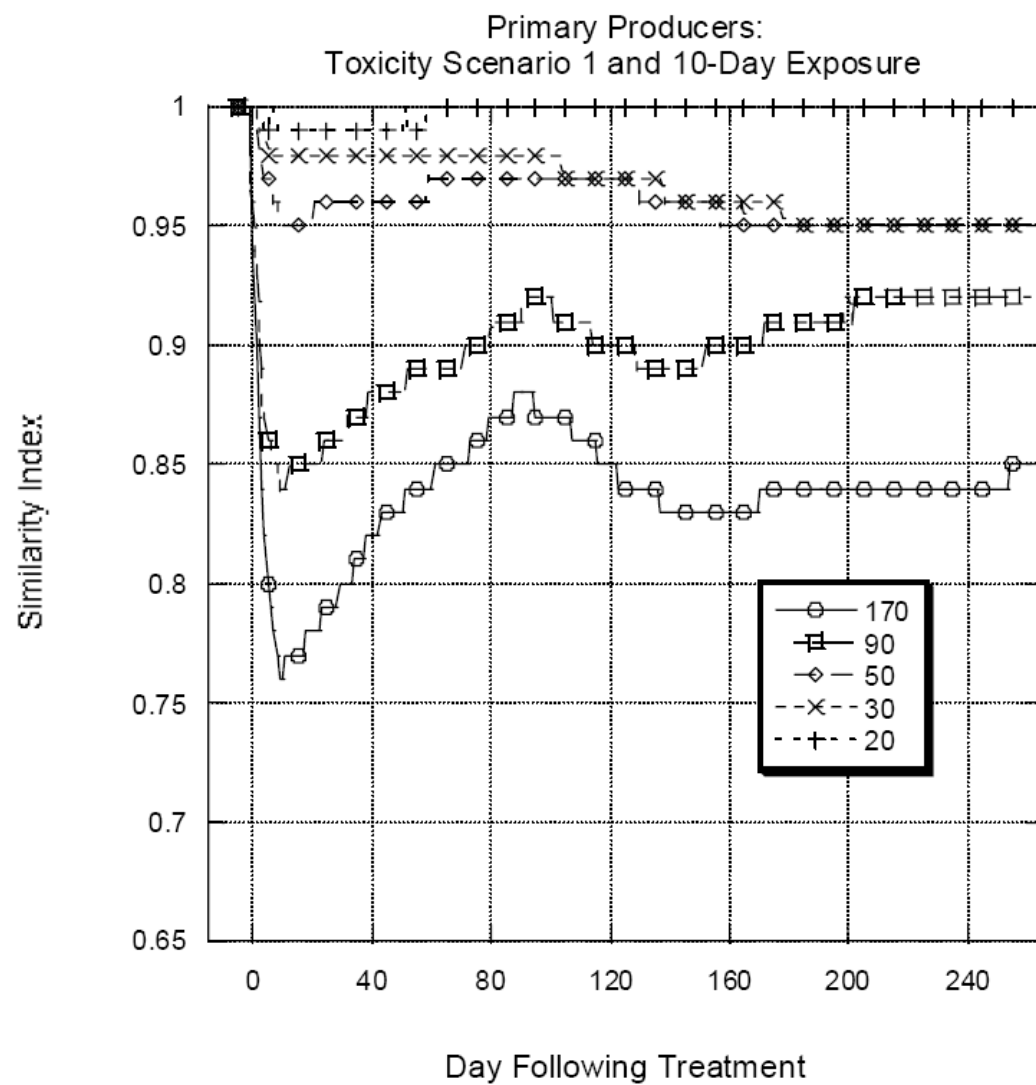
CASM Results: Steinhaus similarity Toxicity Scenario 1 and 5-Day Exposure

