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Review of EPA's Preliminary Ecological Risk Assessment for Atrazine

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REVIEW OF EPA'S ECOLOGICAL RISK ASSESSMENT FOR ATRAZINE

FINAL REPORT

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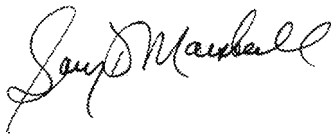

Gary Marshall
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October 4, 2016

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EXECUTIVE SUMMARY

Atrazine is a selective triazine herbicide used for pre-emergent control of many broadleaf and grass weeds in corn, sorghum, sugarcane, and other row and field crops. The United States Environmental Protection Agency (EPA) recently released a draft ecological risk assessment (ERA) for atrazine as part of the registration review process. The draft ERA was overly conservative and contained numerous scientific deficiencies. At the request of the Triazine Network, we reviewed and critiqued the methods and assumptions used in the ERA.

The main issues pertained to data quality and selection, errors, hyper-conservative assumptions, inadequate consideration of the best available science, and the procedures used to calculate the level of concern (LOC) for aquatic plants. In several instances, EPA (2016a) ignored the advice and recommendations provided by five separate Scientific Advisory Panels (SAP). The main issues of the report are summarized below:

- The interpretation of the monitoring data contained a number of shortcomings. Most importantly, a very large dataset currently exists that targeted the most vulnerable watersheds and high use areas. This dataset, the Atrazine Ecological Monitoring Program (AEMP), is a targeted monitoring program required by EPA with daily or near-daily samples and minimal uncertainty of missing peaks. However, EPA (2016a) opted to include a far broader range of available datasets, despite low sampling frequencies, errors, and other issues (e.g., high detection limits). In their interpretation of the data, EPA (2016a) did not account for errors in reporting, duplicate entries, infilling errors for periods when samples were not available, and use of non-detect samples with levels of detection much higher than their proposed LOC.
- EPA (2016a) also used highly conservative modeling to estimate aquatic exposure concentrations. Modeled exposure concentrations were as much as 260-fold higher than corresponding monitoring data.
- Despite the advice and recommendations of SAPs (2009, 2012) and other independent data reviews (Giddings, 2012; Moore et al., 2015, 2016), EPA (2016a) used low quality endpoints that were not scientifically defensible. EPA (2016a) established an LOC for aquatic plants of 3.4 µg/L despite evidence showing no significant effects at concentrations less than 30 µg/L when acceptable studies only are considered. Therefore, EPA's LOC is an order of magnitude lower than the lowest reliable effects concentrations and is overly conservative.
- The effects metrics selected by EPA (2016a) often did not represent the best available data. For example, EPA (2016a) used a flawed (as noted in its own data evaluation record; Bryan et al., 2014) Japanese medaka chronic exposure study (Papoulias et al., 2014) to generate chronic effects metrics for both freshwater and marine fish. This study had a number of limitations including no true negative control, high female to male ratio, low fecundity, and high control mortality. As a result, an updated medaka study was submitted by Syngenta that followed standard testing guidelines and had improved methods (Schneider et al., 2015). This study was available at the time the ERA was being drafted and was more appropriate for use. In addition, a marine fish study was available for atrazine (Cafarella, 2005 [MRID 46648203]). No scientific justification was provided for using a freshwater fish study to interpret risks to marine fish, particularly given the availability of a high-quality marine study.

- To estimate risks to aquatic-phase amphibians, EPA (2016a) ignored previous conclusions of no effects and instead relied on a large dataset primarily comprised of very low quality data where effects were often not observed. Conclusions drawn from that dataset are highly uncertain and likely inaccurate.
- The screening-level wildlife assessment relied on highly conservative assumptions, including wildlife obtaining 100% of their diet from treated fields, wildlife having homogeneous diets several of which are implausible (e.g., 20 g birds foraging only on short grass or broadleaf foliage), outdated food ingestion rate calculations, default body weights that were not representative of the study animals, and a default foliar degradation half-life that was two times higher than the longest half-life observed in the field for atrazine.
- The refined avian assessment also had a number of shortcomings and, further, was not extended to mammals, despite findings of potential risk in the screening-level assessment. For birds, the dermal exposure calculations relied on data for other far more toxic pesticide classes and likely greatly over-estimated the contribution of dermal exposure. EPA (2016a) also opted for worst-case values in determining fraction of time spent on fields and fraction of pesticide retained, rather than using best available data for atrazine.

The above and other issues led to overestimates of acute and chronic risks of atrazine to aquatic and terrestrial biota. In this report, we have made a number of recommendations that would improve EPA's ecological risk assessment for atrazine.

TABLE OF CONTENTS

Page

1.0 INTRODUCTION

Atrazine is a selective triazine herbicide. It was first registered by the United States Department of Agriculture (USDA) in 1958 (SAP, 2012) and is currently used extensively in the United States for pre-emergent control of many broadleaf and grass weeds in corn, sorghum, sugarcane, and other row and field crops, including Christmas tree plantations and conifer forests. Atrazine is applied to over 90% of corn crops in the US and 65% of sorghum and sugarcane crops (EPA, 2016a).

The Environmental Protection Agency (EPA, 2016a) recently released a Preliminary Ecological Risk Assessment for Atrazine. EPA focused their assessment on the parent compound, as degradates have equal or lesser toxicity to terrestrial and aquatic receptors (EPA, 2016a). EPA's standard toolbox of conservative models was used, including the Surface Water Concentration Calculator (SWCC) for aquatic exposure analysis, T-REX, TIM, MCnest, and AEMP monitoring data.

EPA concluded that risks to aquatic and flowering plant communities in heavy use areas are likely and chronic risks to fish, aquatic-phase amphibians and aquatic invertebrates are also likely in high use areas. Risks to birds and mammals are primarily related to chronic exposure.

The Triazine Network retained Intrinsik Environmental Sciences (US), Inc. (Intrinsik) to review and evaluate the preliminary atrazine ERA. The Triazine Network is concerned with a number of effects metrics relied on by EPA (2016a), exposure methods used, and procedures used to calculate the level of concern for aquatic plants. In addition, EPA (2016a) ignored much of the advice and recommendations provided by five separate Scientific Advisory Panels (SAP). The SAPs were comprised of members picked by EPA and were developed to provide expert guidance to EPA on atrazine issues.

Five meetings of SAP members have evaluated the available data for atrazine and offered recommendations and provided direction to EPA to improve future assessments (SAP, 2003, 2007, 2009, 2011, 2012). The SAPs focused on data quality and use of best available methods for estimating risk. Discussion points included the evaluation process for identifying high quality data, the potential for gonadal effects on larval amphibians from atrazine exposure, developing benchmark water quality criteria that are protective of aquatic plants, and initiation of a water monitoring program. The SAPs have identified a number of concerns with EPA's methods for developing a community-level level of concern (LOC) for aquatic plants. The SAPs highlighted incorrect scoring of effects data, use of low quality, unacceptable studies, and poor methods for derivation of the LOC. The SAPs also offered recommendations for improvements to future LOC estimations. However, EPA (2016a) failed to acknowledge most of the recommendations and used poor data and methods to calculate an LOC. A detailed evaluation of the current LOC proposed by EPA (2016a) is presented in this report.

The Triazine Network has also taken issue with the data and methods used in the preliminary ERA (EPA, 2016a) to generate risk conclusions for aquatic and terrestrial receptors. For example, EPA (2016a) used outdated effects metrics for fish and terrestrial plants, when new studies have been performed using current products and improved methods. Similar patterns were noted in the derivation of avian and mammalian effects metrics, and the reliance on invalid study data for aquatic-phase amphibians.

The purpose of this report is to highlight the areas where improvements are needed in the ERA for atrazine.

2.0 AQUATIC RECEPTORS

2.1 Monitoring Data

Since 2004, EPA has overseen the conduct of an extensive monitoring program known as the Atrazine Ecological Monitoring Program (AEMP) in corn and sorghum growing areas and, for a time, in sugarcane growing areas. The program has targeted the most vulnerable watersheds in areas of high atrazine use with daily or near daily sampling of atrazine concentrations. There have also been a large number of monitoring studies conducted for atrazine outside of the AEMP. Many non-AEMP monitoring datasets had low sampling frequencies that prevented an understanding of daily variation in atrazine concentrations. To deal with this deficiency, data infilling was used by EPA (2016a) to create daily chemographs. Incorrect infilling, however, created many errors, particularly in those datasets that had the minimum required sample size of 4 but had less than 12 samples per year and in datasets with no samples for large portions of the year prior to initial sampling. Concerns identified in the interpretation of the monitoring data are reviewed below:

- In each monitoring dataset, EPA projected the first sampling date back to January 1. In cases where the first sampling date also represented the peak concentration (i.e., maximum concentration observed over an entire year), it was assumed that the peak concentration was achieved for the entire time period between January 1 and the first sampling date. When more than 60 days spanned the infilling period, the extrapolation resulted in a 60-day average concentration equal to the peak daily concentration. However, daily monitoring data (e.g., as in the AEMP) indicate that atrazine concentrations rapidly decrease after an initial peak corresponding to the application date(s). Therefore, a 60-day average concentration would and should be much less than the peak concentration observed.
- When only one data point was available for a 60-day period, EPA (2016a) used this single data point to calculate a maximum 60-day average. Again, this resulted in a 60-day average concentration that equaled the peak concentration. This error occurred in 53% of datasets with four to 11 samples per year.
- Extensive data quality errors were identified in the monitoring database used in the ERA.
    - EPA (2016a) incorporated 9,899 duplicates in 42,941 site years of data. Therefore, 23% of the data points used by EPA in their analyses represented duplicate samples. Duplicate occurrences were not removed by EPA prior to their analyses.
    - Duplicate samples have identical latitude and longitude coordinates, and sample time and date stamps. Thus, removal of the duplicate samples should have been an easy exercise.
- EPA (2016a) retained data samples that had levels of detection (LOD) many-fold higher than the level of concern (LOC) that would be protective of aquatic plants as estimated by EPA (2016a).
    - For samples below the level of detection (LOD), EPA (2016a) assigned a concentration of half the LOD to those samples. In one monitoring dataset, the LOD was 100 μg/L (ppb). Samples with concentrations below this LOD were automatically reported as 50 μg/L in the ERA, well above EPA's LOC of 3.4 μg/L. As a result, EPA (2016a) assumed that locations with concentrations reported as

50 µg/L were at risk from atrazine exposure even though atrazine was not detected at these locations.
    - Atrazine monitoring programs routinely have levels of detection of <0.1 µg/L. Clearly, datasets with levels of detection that are 4 or more orders of magnitude higher than routine levels of detection should have been excluded from EPA's analysis of available monitoring data. Such datasets are of unacceptable scientific quality.
- Errors in reporting analytical units.
    - Samples with known unit reporting errors were found in EPA's draft ecological risk assessment for atrazine. For example, samples originally reported as ng/L were erroneously cited in EPA's assessment with the same numeric value but in units of µg/L.

EPA should have relied solely on the AEMP in its analysis of available monitoring data. The AEMP is a targeted monitoring program required by EPA in their 2003 interim registration document for atrazine. The program was designed to sample the most vulnerable agricultural headwater drainage basins in the Midwestern and southern United States with the highest atrazine use. The 70 selected watersheds represent the top 20th percentile for vulnerability to runoff as predicted by EPA's watershed regressions model for pesticides (WARP). The AEMP database contains samples from daily or near-daily sampling efforts between 2004 and 2015. The program has determined worst-case exposure with minimal uncertainty of missing peak concentrations because of the daily frequency of sampling over multiple years.

2.2 Aquatic Exposure Modeling

Despite the availability of the comprehensive AEMP dataset for atrazine (i.e., 288 site-years of daily or nearly daily data from 70 most vulnerable watersheds between 2004 and 2015), EPA (2016a) used their Surface Water Concentration Calculator (SWCC) model to predict atrazine concentrations for a variety of regions and use patterns. According to EPA (2016a), the SWCC model scenarios are intended to be conservative and represent the 90th percentile most vulnerable sites for first-order streams and static water bodies adjacent to atrazine use areas.

However, the SWCC significantly over-predicted the 1-in-10-year peak daily and 60-day average concentrations from all available monitoring data by as much as 260-fold. In considering the most vulnerable watershed sampled in the AEMP, the SWCC over-predicted 1-in-10-year peak daily and 60-day average concentrations by 12-fold.

EPA's SWCC and other screening-level models should not be used to make regulatory decisions. Adequate, targeted monitoring data exist that can be used to support atrazine use. To evaluate specific use patterns, EPA should consider using more refined watershed models such as the Soil Water Assessment Tool (SWAT).

EPA's standard screening-level models are not designed to identify the specific geographical locations where atrazine might truly pose a risk to aquatic organisms, a seemingly more useful approach for decision making. Refined watershed level modeling is a superior approach for such assessments because it provides exposure predictions at a fine geographical resolution with the ability to simultaneously simulate varying site-specific weather, soil, environmental, and cropping conditions within a watershed. Watershed modeling also simulates variation in pesticide application timing, and proximity of treated fields to surface water bodies and riparian

areas. Moreover, watershed level modeling simulates flowing and non-flowing water bodies as well as site-specific hydrologic conditions, water body depths and water body geometries. Examples of available watershed models that could have been used in the atrazine risk assessment include the SWAT, the Agricultural Policy EXtension (APEX) model, the Hydrologic Simulation Program-Fortran (HSPF) model and the Pesticide Root Zone Model – RIVerine Water Quality (PRZM-RIVWQ) model. All of these models, particularly the SWAT model, have undergone validation testing and have been shown to perform well.

Details on the aquatic exposure modeling effort are further discussed in a response document prepared by Waterborne on behalf of Syngenta that was submitted to the docket in October, 2016.

2.3 Derivation of a Level of Concern

As an herbicide, plants are expected to be more sensitive to atrazine than other receptor groups. Therefore, to ensure that atrazine concentrations in watersheds will not cause ecologically-significant effects to aquatic plant communities, EPA (2016a) developed a community-level level of concern (LOC). The LOC was compared to monitoring data to determine which watersheds have atrazine concentrations that could cause adverse effects to aquatic plants. Although the LOC was designed to be protective of aquatic plants communities, it's conservative nature was expected to also make it protective of other receptor communities (e.g., fish, invertebrates), which are less sensitive to atrazine exposure.

In the last several years, EPA has made multiple attempts to define a level of concern for community-level effects of atrazine to aquatic plants. Except for the most recent LOC of 3.4 μg/L in the draft EPA (2016a) assessment, the proposed LOCs have been thoroughly reviewed by EPA's own Scientific Advisory Panels (SAP, 2007, 2009, 2012). Notably, none of EPA's proposed LOCs have been formally accepted or endorsed. In fact, the latest SAP (2012) review strongly recommended increasing the proposed LOC of 4-7 μg/L (EPA, 2007) because many of the mesocosm studies purportedly showing effects at low concentrations were either of unacceptable quality or did not actually cause effects of ecological significance. In its draft assessment, however, EPA (2016a) ignored the advice of the SAP (2012) and the recommendations of other reviewers (e.g., Giddings, 2012; Moore et al., 2015, 2016) by continuing to use poor quality mesocosm study results to derive the lowest LOC that has been proposed to date. In the sections that follow, we comment on two major aspects of EPA's development of an LOC for atrazine, i.e., data selection and method of derivation.

2.3.1 Data Selection

The evaluation of available cosm studies and scoring of results are critical to the calculation of the level of concern (SAP, 2012). EPA (2016a) performed a preliminary screen to reject studies that evaluated mixtures or multi-active ingredients, studies that did not report exposure concentrations, studies that did not evaluate an aquatic plant community, and studies not presented in English. Studies meeting the preliminary criteria were further evaluated and rated as invalid if they did not contain basic elements, including use of controls and at least two replicates per treatment group (EPA, 2016a).

The SAP (2012) recommended evaluating cosm studies using a standard set of scoring criteria. The SAP (2009, 2012) also recommended that EPA re-evaluate and re-score all cosm studies

where effects were observed at concentrations less than 30 µg/L because of weaknesses in study design and data interpretation (see also Giddings, 2012). The SAP (2012) identified a number of studies that were incorrectly scored by EPA as having effects when in fact, no effects were observed during the studies or the studies were clearly of poor quality. Further, the SAP (2012) requested that the LOC be recalculated once the studies were re-scored.

In particular, the SAP (2012) recommended that 11 studies be re-evaluated. Those studies were re-evaluated by EPA (2016a), Giddings (2012) and Moore et al. (2015, 2016). Additional studies published since the SAP (2012) report were also evaluated. The results of the various evaluations are summarized below.

Lampert et al., 1989 [MRID 47543511]

Lampert et al. (1989) performed enclosure experiments with a natural plankton community. The communities were housed in plastic bags suspended in Lake Schohsee in northern West Germany. Bags were 1 meter by 2.70 meters in size and contained 1.70 m^3 of water. Bags were filled with lake water screened with 100 µm mesh then inoculated with zooplankton collected with a plankton net. Two bags per concentration were treated with atrazine dissolved in ethanol at concentrations of 0.1, 1, 10, and 100 µg/L and two bags were left untreated. The experiment lasted "no longer than three weeks". More than 90% of applied atrazine was measured 18 days post-application at concentrations of 1 µg/L and higher, but atrazine was not detected after 10 days in the 0.1 µg/L treatment. Similar results were found for all treatments. However, the study authors proposed an effect concentration of 1 µg/L and a NOEC of 0.1 µg/L.

EPA (2008) developed a Data Evaluation Record (DER) for Lampert et al. (1989). The DER highlighted the incomplete study design and lack of description of protocols. The DER critiqued the two replicate study design and lack of statistical analysis. Further, the DER notes that the bag enclosures may have inhibited mixing and recolonization, thus limiting their applicability to the natural environment. The DER concluded that the experiment did "not provide a realistic simulation of environmental conditions". The DER concluded that the study had extremely limited utility and should only be applied within the weight of evidence when conducting a risk assessment (EPA, 2008).

The SAP (2009, 2012) recommended excluding this study because the solvent used to deliver the atrazine likely caused a shift from an algal-dominant community to a bacteria-dominant community. Specifically, the use of high concentrations of ethanol increased bacterial growth and respiration in the enclosures, which led to decreased primary productivity. Further, ethanol was added to treatment enclosures, but not to controls, which prevented comparison of treatment-related effects.

Giddings (2012) also rejected the Lampert et al. (1989) study because of the solvent issue. Effects were first observed seven days after treatment, which is not consistent with atrazine's mode of action, where effects to productivity occur quickly. In addition, the study design did not include solvent controls to rule out solvent-related effects. Giddings (2012) went on to quote Brock et al. (2000), where it was stated that the Lampert et al. (1989) study is a prime example of "sometimes enormous effects" of ethanol on dissolved oxygen concentrations and other cosm properties.

Moore et al. (2015, 2016) rejected the study because of the solvent issue, no recovery period was included, and the low dissolved oxygen concentrations likely contributed to the observed

delayed effects. Additionally, the description of the study methodology was sparse, statistics were not reported, there was limited control and treatment replication, values of most physical-chemical properties (e.g., water temperature, air temperature, water pH, and conductivity) were not reported, and the study was not conducted following any international guidelines. No clear treatment-related effects were observed during the study.

Despite the extensive support for excluding the study, EPA (2016a) retained this study, asserting that the applied ethanol would have adequately vaporized and biodegraded, and thus was not responsible for observed effects on primary productivity. EPA (2016a) reclassified the endpoint as "effect", and actually lowered the effect level to 0.1 µg/L. However, given the reasoning above and the very poor study quality, this study should be rejected from use. Further, no treatment-related effects were observed at any test concentration (0.1 to 100 µg/L), and no effect level should have been designated.

deNoyelles et al., 1982

deNoyelles et al. (1982) treated experimental ponds with 20 or 500 µg/L of atrazine (CO-OP liquid, 41% a.i.). Two ponds per treatment concentration and two control ponds were maintained for 136 days. Ponds were 0.045 ha (0.11 acres) in size and filled with water and plankton from a nearby well, 50 bluegill sunfish (predator), 20 channel catfish (benthic omnivore), and seven gizzard shad (filtering omnivore). The authors reported that phytoplankton growth was depressed in all treatment ponds, followed by changes in species composition.

EPA (2009a) evaluated deNoyelles et al. (1982) for study quality, design and relevance of conclusions. EPA (2009a) was critical of the lack of statistical analyses reported by the study authors for some data comparisons. EPA (2009a) also criticized the limited replication (n = 2) and limited number of test concentrations (n = 2), "unclear presentation of results", and lack of pre-test analytical analyses of test solutions to confirm no presence of residual atrazine. Additionally, aquatic communities were not evaluated to confirm similarities among ponds. EPA (2009a) determined that data from deNoyelles et al. (1982) should be applied qualitatively only.

The SAP (2012) was critical of the effects level estimated by deNoyelles et al. (1982). Although minor effects on biomass and ^{14}C uptake in phytoplankton were observed at 20 µg/L, the confidence intervals overlapped with control levels. Additionally, survival of gizzard shad was not measured and differential survival among treatments may have caused the observed effects on biomass. Thus, the SAP (2012) concluded that the treatment level of 20 µg/L should be categorized as "no effect".

Giddings (2012) re-evaluated the raw data presented by deNoyelles et al. (1982) and Kettle (1987). Giddings (2012) found that only one of two control cosms demonstrated an algal bloom and phytoplankton biomass in one control pond was lower than the 20 µg/L treatment ponds. Re-analysis of the data found no significant difference between control and 20 µg/L treatment ponds. Therefore, 20 µg/L should not be designated as the effect level.

Moore et al. (2015, 2016) rejected the study because there was no recovery phase (70% of original concentration detected at Day 136), study methodology was not adequately described, a concentration-response relationship could not be determined because there were only two treatment concentrations, there was inconsistent shad and carp survival between controls, replication was limited (n = 2), no statistically significant responses were detected shortly after exposure began, and the study did not follow any internationally-recognized guidelines.

EPA (2016a) re-classified this endpoint at 20 µg/L as an "effect". Therefore, EPA (2016a) incorrectly scored this unacceptable study as having an effect at 20 µg/L and used the endpoint in their calculation of an LOC.

deNoyelles et al., 1989

deNoyelles et al. (1989) simulated exposure of pond communities to atrazine. Ponds were 0.04 ha in size and located at the University of Kansas Nelson Environmental Study Area near Lawrence, KS. Ponds were filled with well water and left undisturbed for one year to establish benthic plant and animal communities. Prior to test initiation, ponds were refilled with well water and stocked with gizzard shad (n = 10), channel catfish (n = 20) and bluegill sunfish (n = 50). After the first year, grass carp were also added (n = 4). Atrazine (reagent grade, 97% a.i. and CO-OP Liquid Atrazine, 41% a.i.) was applied once annually to the surface water of the ponds at concentrations of 20, 100, 200, and 500 µg a.i./L and ponds were observed for 805 days. Six and 12 months after each application, the concentration of atrazine had decreased to 70 and 25% of applied, respectively.