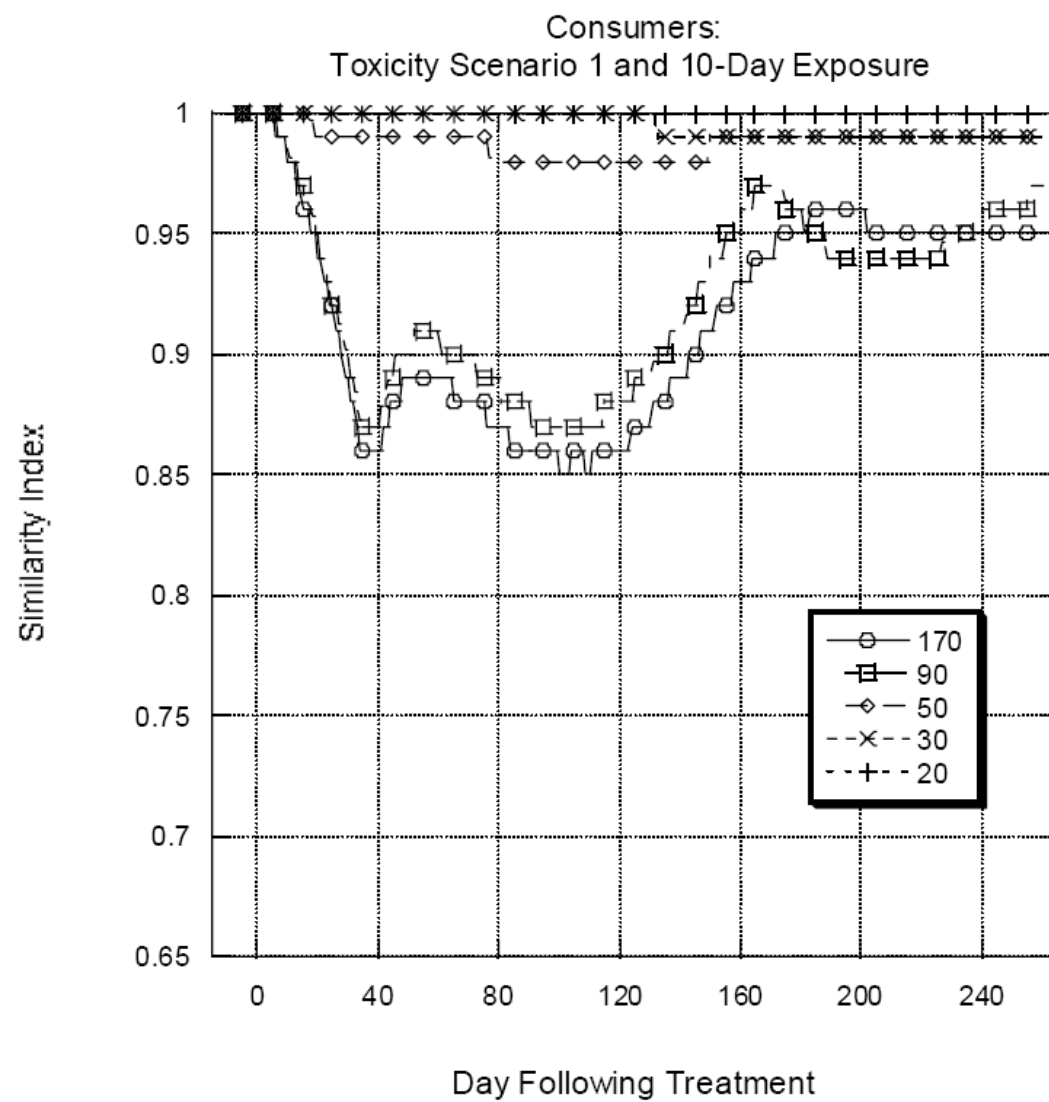


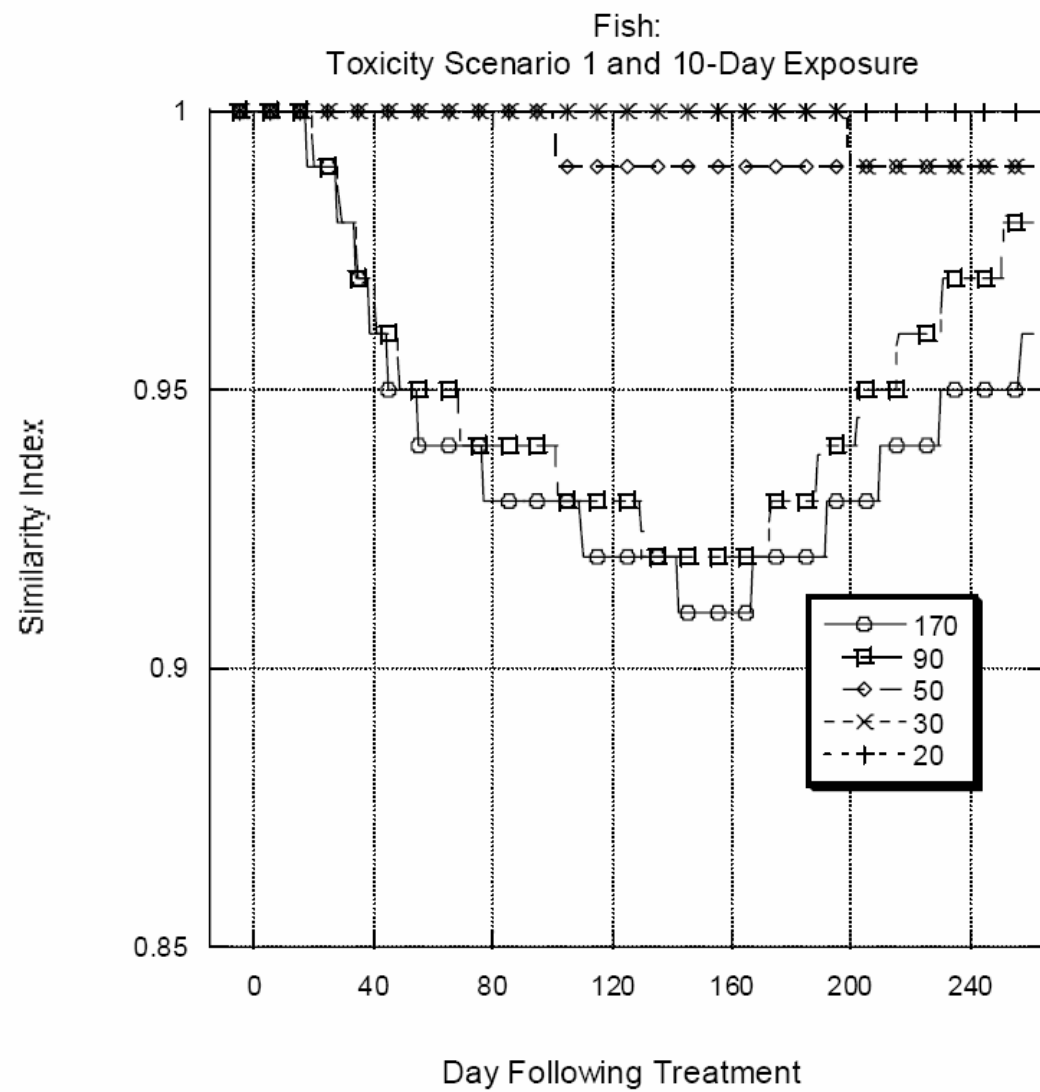