The authors reported that phytoplankton production and biomass were reduced compared to controls for all treatment levels, but recovered by three weeks. Zooplankton, a predatory planktonic insect (*Chaoborus*), channel catfish, and filter-feeding fish (gizzard shad) did not experience indirect effects to survival from a reduction in phytoplankton biomass. However, submerged and emergent macrophytes experienced significant declines in biomass and did not recover. Tadpoles, benthic insect grazers, grass carp, and bluegill sunfish experienced indirect effects as a result of the decline in macrophyte biomass. The authors assigned an effect concentration of 20 µg a.i./L.

The SAP (2009, 2012) was critical of the high stocking density of grass carp (30 fish/ha) and hypothesized that the high density of fish led to observed reductions in aquatic plant biomass. In fact, the SAP (2009, 2012) stated that the effect concentration of 20 µg/L was not treatment-related, but a result of carp grazing and should be re-categorized as "no effect". Additionally, no grass carp were present in one control replicate, which likely led to increased macrophyte biomass in that control replicate.

Criticisms by Giddings (2012) of the deNoyelles et al. (1982) study were also applicable to the deNoyelles et al. (1989) because one study was the extension of the other. High variability among replicates, particularly among control cosms, was of greatest concern. Giddings (2012) evaluated all study data and proposed "no effect" at 20 µg/L because the results were largely inconclusive.

Moore et al. (2015, 2016) rejected this study because there was no recovery phase (70% of original concentration after 6 months), sampling was only performed once during the first treatment year (or other sampling results were not reported), treatments occurred annually, there was no clear concentration-response relationship, timing of measured effects on macrophytes was not reported, and many study condition parameters were not reported.

EPA (2016a) combined the data from deNoyelles et al. (1982) and deNoyelles et al. (1989) to generate one effects endpoint (20 µg/L) for use in calculation of their LOC. However, given the high loading rate of grass carp, inconsistencies of results among control ponds, and study ratings of unacceptable, neither study produced a confident effect concentration and should not have been considered in the derivation of the LOC.

Carney and deNoyelles, 1986

This study was referred to in the main body of EPA's (2016a) draft risk assessment, but was referenced as Carney (1983) in the attachment EPA posted in the atrazine docket. The posted attachment, however, appears to be a document submitted in 2011, prior to the updates suggested by the SAP (2012). Carney (1983) is a master's thesis performed in the same laboratory (University of Kansas) as Carney and deNoyelles (1986). It is unclear how EPA (2016a) used the data from Carney and deNoyelles (1986) and/or Carney (1983) in the calculation of their LOC.

Carney (1983) investigated the effects of atrazine and grass carp on a freshwater macrophyte community. Atrazine (CO-OP liquid; 41% a.i.) was applied annually for two years to static ponds at concentrations of 20, 100, 200, and 500 µg/L. Measured concentrations were 69 to 104% of nominal at the second year of the study. In the first year of the study, there was no clear concentration-response relationship for macrophyte density, but some evidence for effects to community structure at 100 and 200 µg/L. In the second year of the study, there was an apparent concentration-response relationship for macrophyte density. The author reported that atrazine had significant effects on species composition within the macrophyte community, ultimately leading to a domination of Charophytes in the 100 µg/L pond. The author also observed declines in periphyton and emergent macrophyte biomass.

The SAP (2012) criticized the Carney and deNoyelles (1986) study because of the high stocking density of grass carp (20 carp/acre) and notable loss of fish in the control pond. According to the SAP (2012), common testing guidance recommends a stocking density of 2 fish per acre for grass carp to reduce effects from grazing carp. This is an order of magnitude fewer fish than stocked by Carney and deNoyelles (1986) and resulted in complete denudation of macrophytes from one control pond.

Giddings (2012) criticized the addition of grass carp in year two. In the first year of the study, no significant differences were found between control and 20 µg/L ponds. However, after addition of grass carp, differences were observed. In protected areas of the ponds, where no grass carp were permitted, no differences in macrophyte biomass were observed between the control and 20 µg/L treatment in any study year. Therefore, Giddings (2012) concluded that the effects concentration of 20 µg/L should be reclassified as "no effect".

Moore et al. (2015, 2016) rated the Carney (1983) study as not relevant because a recovery phase was not included (69-104% original concentration in second year of study). The study was also scored as unacceptable or supplemental because it only included two replicates, two to four exposure concentrations were evaluated, the study was not performed according to a recognized international guideline, sampling only occurred once in the first year and/or application dates were not provided, no concentration-response was observed during the first year, and there was potential for other stressors in the treated ponds.

Although it is unclear how EPA (2016a) used the Carney and deNoyelles (1986) and Carney (1983) data, we believe that EPA (2016a) maintained an effects concentration of 20 µg/L. However, the effects reported by the study authors at 20 µg/L are likely the result of compounding factors induced by the high stocking density of grass carp in the second year of the study and the lack of consistency among controls. Therefore, the effects concentration of 20 µg/L should be re-categorized as "no effect" or, better yet, completely excluded from the evaluation.

Dewey, 1986

In this study, atrazine was applied to 0.045 ha ponds at the University of Kansas Nelson Environmental Study Area at concentrations of 20, 100, and 500 µg/L. Ponds were stocked with bluegill, gizzard shad, channel catfish, and grass carp, as well as a natural aquatic plant and insect community present in the storage reservoir. Structure of the aquatic insect community was measured using partially submerged funnel emergence traps. The author noted a decrease in macrophyte production with increasing atrazine concentration, and an herbicide resistance of *Chara* sp. up to 100 µg/L. However, no statistical analyses of macrophyte biomass were reported. An effect level of 20 µg/L was estimated based on a significant reduction in emerging insects, insect abundance, and benthic insect species richness, none of which apply to aquatic plants.

The SAP (2012) rejected Dewey (1986) because the presence of high stocking densities of grass carp, gizzard shad, channel catfish, and bluegill likely contributed additional stress to the cosm system. No data on survival of fish were reported to confirm lack of compounding stress on aquatic plant community.

Giddings (2012) noted that the focus of the study was on insect emergence and bluegill reproduction endpoints, not effects on primary producers. Therefore, the study should not be used to calculate an LOC for an aquatic plant community.

Moore et al. (2015, 2016) rejected this study because statistically-significant direct effects on primary productivity were not reported. Further, the study was not conducted according to an internationally recognized guideline, survival of primary producers and fish in controls were not reported, there was high variability among controls, there were only two replicates, many study parameters were not measured and/or reported, and a clear concentration-response relationship for aquatic plants was not demonstrated.

Although it is unclear how EPA (2016a) used the data from Dewey (1986) in their calculation of an LOC, it appears that EPA (2016a) used an effects concentration of 20 µg/L. This effects concentration is inappropriate for use in determining an LOC for an aquatic plant community because it did not evaluate primary productivity, rather it evaluated the effects of atrazine on benthic invertebrates. This study should be rejected from use.

Kettle et al., 1987

Kettle et al. (1987) applied atrazine (CO-OP liquid, 40.8% a.i.) at concentrations of 20 or 500 µg/L to 0.045 ha ponds at the Nelson Environmental Study Area, Lawrence, KS. The ponds were stocked with 50 bluegill, 20 channel catfish, and seven gizzard shad obtained from local ponds and reservoirs. Approximately 70% of nominal concentrations were detected after 136 days. There were no significant differences in mortality of fish among treatments, and analysis of stomach contents of bluegill revealed significantly fewer prey items per stomach compared to bluegill in control ponds. Additionally, the number of retrieved bluegill young per pond was significantly reduced in treated ponds compared to control ponds. Visual observations revealed 60 and 90% declines in macrophyte biomass two months after application in the 20 and 500 µg/L ponds, respectively. No statistical analyses were performed on macrophyte biomass or primary productivity. The authors explicitly stated that the results from the plankton exposures were presented in deNoyelles et al. (1982) (discussed above), and the Kettle et al. (1987) study only presented results for macrophytes and bluegill populations. Moreover, Kettle et al. (1987)

propose an effect concentration for fish of 20 µg/L, but no effect concentration for aquatic plants.

The SAP (2009, 2012) criticized the high stocking densities of fish and the focus on fish survival, rather than effects on primary producers. The SAP (2012) also referenced deNoyelles et al. (1989), a related study that had zero grass carp survival in controls. It is apparent that the studies performed at the Nelson Environmental Study Area were performed in conjunction with one another and had the same flaws. Therefore, low grass carp survival in controls compared to treatment ponds would have positively impacted the availability of plant biomass in controls and negatively affected any comparisons made to control ponds.

Giddings (2012) rejected this study based on a lack of detail on study methods and lack of results for macrophytes. Giddings (2012) compared the Kettle et al. (1987) results to other studies performed at the Nelson Environmental Study Area and found inconsistencies in the results, with no effects at 20 µg/L reported by Carney (1983) and deNoyelles et al. (1982). Further, Carney (1983) and deNoyelles et al. (1982) referred to the macrophyte communities as "sparse" at the start of testing.

Finally, the study was rated not relevant by Moore et al. (2015, 2016) because it focused on fish instead of aquatic plants, there was no quantitative analysis of macrophyte abundance or species richness, and there was no recovery period (70% of original concentration detected at the end of the post-exposure period). The study was rated as unacceptable because it lacked information on study methods, community composition in controls was different from treatment cosms at test start, and the study did not demonstrate a concentration-response relationship for aquatic plants.

It is unclear how EPA (2016a) used data from Kettle et al. (1987) in the calculation of their LOC. However, based solely on the lack of quantitative analysis for primary producers, the data are inappropriate for use in determining a community-level LOC for aquatic plants. The study also lacks a number of crucial study details, and high stocking densities of fish coupled with inconsistencies in community composition among ponds at the start of the test make this study unacceptable for use in risk assessment.

Detenbeck et al., 1996

Detenbeck et al. (1996) treated wetland mesocosms with increasing concentrations of atrazine to simulate Midwestern surface waters during spring runoff. Experimental streams were located at the Monticello Ecological Research Station in Monticello, MN, and supplied with water from the Mississippi River. Flow-through wetland cosms were 230 m in length and contained four pools and four riffles. Stream flow was 76 L/min. Organic debris was allowed to accumulate over the gravel substrate and streams were populated through planting and natural succession. Two wetlands served as treatment cosms and two were controls. Commercial atrazine (AAtrex Nine-O; 85% a.i.) was dissolved in water and applied as a stepped exposure regime with four increasing concentrations; 15, 25, 50, and 75 µg/L. Stepped applications were made every two weeks (four weeks between 50 and 75 µg/L treatments). Periphyton biomass and production, macrophyte cover and growth rates, and growth and development of vertebrates were monitored throughout the study. Atrazine in control wetlands averaged 0.69 µg/L and likely originated from residues present in the Mississippi River. Atrazine concentrations in the outflow of treated wetlands were 78 to 96% of nominal. Resistance of atrazine by periphyton was observed in the 50 and 75 µg/L treatments. Gross productivity was significant reduced at 15

µg/L, and the depth of detritus was two times higher in control wetlands than treated wetlands. A significant reduction in respiration was observed in the 25 µg/L treatment, while a significant increase was observed in the 75 µg/L treatment. High variability in macrophyte biomass was found within treatments, but no significant effects were observed.