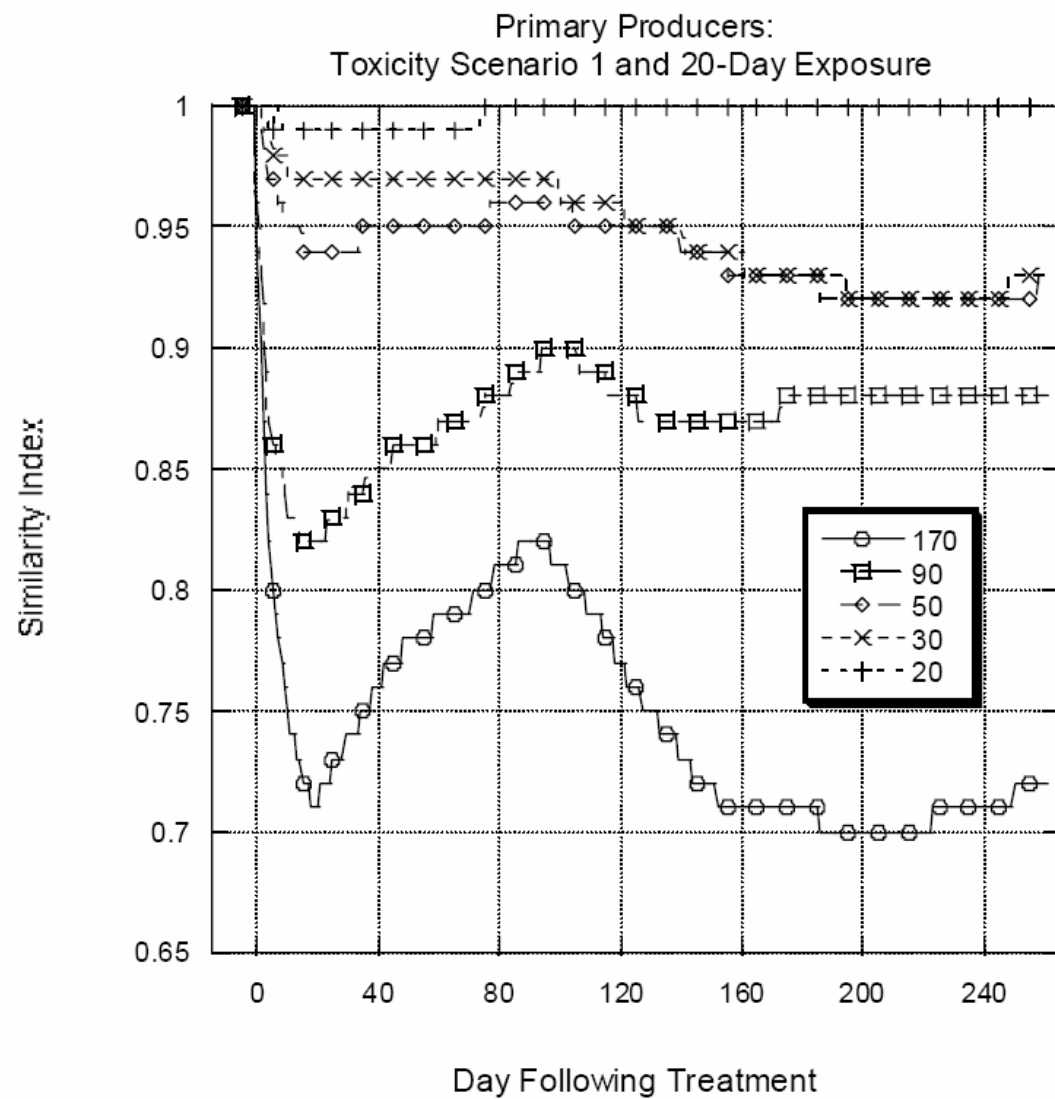
CASM Results: Steinhaus similarity Toxicity Scenario 1 and 10-Day Exposure

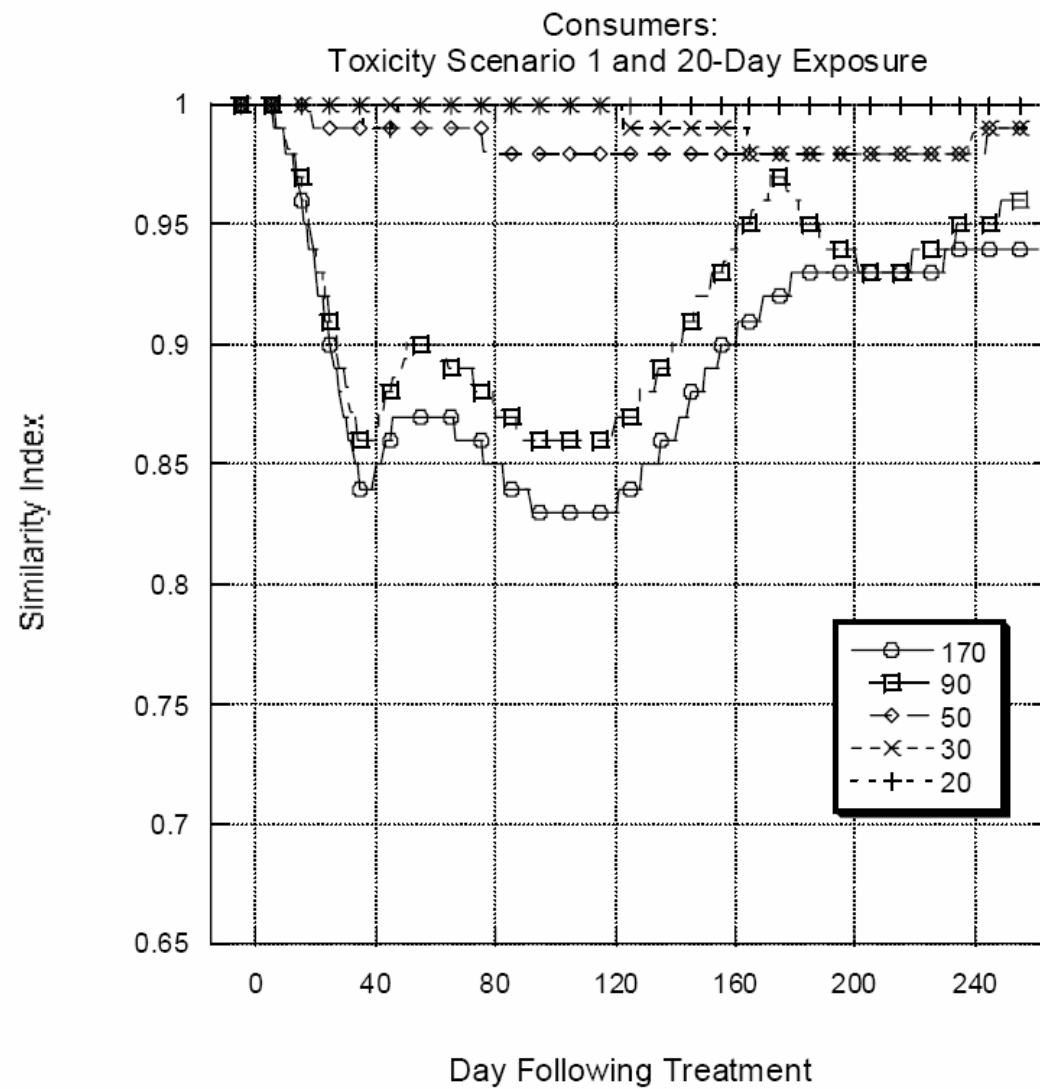


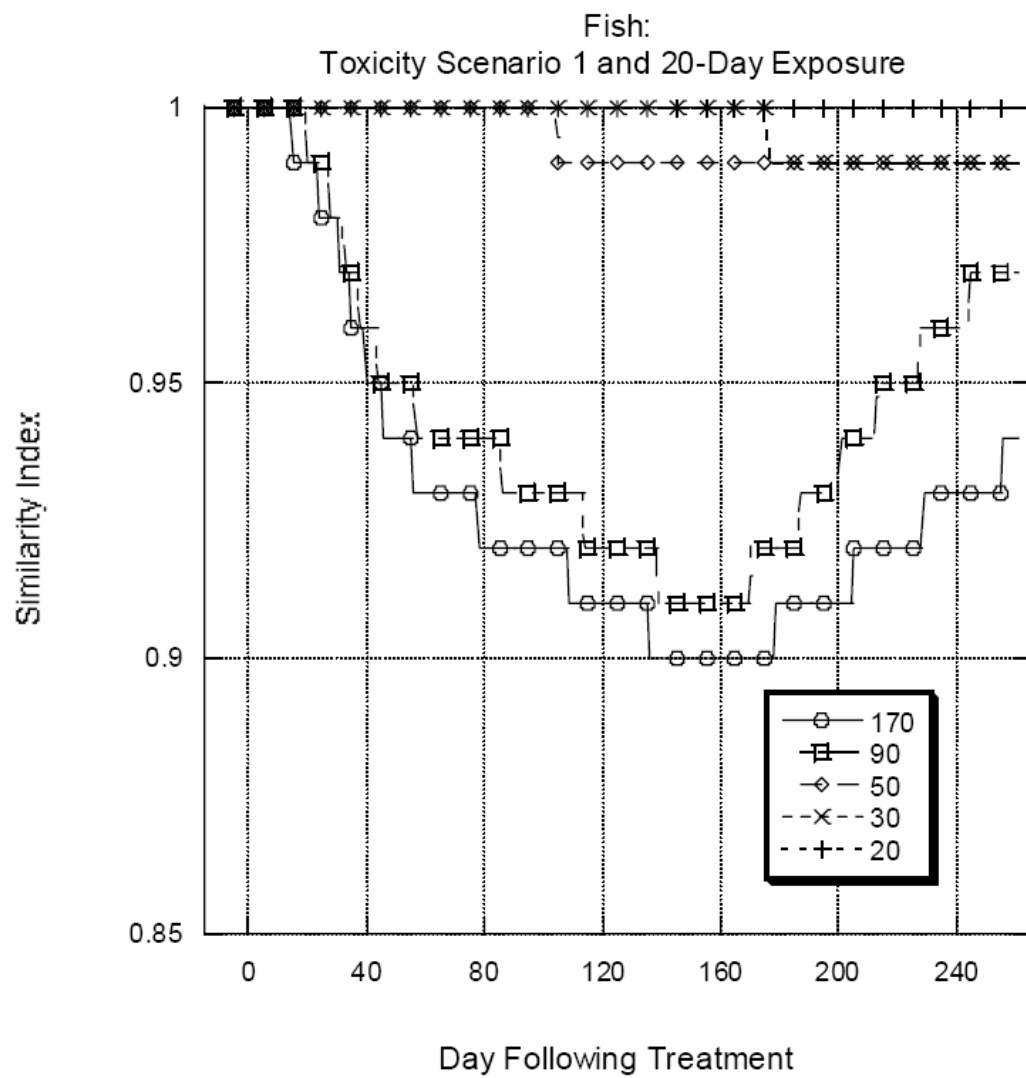




CASM Results: Steinhaus similarity Toxicity Scenario 1 and 20-Day Exposure







Attachment 4: LOC Determination

Appendix 4

Comparison of annual average CASM Steinhaus similarity for a series of chemographs calculated with the Logistic regression vs. actual CASM simulations.