EPA (2009b) evaluated Detenbeck et al. (1996) and criticized the high levels of atrazine detected in control cosms (0.69 µg/L), the limited number of replicates (2 control and 2 treatments), and the high variability in results among treatments. These variables confound the ability to compare potential treatment-related effects to observations made in control cosms. In addition, the study used a very unconventional study design where exposure concentrations were increased in the same treatment cosms to simulate different concentration exposures over time. Therefore, it is unclear if measured concentrations represented peaks or averages. EPA (2009b) rated this study as qualitative and cautioned against use in risk assessments.

The SAP (2012) recommended excluding this study from consideration because the high accumulation of sediment and detritus would have put additional stress on the system and increased respiration rates. Although the authors stated that gross primary productivity (GPP) was impacted at 15 µg/L, the SAP (2012) questioned the validity of the results because no data were presented and GPP returned to control levels at higher concentrations. The SAP (2012) recommended that this study be excluded from consideration.

Giddings (2012) rejected this study because the exposure regime prevented a clear interpretation of concentration-response relationships. In fact, effects observed at low concentrations were not observed at higher concentrations, and treatment-related effects on periphyton and macrophytes were few. For example, effects on chlorophyll and dry weight were not observed, gross productivity was only reduced at 15 µg/L, and respiration was only reduced at 25 µg/L.

Moore et al. (2015, 2016) rejected this study primarily because of the unconventional study design. The two weeks between each stepped exposure were likely insufficient for full recovery of periphyton and macrophytes. Further, atrazine was detected in the control cosm (0.69 µg/L likely the result of agricultural runoff), control results were not reported, only two replicate cosms were employed, and measurements of residual atrazine in pore water and sediment due to stepped exposure were not reported.

EPA (2016a) used four effects endpoints from this study; 15 µg/L for gross productivity, 25 µg/L for respiration and net primary productivity, 50 µg/L for net primary productivity, and 79 µg/L (unclear if this is a nominal or measured concentration) for net primary productivity. However, there is a lack of evidence for a concentration-response relationship and critical failures in the design of the study and reporting of methods prevent confidence in the results. Further, this study has been highly criticized by EPA's own evaluation process (EPA, 2009b), a SAP (2012), Giddings (2012), and Moore et al. (2015, 2016). Therefore, this study should not be considered for use in the calculation of an LOC.

Kosinski, 1984; Kosinski and Merkle, 1984

Kosinski (1984) evaluated the effects of atrazine on species composition and standing crop of periphyton in artificial streams on the roof of a Texas A&M University campus building. The study was primarily designed to test resistance of the periphyton community. Recirculating artificial streams were treated with 0.1, 1, or 10 mg a.i./kg atrazine (Aatrex 80 WP; 100, 1000

and 10,000 µg a.i./L). Twice weekly, 15% of the stream volume was replaced with source water, including algae and nutrients. Half the test streams received an addition of 0.01 mg a.i./kg (10 µg a.i./L) atrazine at each water change to maintain chronic exposure concentrations and observe the potential for resistance. Colonization slides were maintained to measure changes in periphyton. Variability in biomass was high among and within streams. The authors reported significant reductions in primary productivity at 10 and 100 µg a.i./L.

The SAP (2009, 2012) rejected the effect endpoint of 10 µg a.i./L (0.01 mg a.i./kg) used by EPA (2003) and EPA (2016a) because no significant effects were found and there were insufficient data to assign effects at that level. The SAP (2009) highlighted the fact that the results were not supported by the tremendous amount of data available for atrazine. Instead, the SAP (2012) proposed an effect level of 100 µg a.i./L (0.1 mg a.i./kg). Kosinski and Merkle (1984) stated "there was little evidence that exposure to 0.01 mg a.i./kg herbicide during colonization modified the response of the algae to any of the herbicides".

Giddings (2012) also evaluated the data presented by Kosinski (1984) and Kosinski and Merkle (1984), finding that the results during the chronic exposure to 10 µg a.i./L were not consistent and significant effects never occurred on consecutive sampling days. Therefore, the effects at 10 µg/L were slight and transient, and Giddings (2012) proposed an effects score of "0", meaning no effect.

Moore et al. (2015, 2016) rated this study as relevant and supplemental. They also re-evaluated the study data and found a LOEC of 1000 µg a.i./L. Effects at 10 µg a.i./L were transient and only occurred at one sampling time, whereas no significant effects were observed at 100 µg a.i./L. Only at 1000 and 10,000 µg a.i./L were significant effects observed during consecutive sampling effects.

EPA (2016a) selected an effects concentration of 10 µg a.i./L for calculation of their LOC, but based on re-evaluation of the data by others (SAP, 2012; Giddings, 2012; Moore et al., 2015; 2016), a higher effects level is more appropriate.

Seguin et al., 2001

This study evaluated the sensitivity of phytoplankton to atrazine. Large outdoor tanks (3.4 m diameter, 1.2 m depth) capable of holding 5000 L of water were erected at the Ecole Nationale Superieure d'Agronomie de Rennes, France. Approximately 5 cm of natural sediment from a local pond was added to each cosm, along with a mixture of tap and pond water (containing phytoplankton and zooplankton) to a depth of 70 cm. Aquatic macrophytes (*Glyceria maxima*) were planted in two concentric circles in each tank. Atrazine dissolved in acetonitrile was added to three tanks per treatment concentration at rates of 0, 2, and 30 µg/L. There were no statistical differences in gross phytoplankton biomass among treatment and control tanks. Significant decreases in dominant taxonomic groups were only observed for Chlorophyceae. At 2 µg/L, biomass was significantly reduced at Day 17, but returned to control levels by the next sampling time (Day 23). In the 30 µg/L treatment, significant decreases in Chlorophyceae were observed on days 17 and 23, followed by a return to control levels.

EPA (2009c) reviewed Seguin et al. (2001), finding that the limited data provided made study evaluation and determination of relevance of reported results extremely difficult. The reviewer determined that any effects observed at 2 µg/L were transient and not likely treatment-related. The review further critiqued the lack of information on cosm construction, including analytical

measurements of cosm water to confirm that the cosms were not contaminated with atrazine before test start. Successional dynamics and only a three-week cosm colonization period may have confounded the results. The review also highlighted the lack of details provided for the Bray-Curtis dissimilarity index, as the equation and version used, as well as the level of taxa assessed can significantly affect the statistical analyses. EPA (2009c) recommended only using data from Seguin et al. (2001) within a qualitative weight-of-evidence assessment.

The SAP (2012) critiqued the use of acetonitrile as a solvent for ecotoxicological dosing. Seguin et al. (2001) did not report the concentrations used and this solvent is rarely used. Therefore, the effects of acetonitrile on phytoplankton biomass are unknown.

Giddings (2012) also reviewed this study and highlighted the transient nature of the negative effects observed on chlorophytes at 2 µg/L. After a thorough evaluation of the presented data, Giddings (2012) found that possible effects reported at 2 µg/L were slight and transient, and assigned a binary effects score of 0. Giddings (2012) recommended an effects level of 30 µg/L.

Moore et al. (2015, 2016) rated this study as not relevant because concentrations were not measured (or reported) to confirm atrazine levels over time. Data quality was rated as unacceptable or supplemental because a non-standard solvent was used and the quantity was not reported, control results were not reported, concentrations were not measured, limited test concentrations were evaluated, minimal study conditions were reported, and only minimal evidence for a concentration-response relationship was demonstrated. Moore et al. (2015, 2016) found significant effects at only 30 µg/L.

EPA (2016a) used an effects concentration of 2 µg/L in their calculation of an LOC. However, given the low quality of study and evidence of transient, inconsistent effects at 2 µg/L, 30 µg/L is a much more appropriate effects concentration for use in the calculation of an LOC and this value is supported by all reviewers (SAP, 2012; Giddings, 2012; Moore et al., 2015; 2016).

Seguin et al., 2002

Seguin et al. (2002) investigated the effects of atrazine on chlorophyll α production of freshwater phytoplankton communities. Four large, outdoor, circular tanks located at Institut National de la Recherche Agronomique, Rennes, France served as cosms for the study. Tanks were filled with 7 cm of natural sediment and 70 cm of pond water containing phytoplankton. Tanks were stocked with zooplankton collected with nets from local ponds and aquatic macrophytes (*Glyceria maxima*). Tanks were left to colonize over a period of three weeks before addition of atrazine. Two tanks received 30 µg/L atrazine and two tanks were left untreated. Chlorophyll α and dry weight were higher in control tanks than treated tanks nine days after application.

EPA (2009d) reviewed Seguin et al. (2002), finding several limitations in the study. Most notably, background concentrations were not measured in sediment or water prior to test start to confirm that controls were not contaminated. All criticisms were similar to those presented by EPA (2009c) regarding Seguin et al. (2001). Overall, EPA (2009d) recommended that data from Seguin et al. (2002) only be used qualitatively as part of a weight-of-evidence regarding potential effects to aquatic plants.

The SAP (2012) criticized the lack of recovery period included in Seguin et al. (2002). Following a review of the literature, the SAP (2012) concluded that recovery was expected following the 30% reduction in algal biomass observed over 21 days by Seguin et al. (2002). Therefore, SAP

(2012) were concerned about the use of the Seguin et al. (2002) study, but supported the effects concentration of 30 µg/L observed in the study.

Giddings (2012) evaluated Seguin et al. (2002), finding that the lack of study details limited the quality of the study and prevented an in-depth data quality assessment.

Moore et al. (2015, 2016) rated this study as not relevant because atrazine concentrations were not measured and a recovery period could not be established. The study was also rated as unacceptable because limited replication (n = 2) and test concentrations (n =1) were evaluated, minimal study conditions were reported, control results were not reported, and the study did not follow internationally-recognized guidelines.

Although this study is of low quality, effects to biomass, productivity and community structure of phytoplankton were apparent in the 30 µg/L treatment, as noted by EPA (2016a), Moore et al. (2015, 2016) and the SAP (2012). Therefore, inclusion of this endpoint by EPA (2016a) has high uncertainty, but is reasonable.

Baxter et al. (2011)

This study evaluated the effects of atrazine on primary productivity in outdoor microcosms located at the University of Guelph Turfgrass Institute Microcosm Facility, Guelph, ON. Sediment was added to 44% of the surface area of the cosm floors to a depth of 7 cm. Spring water from an adjacent irrigation pond was added to a depth of 1 m. Atrazine (96% a.i.) dissolved in acetone (<0.001% final cosm concentration) was applied to cosms at rates of 1, 10, 30, and 100 µg/L and cosms were observed for 73 days. Shoots of macrophytes, *Myriophyllum spicatum* and *Elodea canadensis,* were planted in pots in the cosms, as well as periphyton substrates. Observations were made on biomass of macrophyte, phytoplankton and periphyton populations. The authors reported no consistent concentration-response relationship, except for a reduction in macrophyte biomass at 100 µg/L.

Giddings (2012) reviewed Baxter et al. (2011) and found no consistent and significant effects on macrophytes, periphyton and phytoplankton over 73 days at concentrations of 1, 10 and 30 µg/L. At 100 µg/L, a significant decrease in wet and dry weight of macrophytes was observed, but effects to periphyton or phytoplankton were not observed.

EPA (2016a) used three effects concentrations from this study; 10, 30 and 100 µg/L. However, significant effects were transient and did not follow a true concentration-response relationship. Shoot weight was reduced 46, 19, and 78% in the 10, 30 and 100 µg/L treatments (Baxter et al., 2011). These results do not follow any trend, but instead show that there is high variation in response. Additionally, no statistically-significant differences were detected between shoot weights of treatment and control plants, except in the 100 µg/L treatment (Baxter et al., 2011). Therefore, an effect level of 100 µg/L is appropriate for macrophytes and >100 µg/L is appropriate for periphyton and phytoplankton.