Total no of CASM runs: 128					1	1	1	1	
99th centile: 1		98th 3		negative	6	2	2	4	
95th centile: 6		trig. (%sim): 5		false positive	120	125	125	123	
Data Set					Percent CASM Effect	Trigger [µg/L]			
		14d	30d	60d	90d	37.7 14d AVG	26.8 30d AVG	17.5 60d AVG	11.8 90d AVG
Honey Creek, 1996, daily ppb original		18.0	13.4	7.8	5.5	1.9 correct	correct	correct	correct
Honey Creek, 1996, daily ppb amplified		36.0	26.8	15.5	10.9	3.6 correct	correct	correct	correct
Honey Creek, 1996, scenario1 amplified		30.3	21.9	13.1	9.0	2.5 correct	correct	correct	correct
Honey Creek, 1996, scenario2 amplified		57.7	37.0	20.8	14.1	5.9 correct	correct	correct	correct
Honey Creek, 1996, scenario3 amplified		30.3	23.7	14.1	9.6	2.5 correct	correct	correct	correct
Honey Creek, 1996, scenario4 amplified		30.0	24.2	14.2	9.7	3.3 correct	correct	correct	correct
Honey Creek, 1997, daily ppb original		22.7	13.5	8.9	6.6	2.6 correct	correct	correct	correct
Honey Creek, 1997, daily ppb amplified		45.3	27.0	17.9	13.1	5.7 correct	correct	correct	correct
Honey Creek, 1997, scenario1 amplified		34.3	20.4	14.6	10.4	4.9 correct	correct	correct	correct
Honey Creek, 1997, scenario2 amplified		36.2	26.8	19.2	14.2	3.9 correct	correct	false positive	false positive
Honey Creek, 1997, scenario3 amplified		55.1	32.6	20.7	15.0	6.0 correct	correct	correct	correct
Honey Creek, 1997, scenario4 amplified		54.3	29.9	18.3	12.9	6.3 correct	correct	correct	correct
Honey Creek, 1998, daily ppb original		21.5	12.9	7.1	4.9	1.7 correct	correct	correct	correct
Honey Creek, 1998, daily ppb amplified		43.0	25.8	14.2	9.9	4.4 false positive	correct	correct	correct
Honey Creek, 1998, scenario1 amplified		37.6	23.0	12.6	8.8	2.5 correct	correct	correct	correct
Honey Creek, 1998, scenario2 amplified		50.6	29.2	15.8	11.0	4.5 false positive	false positive	correct	correct
Honey Creek, 1998, scenario3 amplified		45.9	28.1	15.3	10.6	4.4 false positive	false positive	correct	correct
Honey Creek, 1998, scenario4 amplified		36.1	22.6	12.6	8.8	4.9 correct	correct	correct	correct
Honey Creek, 1999, daily ppb original		25.9	18.7	12.6	9.3	2.6 correct	correct	correct	correct
Honey Creek, 1999, daily ppb amplified		51.7	37.5	25.3	18.6	6.3 correct	correct	correct	correct
Honey Creek, 1999, scenario1 amplified		41.3	31.7	22.0	16.0	5.4 correct	correct	correct	correct
Honey Creek, 1999, scenario2 amplified		56.8	44.7	29.1	20.7	7.1 correct	correct	correct	correct
Honey Creek, 1999, scenario3 amplified		64.7	41.8	27.4	19.3	6.4 correct	correct	correct	correct
Honey Creek, 1999, scenario4 amplified		44.4	31.9	22.2	16.0	6.2 correct	correct	correct	correct
Honey Creek, 2000, daily ppb original		11.8	8.0	5.0	3.5	0.0 correct	correct	correct	correct
Honey Creek, 2000, daily ppb amplified		23.7	16.0	9.9	7.0	3.1 correct	correct	correct	correct
Honey Creek, 2000, scenario1 amplified		28.1	18.5	10.8	7.5	4.0 correct	correct	correct	correct
Honey Creek, 2000, scenario2 amplified		22.3	13.8	8.1	5.7	3.3 correct	correct	correct	correct
Honey Creek, 2000, scenario3 amplified		15.9	11.5	8.0	5.5	0.0 correct	correct	correct	correct
Honey Creek, 2000, scenario4 amplified		31.1	20.9	12.9	8.8	4.0 correct	correct	correct	correct
Honey Creek, 2001, daily ppb original		8.0	5.6	3.6	2.6	0.0 correct	correct	correct	correct
Honey Creek, 2001, daily ppb amplified		16.0	11.2	7.2	5.1	7.0 false negative	false negative	false negative	false negative
Honey Creek, 2001, scenario1 amplified		16.1	11.6	7.4	5.2	0.0 correct	correct	correct	correct
Honey Creek, 2001, scenario2 amplified		13.8	10.2	6.7	4.8	0.0 correct	correct	correct	correct
Honey Creek, 2001, scenario3 amplified		17.6	11.7	7.5	5.4	1.4 correct	correct	correct	correct
Honey Creek, 2001, scenario4 amplified		16.2	11.1	7.1	5.1	1.4 correct	correct	correct	correct
Honey Creek, 2002, daily ppb original		43.4	22.0	14.0	9.6	4.2 false positive	correct	correct	correct
Honey Creek, 2002, daily ppb amplified		86.7	44.0	28.1	19.2	6.7 correct	correct	correct	correct
Honey Creek, 2002, scenario1 amplified		91.3	48.4	29.3	20.0	7.4 correct	correct	correct	correct
Honey Creek, 2002, scenario2 amplified		47.7	27.0	17.7	12.3	6.3 correct	correct	correct	correct
Honey Creek, 2002, scenario3 amplified		35.7	22.2	16.9	11.8	4.3 correct	correct	correct	false positive
Honey Creek, 2002, scenario4 amplified		170.7	83.3	48.5	32.8	9.0 correct	correct	correct	correct
Sandusky, 1995 daily ppb original		9.3	8.7	6.2	4.6	0.0 correct	correct	correct	correct
Sandusky, 1996 daily ppb original		12.5	10.6	7.0	5.3	0.0 correct	correct	correct	correct

Sandusky, 1997, daily ppb original	26.1	16.9	11.5	8.3	2.2	correct	correct	correct	correct
Sandusky, 1998, daily ppb original	20.0	12.1	6.8	5.2	1.6	correct	correct	correct	correct
Sandusky, 2000, daily ppb original	10.2	7.1	4.0	2.9	0.0	correct	correct	correct	correct
Sandusky, 2002, daily ppb original	17.3	12.2	8.6	6.1	1.7	correct	correct	correct	correct
Sandusky, 1995 daily ppb amplified	18.6	17.5	12.4	9.1	2.0	correct	correct	correct	correct
Sandusky, 1996 daily ppb amplified	25.0	21.2	14.1	10.6	3.2	correct	correct	correct	correct
Sandusky, 1997, daily ppb amplified	52.3	33.8	23.0	16.6	7.4	correct	correct	correct	correct
Sandusky, 1998, daily ppb amplified	40.1	24.2	13.6	10.4	4.3	false positive	correct	correct	correct
Sandusky, 2000, daily ppb amplified	20.3	14.1	8.0	5.7	2.0	correct	correct	correct	correct
Sandusky, 2002, daily ppb amplified	86.3	61.0	42.8	30.7	10.1	correct	correct	correct	correct
Sandusky, 2002, scenario1 amplified	98.5	65.8	46.9	33.6	10.5	correct	correct	correct	correct
Sandusky, 2002, scenario2 amplified	64.7	49.4	36.3	26.3	10.0	correct	correct	correct	correct
Sandusky, 2002, scenario3 amplified	78.9	62.4	43.0	30.8	10.4	correct	correct	correct	correct
Sandusky, 2002, scenario4 amplified	96.8	64.4	44.9	32.0	10.2	correct	correct	correct	correct
Miami, 1996, original	4.3	3.0	1.9	1.5	0.0	correct	correct	correct	correct
Miami, 1997, original	15.2	8.1	7.5	5.4	3.8	correct	correct	correct	correct
Miami, 1998, original	4.9	3.5	2.5	2.0	0.0	correct	correct	correct	correct
Miami, 2000, original	8.4	4.6	2.6	1.9	0.0	correct	correct	correct	correct
Miami, 2001, original	9.5	6.1	3.6	2.5	0.0	correct	correct	correct	correct
Miami, 1996, amplified	8.7	5.9	3.8	2.9	0.0	correct	correct	correct	correct
Miami, 1997, amplified	30.4	16.3	15.1	10.7	4.2	correct	correct	correct	correct
Miami, 1998, amplified	9.7	7.1	5.1	4.1	0.0	correct	correct	correct	correct
Miami, 2000, amplified	16.8	9.2	5.2	3.8	2.5	correct	correct	correct	correct
Miami, 2001, amplified	19.0	12.1	7.2	5.1	1.7	correct	correct	correct	correct
Maumee, 1996, original	8.8	7.4	5.7	4.3	0.0	correct	correct	correct	correct
Maumee, 1997, original	18.9	15.0	10.6	7.6	1.7	correct	correct	correct	correct
Maumee, 1998, original	16.6	11.3	6.6	5.1	1.3	correct	correct	correct	correct
Maumee, 2000, original	8.0	6.4	4.0	2.9	0.0	correct	correct	correct	correct
Maumee, 2001, original	14.3	9.6	6.2	4.6	0.4	correct	correct	correct	correct
Maumee, 1996, amplified	17.6	14.8	11.4	8.6	1.0	correct	correct	correct	correct
Maumee, 1997, amplified	37.8	29.9	21.1	15.3	6.1	correct	correct	correct	correct
Maumee, 1998, amplified	33.1	22.6	13.2	10.1	3.3	correct	correct	correct	correct
Maumee, 2000, amplified	15.9	12.8	8.0	5.7	0.4	correct	correct	correct	correct
Maumee, 2001, amplified	28.6	19.2	12.4	9.2	3.2	correct	correct	correct	correct
Muskingum, 1996, original	3.8	3.5	2.3	1.6	0.0	correct	correct	correct	correct
Muskingum, 1997, original	5.2	4.3	2.7	2.0	0.0	correct	correct	correct	correct
Muskingum, 1998, original	2.1	1.8	1.3	1.0	0.0	correct	correct	correct	correct
Muskingum, 2000, original	0.9	0.7	0.5	0.5	0.0	correct	correct	correct	correct
Muskingum, 2001, original	5.9	3.3	2.3	1.6	0.0	correct	correct	correct	correct
Muskingum, 1996, amplified	7.5	7.1	4.6	3.3	0.0	correct	correct	correct	correct
Muskingum, 1997, amplified	10.5	8.7	5.3	4.0	0.0	correct	correct	correct	correct
Muskingum, 1998, amplified	4.2	3.6	2.6	2.0	0.0	correct	correct	correct	correct
Muskingum, 2000, amplified	1.7	1.4	1.0	0.9	0.0	correct	correct	correct	correct
Muskingum, 2001, amplified	11.9	6.5	4.6	3.2	0.0	correct	correct	correct	correct
Raisin, 1996, original	7.9	4.4	2.4	1.8	1.0	correct	correct	correct	correct
Raisin, 1997, original	39.0	19.7	10.9	8.0	4.3	false positive	correct	correct	correct
Raisin, 1998, original	2.7	1.9	1.3	1.0	0.0	correct	correct	correct	correct
Raisin, 2000, original	2.2	2.0	1.4	1.1	0.0	correct	correct	correct	correct
Raisin, 2001, original	10.8	6.2	3.5	2.5	0.8	correct	correct	correct	correct
Raisin, 1996, amplified	15.8	8.9	4.9	3.6	1.1	correct	correct	correct	correct
Raisin, 1997, amplified	77.9	39.5	21.9	16.1	8.4	correct	correct	correct	correct
Raisin, 1998, amplified	5.5	3.8	2.5	2.0	0.0	correct	correct	correct	correct
Raisin, 2000, amplified	4.5	4.0	2.9	2.1	0.0	correct	correct	correct	correct
Raisin, 2001, amplified	21.5	12.4	7.0	4.9	3.0	correct	correct	correct	correct
Rock Creek, 1996, original	10.5	7.5	4.3	2.9	0.7	correct	correct	correct	correct

Rock Creek, 1997, original	16.3	11.1	8.0	5.9	2.1	correct	correct	correct	correct
Rock Creek, 1998, original	17.1	10.1	5.5	3.8	1.2	correct	correct	correct	correct
Rock Creek, 2000, original	10.7	6.4	3.4	2.4	0.4	correct	correct	correct	correct
Rock Creek, 2001, original	10.0	6.2	3.5	2.4	0.0	correct	correct	correct	correct
Rock Creek, 1996, amplified	21.1	15.1	8.5	5.8	2.3	correct	correct	correct	correct
Rock Creek, 1997, amplified	32.6	22.3	16.0	11.8	4.8	correct	correct	correct	false positive
Rock Creek, 1998, amplified	34.1	20.2	11.0	7.6	3.7	correct	correct	correct	correct
Rock Creek, 2000, amplified	21.5	12.9	6.9	4.7	2.4	correct	correct	correct	correct
Rock Creek, 2001, amplified	19.9	12.4	6.9	4.9	2.0	correct	correct	correct	correct
Scioto, 1996, original	7.5	6.1	4.3	3.2	0.0	correct	correct	correct	correct
Scioto, 1997, original	15.7	11.2	9.1	6.7	0.0	correct	correct	correct	correct
Scioto, 1998, original	6.6	5.0	3.5	2.8	0.0	correct	correct	correct	correct
Scioto, 2000, original	5.1	3.3	2.1	1.6	0.0	correct	correct	correct	correct
Scioto, 2001, original	12.0	8.4	5.3	3.9	0.0	correct	correct	correct	correct
Scioto, 1996, amplified	15.0	12.2	8.6	6.4	0.0	correct	correct	correct	correct
Scioto, 1997, amplified	31.5	22.3	18.2	13.5	3.8	correct	correct	false positive	false positive
Scioto, 1998, amplified	13.1	9.9	6.9	5.6	0.0	correct	correct	correct	correct
Scioto, 2000, amplified	10.1	6.6	4.2	3.2	0.0	correct	correct	correct	correct
Scioto, 2001, amplified	24.1	16.8	10.6	7.7	2.8	correct	correct	correct	correct
Lost Creek, 1987, original	12.5	9.1	6.9	5.6	0.7	correct	correct	correct	correct
Lost Creek, 1989, original	4.9	3.0	1.8	1.3	0.0	correct	correct	correct	correct
Lost Creek, 1990, original	8.3	4.8	4.0	3.0	0.0	correct	correct	correct	correct
Lost Creek, 1992, original	3.3	1.6	0.9	0.6	0.0	correct	correct	correct	correct
Lost Creek, 1993, original	10.2	7.2	4.6	3.3	0.4	correct	correct	correct	correct
Lost Creek, 1987, amplified	25.1	18.2	13.8	11.3	4.2	correct	correct	correct	correct
Lost Creek, 1989, amplified	9.8	6.1	3.5	2.7	0.0	correct	correct	correct	correct
Lost Creek, 1990, amplified	16.5	9.7	7.9	6.0	1.9	correct	correct	correct	correct
Lost Creek, 1992, amplified	6.6	3.2	1.8	1.2	0.5	correct	correct	correct	correct
Lost Creek, 1993, amplified	20.4	14.3	9.2	6.5	2.1	correct	correct	correct	correct

Attachment 5: Percent Biomass Reduction for 3 Toxicity Scenarios

Appendix 5

Comparison of simulated change in annual production for phytoplankton, periphyton, macrophytes, zooplankton, benthic invertebrates, and fish for CASM parameterizations using the geometric mean values of EC₅₀ (toxicity scenario 1), the 90th centile (toxicity scenario 2) and the 10th centile (toxicity scenario 3) of the EC₅₀ values.