Pannard et al., 2009

Pannard et al. (2009) evaluated the effects of atrazine on a controlled freshwater wetland phytoplankton community. Cosms were stocked with plankton collected from a freshwater wetland in Brittany, France and maintained under semi-continuous culture in the lab. At the start of the study, the phytoplankton community consisted of eight species and was dominated by *Oocystis* sp. and *Selenastrum bibraianum*. Before application of atrazine, the phytoplankton

community was "starved" by making the community phosphorus-deficient. On Day 0, atrazine was applied once weekly to cosms at rates of 0.1, 1, and 10 µg/L, along with 52.6 µg/L phosphorus. Cosms consisted of 500 mL Erlenmeyer bottles containing 240 mL water with phytoplankton community. Discontinuous fresh input of culture was made once weekly. The cosms were observed for seven weeks after the initial application to evaluate potential changes in photosynthetic activity, biomass and community structure. The authors noted significant effects on primary production and community structure in all treatments. However, there were no differences in chlorophyll α (biomass estimator) among treated and control cosms. Notably, the Simpson's index of diversity was significantly higher in treatments than controls.

This study was not evaluated by the SAP (2012), Giddings (2012) or Moore et al. (2015, 2016). However, the study was evaluated here following the guidance outlined in Moore et al. (2015, 2016). See Appendix A for the full study evaluation. The study was rated as not relevant because it did not include a recovery period and was rated unacceptable for data quality because the composition of species in controls decreased 40 to 92% over the course of the study, limited results were presented for sampling times and endpoints, limited study criteria were reported, and test concentrations were not measured. Additionally, the depletion of phosphorus at test start then addition of phosphorus throughout the study muddled possible treatment-related effects. This study focused more on the effects of phosphorus supply on biological productivity and less on the effects of atrazine. As a result, phosphorus availability was an additional stressor.

It is unclear how EPA (2016a) used the data from Pannard et al. (2009) in their ERA for atrazine, but EPA (2016a) did state that significant effects were observed at all test concentrations and a significant shift in community composition was observed at and above 1.0 µg/L. However, the large decrease in control cell density between test start and test end and the lack of effect on phytoplankton biomass from atrazine exposure, shows the presence of an additional stressor on the study system and poor study design. Thus, an effects concentration of 1.0 µg/L is not supported and should not be used in the derivation of an LOC. The study is critically flawed and should have been rejected by EPA (2016a).

King et al., 2014

King et al. (2014) simulated atrazine chemographs in streams of agriculture catchments receiving pulsed inputs. The system included riffles, pools and glides, and water was drawn from the North Bosque River then filtered through a wetland before reaching the study tank. Colonization of benthos taken from the North Bosque River was allowed for 30 days prior to test start. Twelve pots of *Ceratophyllum demersumi* were secured into the ponds and wild caught minnows were added to the glides. Nominal concentrations were selected to achieve 60-day mean concentrations of 10, 20, and 30 µg a.i./L. Streams were dosed with daily pulses of 50, 100, and 150 µg a.i./L for four days, followed by seven days of no dosing. The 11-day cycle of dosing was repeated three times, followed by a recovery period of 26 days. Mean 60-day measured concentrations were 0.07 10.7, 20.9, and 31.0 µg a.i./L atrazine for the control, 10, 20, and 30 µg a.i./L treatments, respectively. The authors concluded that no significant effects on structural endpoints were observed at concentrations up to 30 µg/L and only transient functional effects were observed at 30 µg/L. Full recovery was observed by day 60.

The study was evaluated by EPA (2016b), who determined that the statistical approaches used by King et al. (2014) were not appropriate for evaluating significant changes in community

structure. EPA (2016b) also asserted that statistically and biologically significant effects were observed without subsequent recovery. EPA (2016b) determined an effects concentration of 10 µg a.i./L based on biologically significant decreases in metaphyton production, periphyton biomass, and dissolved oxygen. EPA (2016b) also determined that King et al. (2014) had limited use in the calculation of an LOC because of the mitigating effects of high nitrogen and phosphorus levels present in the study.

EPA (2016a) misinterpreted this study. Although EPA (2016a) asserted that nutrient levels were too high, they were not. The study was specifically designed to represent 2nd and 3rd order Midwestern streams in corn-growing areas and nutrient levels were consistent with those locations. Further, the study design was based on recommendations of the SAP (2012) and is state-of-the-art, including the statistical analyses conducted by King et al. (2014). The results indicate only transient effects at the highest test concentration and thus all test concentrations from this study should have been scored by EPA (2016a) as a zero in deriving the LOC.

Data Conclusions

EPA (2016a) stated:

> "those freshwater and estuarine/marine monitoring sites with a 60-day running average at or above 3.4 µg/L have atrazine concentrations that are above the CELOC, and that ecologically significant changes in aquatic plant community structure, function, and/or productivity would be expected."

This conclusion is untrue. The results of Giddings (2012), two SAPs (2009, 2012) and Moore et al. (2015, 2016) demonstrate that no statistically significant effects occurred at atrazine concentrations less than 30 µg/L. Moore et al. (2015) also evaluated new cosm studies published since the SAP (2012) review (Baxter et al., 2013; Choung et al., 2013; Halstead et al., 2014; Knauer and Hommen, 2012; Murdock and Wetzel, 2012). In these studies, no effects were observed at concentrations less than 30 µg/L. Therefore, EPA's 60-day LOC of 3.4 µg/L is almost an order of magnitude lower than the lowest reliable effects concentrations observed in mesocosm studies, and is overly conservative.

EPA (2016a) did not account for recovery. However, the mode of action of atrazine is reversible upon removal of atrazine exposure at the target site of both terrestrial and aquatic plants (Brain et al., 2012a,b; Brockway et al., 1984; Hughes et al., 1988; Jensen et al., 1977; Jones et al., 1986; Juttner et al., 1995; Klaine et al., 1996; Mohammad et al., 2008, 2010; Moorhead and Kosinski, 1986; Shimabukuro et al., 1970; Stay et al., 1985, 1989; Vallotton et al., 2008). Monitoring data from the AEMP have shown that the median duration of concentration peaks greater than 15 µg/L is 2 days (Brain, 2012). Atrazine is likely to enter natural systems as pulses during runoff events. Independent of degradation, water dynamics, flow and dilution would disperse the atrazine away from the point of input and decrease the concentration quite rapidly, particularly in larger flowing waterbodies. Decreasing concentration levels would immediately allow for recovery of aquatic communities (Brain, 2012). The recovery potential of plant communities is further reviewed by Brain (2012), where the influences of exposure magnitude and duration are shown to have limited impact on recovery. Brain (2012) concluded that, "relative to worst-case environmentally measured durations and concentrations, aquatic plants would be expected to fully recover from episodic atrazine exposures."

2.3.2 *Method of Derivation*

EPA (2016a) used the Plant Assemblage Toxicity Index (PATI) method to derive an LOC for aquatic plants. The PATI uses singles species toxicity studies to derive an index against which cosm data and monitoring chemographs are compared. PATI inputs are at the population level of biological organization and the method makes binary predictions (e.g., effect or no effect) regarding community-level effects. The PATI method is very sensitive to changes in inputs (e.g., scoring of cosm studies).

The first step of the PATI method is to develop an effects index based on single species toxicity data and a species sensitivity distribution (SSD). However, for chemicals with highly variable exposure profiles (e.g., atrazine), SSDs have several major shortcomings:

- The inputs (i.e., EC50s) to an SSD are generally from one to four-day toxicity tests with constant exposure concentrations. If an LOC was derived from an SSD percentile (e.g., 5th percentile), what is the appropriate exposure duration for judging risk? Is it the peak exposure concentration? In this case, the assumption would be that high peaks of short duration pose more risk than short peaks that last much longer. Such an assumption has no scientific basis, as risk is a function of both magnitude and duration of exposure. Calculating an average exposure duration (e.g., 4 days to approximately match durations of toxicity studies) can also be problematic for situations in which atrazine exposures are considerably longer. Thus, an SSD-based LOC likely has limitations for determining whether a particular atrazine chemograph is or is not an issue for aquatic plant communities.
- Even if exposure durations in the field were comparable to those used in toxicity tests, SSDs have other limitations for determining community-level impacts for a particular environmental concentration. The HC5, for example, is the concentration that will cause a 50% impact (e.g., reduced growth rate) for the 5th percentile species on the SSD. However, more sensitive species would experience greater than 50% impact and slightly more tolerant species would also experience some impact though less than 50% impact.

There are two major assumptions in the PATI calculations: (1) the sensitivity distribution is representative of the range of plant sensitivities in waterbodies near where atrazine is used, and (2) all species are weighted equally. In reality, natural aquatic plant communities have species that are more and less dominant than others.

Next, a cumulative PATI distribution is constructed, taking into account exposure duration. However, the cumulative PATI assumes: (1) there is no residual toxicity from previous days and toxicity is only a function of the current day's exposure; (2) over time there is no replacement of sensitive individuals by more tolerant individuals within a species and no replacement of sensitive species by more tolerant species; and (3) a specific reduction in growth over one day is equivalent to half that reduction persisting over two days or a quarter of that reduction persisting over four days.

Finally, the cumulative PATI is calibrated with results from cosm studies. This process involves a number of assumptions, including: (1) environmental conditions affecting exposure, sensitivity and/or recovery of aquatic plant communities are unimportant; (2) the disparity in exposure magnitudes and durations across available cosm studies can be accounted for by normalizing to

units of cumulative PATI (% effect-days); and (3) the magnitudes of effects observed in the cosm studies are unimportant to derivation of the LOC_{PATI}. Therefore, the PATI process is very sensitive to the assumed assessment period and the determination of effect versus no effect for cosm studies.

The SAP (2012) expressed concerns over the reliance on the PATI method by EPA (2003, 2012) to develop the LOC, as well as the scheme used by EPA to score cosm data. Most importantly, the SAP (2012) panel disagreed with the selection process used to determine the cosm dataset employed to calculate the LOC, as the PATI method is highly sensitive to which cosm studies are included in calculations. The SAP (2012) expressed minimal confidence in the LOC of 4 to 7 µg/L calculated by EPA (2003) in their IRED.

Weaknesses of the PATI method that were highlighted by the SAPs (2009, 2012) included the following:

- The method does not account for environmental conditions in the receiving environment including several that could significantly affect exposure to atrazine, atrazine toxicity and post exposure recovery rates (e.g., nutrient levels, total suspended solids).
- The cumulative PATI does not correspond to the fractional change in aquatic plant community biomass over time. This point is readily apparent when one considers the $PATI_{60\,d}$ LOCs calculated for various cosm datasets. All of the values are greater than 100% effect-days which would imply complete elimination of primary producers over the course of a 60-day exposure. This was obviously not the case in the vast majority of cosm treatments even in treatments with $PATI_{60\,d}$ values well in excess of 1000% effect-days. There are several reasons to explain the discrepancy between cumulative PATI and fractional change in aquatic plant community biomass over time. First, the PATI method assumes that sensitivities of populations and the plant community do not change over time. However, the most sensitive individuals and populations are likely replaced by more tolerant individuals and populations during a long-term atrazine exposure. Thus, overall community growth rate on a daily basis is less affected as time goes by even if atrazine concentrations are constant. Second, the PATI method assumes that there is a linear relationship between index values and community growth rate. This is likely not the case for plant communities that are not in the exponential growth phase. High PATI values may have little impact on plant communities at or near carrying capacity because effects due to atrazine may be offset by release from self-shading and nutrient depletion (Schafer et al., 1994). Third, plants may adapt to atrazine exposure over time by, for example, increasing chlorophyll content to compensate for reduced photosynthesis (see discussion in EPA, 2012a). Such adaptations are likely important at low to modest exposure concentrations. Thus, the assumption that long exposures at low concentrations (e.g., daily PATI = 5% effect-days for 20 days) are of equal concern to short exposures at high concentrations (e.g., daily PATI = 100% effect-days for 1 day) is very tenuous.
- There is no means of evaluating model performance with respect to estimates of toxic effects. Because the PATI is a relative index of effect (see preceding bullet), there is no way to directly test or evaluate model performance using community cosm or field test data not used in the calibration step. The risk of making a wrong decision in regulatory decision making depends on the reliability of the model predictions. Therefore, there is a

strong need to establish the validity of the PATI method, but unfortunately no way to do so.