Table 1. Estimated percent decreases ^a in total annual production of phytoplankton (264 g C m ⁻²) for a generic 2 nd -3 rd order Midwestern stream.					
	Atrazine concentration (µg/L)				
Exposure (days) ^b	20	30	50	90	170
1	0.3 ^c (0.2 – 1.2) ^d	0.1 (0.1 – 1.1)	1.5 (1.5 – 7.5)	12.5 (7.0 – 12.5)	13.8 (8.2 – 14.1)
3	0.9 (0.6 – 2.6)	0.4 (0.3 – 2.1)	1.6 (1.8 – 12.3)	21.9 (12.3 – 21.9)	24.2 (14.8 – 24.8)
5	2.3 (1.3 – 3.9)	1.0 (0.4 – 2.7)	1.5 (1.8 – 14.1)	25.9 (14.8 – 25.9)	28.5 (17.8 – 29.2)
10	1.2 (0.8 – 2.9)	1.1 (2.2 – 0.8)	1.8 (0.9 – 14.4)	29.3 (16.5 – 29.3)	32.0 (19.5 – 32.7)
20	2.1 (0 – 3.9)	0.2 (1.5 – 1.6)	2.0 (1.0 – 14.6)	30.0 (17.4 – 30.0)	33.0 (21.3 – 33.9)
60	2.5 (0.3 – 4.3)	0.1 (1.2 – 1.9)	2.8 (1.8 – 13.1)	24.8 (15.9 – 24.8)	33.3 (21.4 – 33.9)
130	2.5 (0.3 – 4.3)	0.1 (1.2 – 1.9)	7.9 (9.6 – 0.1)	22.7 (2.9 – 23.9)	33.3 (21.4 – 33.9)
260	2.5 (0.3 – 4.3)	0.1 (1.2 – 1.9)	9.5 (11.3 – 1.7)	27.2 (7.6 – 28.4)	33.3 (21.4 – 33.9)

^aBased on the mean values of 100 Monte Carlo simulations using the Comprehensive Aquatic Systems Model (CASM)
^bConsecutive days of constant exposure beginning on model day 105 (April 15)
^cResults using the geometric mean values of EC₅₀ assigned to modeled populations (Toxicity Scenario 1)
^dResults using the 90th and 10th percentile estimates of the geometric mean of the EC₅₀ values (Toxicity Scenarios 3 and 2)

Table 2. Estimated percent decreases ^a in total annual production of periphyton (5,124 g C m ⁻²) for a generic 2 nd -3 rd order Midwestern stream.					
	Atrazine concentration (µg/L)				
Exposure (days) ^b	20	30	50	90	170
1	0.1 ^c (0.1 – 0) ^d	0.1 (0.1 – 0)	0.1 (0.1 – 2.7)	3.8 (1.3 – 3.8)	3.8 (1.4 – 3.7)
3	0 (0.1 – 0.2)	0.2 (0.2 – 0)	0.5 (0.5 – 5.4)	7.8 (3.5 – 7.9)	7.6 (3.4 – 7.2)
5	0.1 (0.2 – 0.1)	0.4 (0.6 – 0.3)	1.2 (1.1 – 7.2)	10.5 (5.2 – 10.6)	10.0 (5.1 – 9.5)
10	0.2 (0.3 – 0.3)	0.6 (0.9 – 0.5)	2.1 (1.9 – 9.5)	14.7 (8.0 – 14.8)	13.5 (7.6 – 12.4)
20	0.1 (0.3 – 0.2)	0.8 (1.1 – 0.7)	2.8 (2.6 – 10.8)	16.7 (9.7 – 16.9)	14.7 (8.9 – 12.4)
60	0.1 (0.7 – 0)	1.4 (1.8 – 1.4)	4.3 (4.1 – 12.4)	19.2 (11.9 – 19.5)	14.4 (10.1 – 7.4)
130	0.3 (0.8 – 0.2)	1.7 (2.1 – 1.7)	5.0 (4.9 – 13.5)	21.7 (13.3 – 22.0)	12.8 (10.2 – 2.5)
260	0.5 (1.0 – 0.4)	2.0 (2.4 – 2.0)	5.5 (5.4 – 14.0)	22.3 (13.8 – 22.6)	13.0 (10.7 – 5.5)

^aBased on the mean values of 100 Monte Carlo simulations using the Comprehensive Aquatic Systems Model (CASM)
^bConsecutive days of constant exposure beginning on model day 105 (April 15)
^cResults using the geometric mean values of EC₅₀ assigned to modeled populations (Toxicity Scenario 1)
^dResults using the 90th and 10th percentile estimates of the geometric mean of the EC₅₀ values (Toxicity Scenarios 3 and 2)

Table 3. Estimated percent decreases ^a in total annual production of macrophytes (8,478 g C m ⁻²) for a generic 2 nd -3 rd order Midwestern stream.					
Exposure (days) ^b	Atrazine concentration (µg/L)				
	20	30	50	90	170
1	0 ^c (0 – 0) ^d	0.3 (0.3 – 0.3)	0.2 (0.2 – 0.4)	1.4 (-0.4 ^e – 1.0)	0.6 (0.8 – 0.6)
3	0 (0 – 0)	1.0 (1.0 – 1.0)	0.7 (0.7 – 0)	2.4 (0.5 – 1.3)	2.9 (2.8 – 2.9)
5	0 (0 – 0)	1.5 (1.5 – 1.5)	1.2 (1.2 – 0.7)	2.8 (0.4 – 1.1)	5.3 (4.5 – 5.3)
10	0.2 (0.2 – 0.2)	2.7 (2.7 – 2.7)	2.4 (2.4 – 2.6)	2.8 (0.3 – 0)	10.5 (8.2 – 10.5)
20	0.2 (0.1 – 0.2)	4.0 (3.9 – 4.0)	3.7 (3.7 – 5.0)	2.0 (1.3 – 2.2)	17.5 (12.6 – 17.6)
60	0.2 (0.1 – 0.2)	5.5 (5.5 – 5.5)	5.6 (5.5 – 8.4)	0.3 (3.4 – 5.5)	27.7 (18.1 – 28.0)
130	0.2 (0.1 – 0.2)	6.0 (5.9 – 6.0)	6.1 (6.1 – 9.4)	0.3 (4.0 – 6.5)	29.9 (18.3 – 30.4)
260	0.2 (0.1 – 0.2)	6.1 (6.1 – 6.1)	6.2 (6.2 – 9.6)	0.4 (4.1 – 6.7)	27.2 (15.2 – 27.5)

^aBased on the mean values of 100 Monte Carlo simulations using the Comprehensive Aquatic Systems Model (CASIM)
^bConsecutive days of constant exposure beginning on model day 105 (April 15)
^cResults using the geometric mean values of EC₅₀ assigned to modeled populations (Toxicity Scenario 1)
^dResults using the 90th and 10th percentile estimates of the geometric mean of the EC₅₀ values (Toxicity Scenarios 3 and 2)
^eValues in italics indicate percent increase in total annual production

Table 4. Estimated percent decreases ^a in total annual production of zooplankton (19 g C m ⁻²) for a generic 2 nd -3 rd order Midwestern stream.					
Exposure (days) ^b	Atrazine concentration (µg/L)				
	20	30	50	90	170
1	0.2 ^c (0.2 – 1.6) ^d	0.1 (0.1 – 1.5)	2.2 (2.3 – 8.0)	12.4 (6.7 – 12.4)	13.2 (7.3 – 13.4)
3	0.8 (0.5 – 3.3)	0.3 (0.1 – 2.8)	2.9 (3.1 – 13.9)	23.4 (11.6 – 23.4)	25.2 (13.0 – 25.6)
5	2.1 (1.0 – 4.7)	0.8 (0.2 – 3.5)	3.0 (3.3 – 16.4)	29.3 (14.1 – 29.3)	31.8 (16.0 – 32.6)
10	0.8 (1.5 – 3.9)	1.44 ^e (2.9 – 1.6)	0.3 (0.7 – 17.5)	36.1 (15.9 – 36.1)	39.4 (17.2 – 40.6)
20	2.3 (0.5 – 5.2)	0.4 (2.02 – 2.7)	0.5 (0.7 – 18.1)	39.0 (17.3 – 38.9)	43.5 (19.7 – 44.8)
60	3.3 (0.5 – 6.3)	0.5 (1.2 – 3.4)	2.1 (0.8 – 15.9)	34.3 (14.5 – 34.2)	43.3 (19.8 – 44.9)
130	3.3 (0.5 – 6.3)	0.5 (1.2 – 3.4)	3.0 (2.1 – 13.6)	24.2 (10.6 – 24.9)	43.3 (19.8 – 44.9)
260	3.3 (0.5 – 6.3)	0.5 (1.2 – 3.4)	2.8 (1.9 – 13.8)	24.6 (11.2 – 25.5)	43.3 (19.8 – 44.9)

^aBased on the mean values of 100 Monte Carlo simulations using the Comprehensive Aquatic Systems Model (CASIM)
^bConsecutive days of constant exposure beginning on model day 105 (April 15)
^cResults using the geometric mean values of EC₅₀ assigned to modeled populations (Toxicity Scenario 1)
^dResults using the 90th and 10th percentile estimates of the geometric mean of the EC₅₀ values (Toxicity Scenarios 3 and 2)
^eValues in italics indicate percent increase in total annual production

Table 5. Estimated percent decrease ^a in total annual production of benthic invertebrates (371 g C m ⁻²) for a generic 2 nd -3 rd order Midwestern stream.					
Exposure (days) ^b	Atrazine concentration (µg/L)				
	20	30	50	90	170
1	0.1 (0.1 – 0)	0.4 (0.4 – 0.2)	0.3 (0.3 – 1.5)	1.6 (0.8 – 1.8)	2.6 (1.4 – 2.6)
3	0 (0 – 0.1 ^c)	0.7 (0.7 – 0.5)	1.0 (1.0 – 3.5)	3.5 (2.1 – 4.1)	6.1 (3.8 – 5.9)
5	0 (0.1 – 0.2)	1.1 (1.2 – 1.0)	1.7 (1.7 – 4.9)	4.9 (3.2 – 5.8)	8.9 (5.9 – 8.5)
10	0 (0.3 – 0.1)	1.9 (2.1 – 1.8)	3.1 (2.9 – 7.5)	7.5 (5.4 – 9.1)	13.9 (9.7 – 13.4)
20	0.1 (0.3 – 0.1)	2.8 (2.9 – 2.7)	4.3 (4.2 – 9.8)	9.3 (7.2 – 11.8)	19.2 (13.2 – 18.0)
60	0.3 (0.6 – 0.1)	4.3 (4.5 – 4.2)	6.6 (6.5 – 13.3)	12.7 (10.2 – 16.2)	26.2 (17.5 – 22.5)
130	0.4 (0.7 – 0.3)	4.9 (5.2 – 4.9)	7.9 (8.0 – 15.6)	17.7 (12.9 – 21.4)	28.1 (17.9 – 23.8)
260	0.6 (0.9 – 0.5)	5.2 (5.4 – 5.1)	8.3 (8.5 – 16.1)	18.5 (13.5 – 22.1)	31.3 (19.7 – 33.4)
^a Based on the mean values of 100 Monte Carlo simulations using the Comprehensive Aquatic Systems Model (CASM)					
^b Consecutive days of constant exposure beginning on model day 105 (April 15)					
^c Results using the geometric mean values of EC ₅₀ assigned to modeled populations (Toxicity Scenario 1)					
^d Results using the 90 th and 10 th percentile estimates of the geometric mean of the EC ₅₀ values (Toxicity Scenarios 3 and 2)					
^e Values in italics indicate percent increase in total annual production					