- The use of test results from tolerant species weakens the reliability of the cumulative PATI score in stage 2. As a result, the SAP (2012) recommended testing of a broader range of primary producer species. However, we do not agree that inclusion of some tolerant species is a weakness of the PATI methodology. First, primary producers in water bodies close to atrazine use areas are likely "tolerant" of various stresses stemming from being present in agro-ecosystems including frequent exposure to pesticides. It is a generally accepted notion among ecologists that systems with a history of disturbance are more likely to recover quickly from a disturbance, particularly those that mimic historical disturbance events (Denslow, 1985; Rapport et al., 1985). Thus, inclusion of atrazine-tolerant species in the cumulative PATI score is appropriate. Second, and more importantly, the sensitivity analyses conducted by EPA (2012a) demonstrated that the taxonomic composition of the dataset used to estimate the cumulative PATI in stage 2 has little impact on estimates of atrazine risk for different chemographs. The stage 3 calibration to cosm results is far more important than the stage 2 calculation of the cumulative PATI.
- The method does not adequately account for interactions between and within aquatic plant species. This point is true for calculation of daily or cumulative PATI scores. That said, calibration of the PATI scores to the results of cosm studies implicitly accounts for interactions between and within aquatic plant species.
- The PATI methodology is highly sensitive to the cosm data selected. Therefore, mis-scoring of effects or no effects and/or inclusion of poor quality data could have large implications on the estimated LOC.
- Use of binary decisions for each cosm treatment (no effect, effect) results in a large loss of information regarding magnitude and duration of responses across a range of community-level endpoints.
- The EPA (2012a, 2016a) definition of "recovery" in cosm studies (i.e., recovery to pre-exposure conditions for populations) is unrealistic because aquatic plant communities are dynamic and change throughout the growing season. As noted by Landis et al. (1996, 1998) and others (e.g., Chapin et al., 1996), recovery is not a fundamental property of ecosystems although they may have characteristic patterns and boundaries that are set by the prevailing environmental conditions. Thus, expecting populations in cosm studies to recover to pre-exposure conditions is misguided. Rather, change to the general structure and function observed in control communities during the post exposure period is a more realistic indication of "recovery".

As outlined above, the PATI method has a number of uncertainties and weaknesses. Instead of relying on a single method, Moore et al. (2015, 2016) described a weight-of-evidence approach for deriving an LOC protective of aquatic plant communities for atrazine. The weight-of-evidence approach included four methods (PATI, Comprehensive Aquatic Systems Model for atrazine [CASM], EPA's water quality criteria method, and direct interpretation of cosm studies) to independently calculate LOCs and then used a weighted calculation to estimate a community-level LOC. The PATI method was described above. The CASM is a bioenergetics-based aquatic

food web model that was previously used by EPA (2003, 2007). CASM simulates daily changes in biomass production of aquatic primary producer and consumer communities, as well as changes in water quality parameters over a model year (Bartell et al., 2009). The Water Quality Criteria (WQC) method is a standardized approach used by EPA (1985) to establish aquatic benchmarks. The LOC is estimated from a species or genus sensitivity distribution. For atrazine, Moore et al. (2015, 2016) calculated a genus sensitivity distribution using specific growth rate of aquatic plants. Finally, Moore et al. (2015, 2016) calculated an LOC directly from the results of cosm studies. All data points were first classified using the Brock et al. (2000) 5-point system then plotted by exposure concentration and duration to estimate 30- and 60-day LOCs.

The LOCs calculated with the four methods ranged from 19.6 to 26 µg/L and the weighted LOC was 23.6 µg/L. This LOC is consistent with the results of all acceptable cosm studies, which indicate that negative effects are not likely at concentrations less than 30 µg/L. The LOC calculated by Giddings (2012) and Moore et al. (2015, 2016) using the PATI method was 25 µg/L, which is almost an order or magnitude higher than the LOC calculated by EPA (2016a). Therefore, it is clear that correct interpretation and scoring of cosm results strongly influences the calculation of an LOC. By using invalid data and mis-scoring key effects endpoints, EPA (2016a) generated an extremely conservative LOC. We recommend that EPA re-evaluate the data used and employ a weight of evidence approach to estimate a more scientifically defensible LOC for aquatic plants.

2.4 Fish

2.4.1 Freshwater Fish

EPA (2016a) selected a chronic exposure study using Japanese medaka (*Oryzias latipes*) (Papoulias et al., 2014) as the basis of their chronic effects metrics for both freshwater and estuarine/marine fish. Papoulias et al. (2014) exposed breeding groups of one male and four females to atrazine in a static renewal system for up to 38 days. Nominal test concentrations were 0.5, 5.0, and 50 µg/L. The authors found reduced egg production at all treatment levels.

The study (Papoulias et al., 2014) was flawed, as EPA noted in its own DER (Bryan et al., 2014). The study did not follow a standard guideline, had high intra-treatment variability, particularly in the solvent control treatment, and did not demonstrate a clear concentration-response relationship (Bryan et al., 2014). EPA further highlighted other limitations of the study, including lack of a true negative control treatment, a high female to male ratio (4:1 versus 1:1), no results reported for time zero sampling, low fecundity in the control treatment (9.7 eggs/female/day), and high mortality in the solvent control. The study authors found significant effects to egg production and reproduction at all treatment levels (NOEC < 0.5 µg/L), but EPA (2016a; Bryan et al., 2014) re-evaluated the study data and determined a NOEC of 5 µg/L and a LOEC of 50 µg/L. In fact, all differences in egg production were within the variability in the dataset, high variability was noted throughout the test, and only weak statistical evidence of an atrazine effect was observed at 50 µg/L (Bryan et al., 2014).

Because of the many flaws in the Papoulias et al. (2014) study, Syngenta sponsored a GLP study on Japanese medaka that followed standard testing protocols (GLP; OCSPP Guideline 890.1350; OECD 229) to provide a higher quality study for future use in risk assessments (Schneider et al., 2015 [MRID 49694001]).

Schneider et al. (2015) exposed Japanese medaka to atrazine under flow-through conditions for 21 days. Nominal exposure concentrations were 0, 0.49, 4.9, and 49 µg a.i./L, which corresponded to mean measured concentrations of <0.125, 0.59, 5.4, and 53 µg a.i./L, respectively. Schneider et al. (2015) found no significant effects on fecundity or fertility at any treatment level. Control fecundity was 40.9 eggs/female/day/replicate, compared to 9.7 eggs/female/day in Papoulias et al. (2014). Mean control fertility in Papoulias et al. (2014) was 62%, while mean control fertility in Schneider et al. (2015) was 91.7%. Only the Schneider et al. (2015) study meets the OECD guidelines, which require ≥80% fertility.

Although medaka is not a recommended species in the OCSPP guideline, it is a recommended species for OECD 229 and the purpose of the study was to replicate the original non-guideline study performed by Papoulias et al. (2014). Schneider et al. (2015) made several improvements to the study design used by Papoulias et al. (2014), including use of a flow-through exposure system, achieving much higher fecundity in controls, no reliance on solvent, and alignment with recommended guidelines. Therefore, Schneider et al. (2015) offers significantly higher data quality than the Papoulias et al. (2014) study relied upon by EPA (2016a) in their ERA for atrazine. Schneider et al. (2015) were not able to reproduce the results of Papoulias et al. (2014). In fact, the higher quality study found no treatment-related effects at the test concentrations previously used by Papoulias et al. (2014), further decreasing the value of the effects metric used in the ERA.

EPA also evaluated Schneider et al. (2015) in a DER (Marton et al., 2015). The reviewers determined that the study was scientifically sound and the methods used were generally consistent with OCSPP guideline 890.1350. Marton et al. (2015) agreed with the study authors that no significant treatment-related effects were observed at any test concentration. Therefore, we recommend that EPA re-consider the effects metric used in their ERA and rely on high quality data, instead of merely using the lowest NOEC available.

2.4.2 Estuarine/Marine Fish

EPA (2016a) used the Japanese medaka study listed above (Papoulias et al., 2014) as a surrogate effects metric for chronic effects to estuarine/marine fish. EPA (2016) provided no support or reasoning for their choice and provided no evidence indicating that freshwater and estuarine/marine fish have similar sensitivities to atrazine. Further, EPA (2016) ignored a chronic marine fish study that is available for sheepshead minnow (Cafarella, 2005 [MRID 46648203]).

Under GLP conditions, Cafarella (2005) exposed fertilized sheepshead minnow eggs from 26-hours post-fertilization to 28 days after hatch to concentrations of atrazine (FL-881692; 97.1% purity) ranging from 200 to 3200 µg a.i./L. Mean measured concentrations were 150, 300, 570, 1100, and 2200 µg a.i./L. Larval length and wet weight were the most sensitive endpoints, resulting in a NOEC of 1100 µg a.i./L and LOEC of 2200 µg a.i./L.

EPA previously scored the Cafarella (2005) study as supplemental because it did not fulfill guideline requirements (Volz, 2006 [MRID 46952604]). The flaws highlighted by EPA included: the study only maintained two replicate aquaria, did not assess time-to-hatch, and the study duration was 28 days post-hatch instead of the recommended 32 days. Syngenta subsequently amended the reported and provided support for re-scoring of the study (Volz, 2006). The issues are addressed below:

- Description of test substance: Provided to EPA in amended report.
- Replication: Current OPPTS Guidelines (850.1400) recommend at least two replicates, with 60 eggs total. Cafarella (2005) had 40 eggs per replicate, and thus 80 eggs per treatment. The guideline requirements were met.
- Duration: OPPTS Guideline 850.1400 clearly states that the study should last 28 days post-hatch for sheepshead minnow. This duration was used in the original study (Cafarella, 2005).
- Reporting of dilution water analysis: Provided to EPA in amended report.
- pH range: According to OPPTS Guideline 850.1400, a pH range of >7.5 to <8.5 is appropriate for marine testing. This is consistent with the pH range of 7.8 to 8.2 measured by Cafarella (2005).
- Time-to-hatch: Although treatment effects on time-to-hatch were not directly evaluated to avoid injuring newly hatch fry, percent hatch was determined on Day 5. On Day 5, percent hatch among treatments and controls was not significantly different, indicating that atrazine did not affect hatch.

Based on data provided in the amended report and consistencies between the original study methods and OPPTS guideline 850.1400, this study is appropriate for use in a risk assessment and should have been used by EPA in their atrazine assessment for estuarine and marine fish. Further, the NOEC for sheepshead minnow (1100 µg a.i./L; Cafarella, 2005) is 220 times higher than the NOEC (5 µg/L; Papoulias et al., 2014) used by EPA (2016a) and at least 22 times higher than the NOEC (>53 µg/L) determined by Schneider et al. (2015). For the reasons described above, EPA (2016a) had no scientific justification for relying on a poorly conducted freshwater fish study as the basis for the chronic effects metric for estuarine and marine fish, particularly given the availability of a well-conducted chronic study on sheepshead minnow.

2.5 Aquatic-phase Amphibians

In 2004, EPA initiated a data call-in (DCI) request for studies related to gonadal development of aquatic-phase amphibians. Following submission of a registrant-sponsored study on *Xenopus laevis* (Kloas et al., 2009), EPA (2012a) discounted all other data, which were deemed low quality, and focused all risk assessments on the high quality DCI study. Ultimately, based on the DCI study, EPA (2012a) determined that effects to aquatic-phase amphibians and reptiles from exposure to atrazine are unlikely.

Although the SAP (2012) deemed the study robust and agreed that this was the only study of high quality that should be used quantitatively, it had a number of concerns with reliance on only this study because the study employed a flow-through exposure regime that is unlike the natural environment of frogs and unlikely to include exposure to potential atrazine degradates and transformation products, and use of an insensitive strain of *Xenopus* that may not be indicative of the risks posed to other amphibians. Previous SAPs (2003, 2007) agreed that atrazine likely had limited potential for negative effects to aquatic-phase amphibians. However, the most recent SAP (2012) suggested that EPA reconsider the results of some of the previously eliminated studies in a weight-of-evidence assessment because results for one species (*Xenopus laevis*) from the only high quality study may not be protective of all species. Therefore, in their draft ERA, EPA (2016a) shifted its approach and instead included all available toxicity data for aquatic-phase amphibians in a weight-of-evidence assessment.

EPA (2007) listed several requirements for studies to be of acceptable quality. Studies were rated as invalid if they included any of the following:

1. Nominal atrazine concentrations not reported;
2. Insufficient replication (n = 2 or lower);
3. No use of control treatments and solvent controls if solvent used to dissolve test chemical;
4. Test chemical contamination of controls;
5. >30% control mortality;
6. >0.05% solvent concentration;
7. Sufficient data and statistical analyses not reported;
8. Presence of other stressors in controls and/or treatments; and
9. Confounding effects in controls (e.g., high incidence of intersex or skewed sex ratio).

Studies that were considered valid were further evaluated to determine their level of quality. Acceptable (or quantitative) studies also contained the following elements:

1. If a solvent was used, included both negative and solvent control groups;
2. No significant differences between negative and solvent control groups;
3. <0.01% solvent concentration and no use of DMSO as a solvent;
4. No use of plastic test vessels or vessel that have the potential to leach;
5. Use of a technical product;
6. Use of recommended loading rate (e.g., <1 tadpole/L/day); and
7. Use of laboratory-reared organisms.

EPA (2007) evaluated 75 open literature studies. Possible contamination of controls was not determined in 59% of studies and at least 40% of studies had loading rates higher than recommended (>1 tadpole/L/day). Among other uncertainties, water quality parameters were often not thoroughly reported and test organisms were often wild-caught with no indication of past exposure. All studies classified as invalid or valid (qualitative) with a low level of confidence were excluded, so as not to mask the ability to discern effects based on study quality. This left 10 studies for consideration in determining risk of atrazine to aquatic-phase amphibians. Following a thorough review of all data, EPA (2007) concluded that the available data were not sufficient to make inferences on risk to aquatic-phase amphibians.

EPA (2016a) incorporated 55 studies into their weight-of-evidence analysis. Only studies rated as invalid (see above) were excluded from the analysis. This left one quantitative and 54 qualitative studies, spanning a range of data qualities. However, EPA (2016a) noted that studies often did not report and/or measure atrazine concentrations, used wild-caught organisms, and did not adequately report or maintain physical and chemical test characteristics.

The mortality data used by EPA (2016a) in their weight-of-evidence analysis were constructed from one quantitative study, two studies rated qualitative with high data quality, four studies rated qualitative with medium data quality, and 18 studies rated qualitative with low data quality. An additional 10 qualitative studies were included, but did not include data quality rankings. Therefore, of the 25 studies with data quality rankings, 18 (72%) were of low quality. Additionally, of the 44 data points used in their mortality data array, 32 (72%) represented studies that had no effects at the test concentrations evaluated, and spanned the range of concentrations included in the array.

A similar story occurred in the amphibian development data array, which included one quantitative study, two studies rated qualitative with high data quality, two studies rated qualitative with medium data quality, and 12 studies rated qualitative with low data quality. An additional six qualitative studies were included, but did not include data quality rankings. Therefore, of the 17 studies with data quality rankings, 12 (71%) were of low quality, and 28 of 41 (68%) data points were endpoints with no effects (i.e., unbounded). Similarly, for growth and reproductive, 13 of 17 (76%) and 10 or 12 (83%) of studies with data quality rankings, respectively, were of low quality. For growth, 29 of 51 (57%) data points were unbounded, and for reproduction, 18 of 31 (58%) data points were unbounded.

Despite having the highest percentage of unbounded effects data (72%), mortality data were weighted by EPA (2016a) as having the highest degree of confidence of all lines of evidence (mortality, growth, development, reproduction). Additionally, more than 70% of ranked data points for all lines of evidence were of low quality. That is very concerning as data quality is essential for accurate interpretation of risk. In fact, by their own admission, EPA (2016a) overlooked study quality in favor of evaluating the entire range of data. EPA (2016a) also pointed out that many studies with the lowest data points were of dubious quality and results could not be replicated in other studies with the same species. Therefore, reliance on a large dataset primarily comprised of very low quality data and in many cases, studies where no effects were observed, is overly conservative and careless when used to predict risks to amphibians. Conclusions drawn from the data presented by EPA (2016a) are highly uncertain and likely inaccurate.

Finally, EPA (2016a) compared their weight-of-evidence predictions for aquatic-phase amphibians to their LOC for aquatic plants. The LOC was reviewed in detail above. Despite using an LOC that is an order of magnitude lower than effect concentrations observed in cosm studies and nearly an order of magnitude lower than the LOC predicted by others (Moore et al., 2015, 2016; Giddings, 2012), EPA (2016a) believes that their LOC (3.4 µg/L) is protective of potential direct and indirect effects to aquatic-phase amphibians. Therefore, use of higher quality test data and fewer no effects concentrations would likely be further protective of amphibians.

This position is further supported by Hanson et al. (2016), who updated the quantitative weight-of-evidence assessment and determined that atrazine does not pose a significant risk of adverse effects to amphibians. Hanson et al. (2016) reviewed available data and scored studies by relevance and quality. Ten study characteristics were scored on a scale of zero to four. For example, a study with a score of four in each category would be considered to show "strong evidence of adverse effects", while a score of one would show "weak evidence of no adverse effects". Mean scores for each category and endpoint were determined to estimate the average response. No endpoint resulted in mean score representing evidence of adverse effects.

3.0 TERRESTRIAL RECEPTORS

3.1 Terrestrial Plants

For terrestrial plants, EPA (2016a) chose effects endpoints from Chetram (1989a,b). Both the seedling emergence and vegetative vigor studies were performed according to GLP and evaluated the effects of atrazine on ten crops: soybean, lettuce, carrot, tomato, cucumber, cabbage, oat, ryegrass, corn, and onion. In the seedling emergence study (Chetram, 1989a), atrazine (FL-850612; 97.7% purity) was applied to bare soil at rates of 0.0025 to 4.0 lb a.i./A. The most sensitive metric was plant dry weight, with NOECs for oat and carrot of 0.0025 lb a.i./A (Chetram, 1989a). For the vegetative vigor study (Chetram, 1989b), atrazine (FL-850612) was applied to the foliage of seedlings in the 1-3 true leaf stage at rates of 0.0025 to 4.0 lb a.i./A. Plant dry weight was again the most sensitive metric, resulting in a NOEC of 0.0025 lb a.i./A for cabbage (Chetram, 1989b). The Chetram (1989a,b) studies are outdated (see discussion below) and did not evaluate recovery. A recovery phase is an important study design component for herbicides such as atrazine that have a reversible mode of action following cessation of exposure. In fact, European Food Safety Authority (EFSA, 2012) guidance recommends that the potential for ecological recovery be integrated into a risk assessment. For non-target terrestrial plants, EFSA evaluates recovery at the population level for germination (seedling emergence), biomass, and vegetative vigor.

Recently, new seedling emergence (Martin, 2015a [MRID 49639102]) and vegetative vigor (Martin, 2015b [MRID 49639101]) studies were performed using a current product (Atrazine SC; 43.0% a.i.). The new studies followed GLP, OCSPP Guideline 850.4100 and OECD 208. The Martin (2015a,b) studies applied a typical end-use product, which is required for OCSPP 850.4100 guidelines. Conversely, the Chetram (1989a,b) studies applied technical atrazine which is not what terrestrial plants would be exposed to from spray drift in the field. Terrestrial plants are exposed to end-use products, as exposure is the result of spray drift to non-target areas during and shortly after application.

The Martin (2015a,b) studies included a recovery phase to determine the long-term effects of atrazine application on terrestrial plants. In the seedling emergence study, Martin (2015a) applied Atrazine SC to bare ground and observed plants for 28 days after 50% emergence in controls. After 14 days, the most sensitive monocot was oat, with a NOEC of 0.021 lb a.i./A and an EC25 of 0.0456 lb a.i./A based on dry weight. The most sensitive dicot was cabbage, with a NOEC of 0.097 lb a.i./A and an EC25 of 0.0299 lb a.i./A based on dry weight. After 28 days, the most sensitive monocot was onion, with a NOEC of 0.025 lb a.i./A and an EC25 of 0.0341 lb a.i./A based on dry weight, and the most sensitive dicot was cabbage, with a NOEC of 0.0099 lb a.i./A and an EC25 of 0.0177 lb a.i./A based on dry weight. Recovery was analyzed for the growth rate endpoint. Martin (2015a) observed recovery in cabbage shoot length growth rate and in tomato shoot length and dry weight growth rates. Other species either exhibited no recovery or recovery was non-significant.

In the vegetative vigor study, Martin (2015b) applied Atrazine SC to crops. Observations were made at 21 and 42 days after application to determine effects and the potential for recovery. After 21 days, the most sensitive monocot was onion, with a NOEC of <0.044 lb a.i./A and an EC25 of 0.0379 lb a.i./A based on dry weight. The most sensitive dicot was cucumber, with a NOEC of <0.0099 lb a.i./A and an EC25 of 0.0145 lb a.i./A based on dry weight. After 42 days,

the most sensitive monocot was onion, with a NOEC of 0.092 lb a.i./A and an EC25 of 0.0996 lb a.i./A based on dry weight. The most sensitive dicot was soybean, with a NOEC of <0.0044 lb a.i./A and an EC25 of 0.00397 lb a.i./A based on dry weight. Recovery of vegetative vigor was analyzed using growth rate. Recovery was observed for all species, except for corn (no concentration-response relationship at up to six times registered application rate; 2.0 lb a.i./A) and shoot dry weight for oat.

When evaluating the effects of an herbicide on the terrestrial environment, the potential for recovery must be considered. Agro-ecosystems in dynamic environments are likely to recover from disturbances (e.g., pesticide applications), especially disturbances that mimic historical events (e.g., previous pesticide applications; Denslow, 1985; Rapport et al., 1985; Moore, 1998).

Recent studies with terrestrial plants suggest that tested species generally recover following single and repeated exposures to atrazine at environmentally relevant concentrations. Dalton and Boutin (2010) applied atrazine to microcosms and single species to determine the potential for effects and recovery. Nine terrestrial and seven wetland plant species (1 monocot and 15 dicots) found in Eastern Ontario and Western Quebec were evaluated. The study compared EC25s for percent of control biomass in different experimental systems (short- and long-term greenhouse and outdoor microcosm environments). Short-term experiments were 28 days and long-term experiments spanned 60 and 70 days for terrestrial and wetland microcosms, respectively. AAtrex® 480 (Syngenta Crop Protection; 470.4 g a.i./L) was applied at doses selected to achieve 20 to 80% effect in target species.

Some recovery was observed in the long-term microcosms, and EC25s for total microcosm biomass were higher in longer-term microcosms than in the 28-day greenhouse microcosms. Overall, EC25s were within an order of magnitude of one another, with total microcosm biomass EC25s ranging from approximately 0.09 to 0.45 lb a.i./A. These values are within the range of single-species growth EC25s reported in the Tier II atrazine studies (Chetram, 1989a,b; Martin, 2015a,b).

There is no scientific justification for EPA (2016a) excluding two high quality terrestrial plant studies that follow standard guidelines and use a current product (i.e., Martin et al., 2015a,b). In addition, it is critical that recovery be considered for herbicides such as atrazine. Herbicides are developed to eradicate unwanted plants. Therefore, it is understandable that spray drift and runoff to non-target areas could cause negative effects to non-target plants. However, if recovery is possible, it could mitigate the initial effects of atrazine and show that non-target plants are able to tolerate higher concentrations than initially assumed using shorter toxicity tests.

3.2 Wildlife

Included below is a brief summary of the key issues identified in the bird and mammal risk assessments performed by EPA (2016a). Details are further discussed in a response document submitted to the docket by Syngenta (Olson et al., 2016).

3.2.1 Exposure Assessment

EPA (2016a) used T-REX version 1.5.2 in their screening-level assessment to estimate risks to birds and mammals potentially exposed to atrazine. However, T-REX uses a number of highly conservative assumptions to estimate risk, including the following:

- One residue unit dose is used for all arthropods, despite varying residues based on location and behavior of the arthropods. For example, flying insects are likely to have much lower residues than crop-dwelling insects. Therefore, residues ingested by birds that consume only flying insects may be several fold lower than residues ingested by birds that consume soil surface or crop-dwelling arthropods. For realistic dietary exposure estimates, residues should be quantified for different invertebrate groups using available pesticide data.
- The model assumes that wildlife obtain 100% of their daily diet from treated locations immediately after application, while in reality most species forage on and off the field and will vacate the area during application, only returning after the disturbance has ceased.
- The model does not allow for analysis of mixed diets and instead considers homogeneous diets, several of which are implausible (e.g., 20 g bird consuming only foliage; Sullivan and Wisk, 2012).
- Food ingestion rate is estimated in T-REX with allometric equations derived from Nagy (1987). However, more up-to-date equations are currently available (e.g., Nagy et al., 1999). Further, the calculations should be based on field metabolic rates, gross energies of dietary items, and assimilation efficiencies of the dietary items consumed.

In its assessment, EPA (2016a) used a default 35-day foliar dissipation half-life, despite acknowledging appropriate residue studies for atrazine. These residue studies reported a maximum observed half-life of 17 days. EPA (2012b) guidance directs risk assessors to estimate a 90% upper confidence limit on a mean half-life when there are three or more half-lives available. EPA (2016a) failed to follow their own guidance, citing degradates of atrazine as the primary reason. However, no data were presented by EPA (2016a) that support a longer half-life for atrazine or that describe the nature of residues on foliage. Further, a field study investigating atrazine residues on grain sorghum supports foliar dissipation half-lives between four and five days for "equivalently toxic" residues (Selman, 1995).

3.2.2 Effects Assessment

3.2.2.1 Birds

EPA (2016a) selected an acute LD50 of 783 mg a.i./kg bw and a slope of 2.263 to estimate acute risks to birds. Although the original study reported an oral LD50 of 940 mg a.i./kg bw with a corresponding slope of 2.263 (Fink, 1976 [MRID 00024721]), the LD50 used by EPA (2016a) was recalculated from raw data provided in the original study. EPA (2016a) re-analyzed the raw data using probit analysis, resulting in an LD50 of 783 mg a.i./kg bw and a probit slope of 3.836. However, when generating effects metrics for the ERA, EPA (2016a) paired the recalculated LD50 with the probit slope reported in the original study. This is incorrect. The correct LD50 and slope should be 783 mg a.i./kg bw and 3.836, respectively.

To estimate chronic risks to birds, EPA (2016a) selected a NOEC of <75 mg a.i./kg diet for hatchling weight and LOEC of 225 mg a.i./kg diet for egg production and food consumption

(Pedersen and DuCharme, 1992 [MRID 42547101]). A number of issues have been identified with the use of these endpoints, and include the following:

- When analyzing the raw data, the dead female in the 225 mg a.i./kg diet group should have been excluded from analyses for reproduction, so as not to skew the results.
- Data for egg production were analyzed using the William's Multiple Comparison Test, which is only acceptable for continuous data (Piegorsch and Bailer, 1997). It would have been more appropriate to use the Dunnett's test (Zar, 2010). Use of the Dunnett's test would have resulted in a NOEC of 225 mg a.i./kg diet and LOEC of 625 mg a.i./kg diet. When calculated with the appropriate statistical analyses, the endpoints are at least three-fold higher.
- The NOEC selected by EPA (2016a) for hatchling weight is likely the result of inherent variability in hatchling weights and not ecologically significant. In fact, the original study authors (Pedersen and DuCharme, 1992 [MRID 42547101]) noted that the lower hatchling weight values observed on day 1 were likely attributable to normal biological variation not atrazine exposure. Statistical analyses showed large variations among birds within treatments, including within controls. Therefore, the statistical significance identified by EPA (2016a) is likely trivial from an ecological standpoint and a more appropriate endpoint showing clear a concentration-response relationship should be selected.
- Estimates of food consumption were calculated incorrectly by EPA (2016a). Food consumption depends on a number of factors, particularly body weight. Therefore, food consumption is generally normalized to body weight. However, not only did EPA (2016a) fail to normalize the data, but they also included data points for dead birds and capped the food limit at 4600 g per cage per week for reasons unstated. This led to inaccurate food consumption calculations and artificial censoring of the data. If the raw data are recalculated to normalize for body weight and exclude dead individuals, the NOEL is ≥675 mg a.i./kg diet.

3.2.2.2 Mammals

EPA (2016a) selected a chronic NOEL of 3.7 mg a.i./kg bw/d to estimate long-term effects to mammals. EPA (2016a) stated that their endpoint was calculated from a NOEC of 50 mg a.i./kg diet reported by Mainiero et al. (1987 [MRID 40431303]). However, the selection of the chronic NOEL was not discussed anywhere in the assessment and we could not replicate the calculations, despite access to raw data. Therefore, the accuracy of the NOEL is unknown.

3.2.3 Risk Characterization

While characterizing risk to birds and mammals, EPA (2016a) used default body weights for test species. The default body weights for birds and mammals are 178 g (northern bobwhite) and 350 g (rat), respectively. However, the actual body weights of animals tested in the corresponding toxicity studies were considerably lower. The northern bobwhite chicks used by Fink (1976 [MRID 00024721]) weighed 33 to 35 g, while the rats used by Sachsse and Bathe (1975 [MRID 00024706]) weighed 160 to 180 g. In risk calculations, EPA (2016a) scaled all effects metrics to body weight. For example, the adjusted LD50 for birds was 516 mg a.i./kg bw. However, if the mean body weight corresponding to the study animals is used, the adjusted

LD50 is 564 mg a.i./kg bw. Discrepancies in adjusted LD50 calculations could have significant impacts on risk calculations.

No other lines of evidence were discussed by EPA (2016a) to validate their conclusions. Some additional information should have included:

- The lack of incident reports for birds or mammals associated with atrazine exposure despite many decades of widespread use.
- Potential avoidance, as is often observed with other pesticides at high doses.
- Comparison of acute oral gavage and acute dietary risk estimates and the reasons why the former are overly conservative (i.e., oral gavage is a worst-case exposure for birds and does not represent how exposure occurs in the field).
- The implications of their assessment being based on sprayed vegetation rather than sprayed soil, which is the predominant timing of application for atrazine in corn.

3.2.4 Refined Risk Assessment

The refined risk assessment for birds was conducted using the TIM and MCnest models. However, there are numerous issues. Many of the input values chosen by EPA (2016a) did not follow their guidance or were not appropriately determined from studies and the models incorporated a number of overly conservative assumptions that are inappropriate for a refined assessment. Some of the issues are described below and further reviewed by Olson et al. (2016):

The Assumptions in the ERA were Not Supported by Best Available Data

- EPA used a dermal effects ratio for birds that relied on data for organophosphates and carbamates, the applicability of which to atrazine are unknown. As a result of the effects ratio, 80% of the predicted exposure was contributed by dermal contact. However, birds have feathers that will intercept and significantly decrease exposure, most birds will leave a treated area during application, and atrazine becomes rainfast within one to two hours. Therefore, contact exposure is likely to be much lower.
- A dermal effects ratio for atrazine and related chemicals (i.e., triazines) is much more applicable. For example, an effects ratio produced from data for triazine compounds reduces the estimated mortality of vesper sparrows by two thirds.
- EPA did not incorporate the vast amount of field data showing the fraction of time birds spend on treated fields. Fitting distributions to the available data, rather than using worst case scenarios, is a more applicable approach for estimating the potential for exposure.
- EPA assumed a default foliar half-life of 35 days, despite a number of field studies demonstrating a half-life of 17 days or less for atrazine. Use of the default half-life was not supported and did not follow EPA's own guidance.

The ERA Contained Significant Errors that Impacted Risk Estimates

- To estimate acute risks to birds, EPA used an LD50 and slope that were calculated using different methods. When the LD50 was paired with the appropriate slope, mortality estimates were reduced by almost 50%.
- To estimate chronic risks to birds, EPA made several errors in the selection and use of statistical analyzes. When the correct statistics are used, the NOEC increases from <75

mg a.i./kg-diet to 225 mg a.i./kg-diet. This has a significant impact on chronic risk estimates.

The ERA was Hyper-Conservative

- EPA selected an arbitrary hourly fraction of pesticide retained rather than calculating one from available data. The calculated value is lower, which decreases the potential for toxicity over long-term or multiple exposures.
- Instead of using average exposure values over the duration required to elicit reproductive effects in birds, EPA used one-day peak exposure values. This greatly increased the exposure estimates.
- EPA assumed complete nest failure when exposure exceeded the no observed effect concentration (NOEC), despite studies showing only a 24% reduction in clutch size at the LOEC and modeling showing no impacts on reproductive success when the clutch size is lowered by 22%.

The ERA for Mammals was Inadequate

- Despite identifying potential risk concerns for mammals, EPA did not consider any exposure refinements for mammals and no other lines of evidence were discussed.

The issues summarized above led to significant overestimates of acute and chronic risks for birds and mammals potentially exposed to atrazine. Using the vesper sparrow as an example, EPA's conservative assumptions predicted 21.8% mortality in treated corn fields. However, this is not supported by 50 years of incident data or field studies. When best available data are applied, negligible risks are predicted for birds and mammals as described in Olson et al. (2016).

4.0 CONCLUSIONS

The draft risk assessment recently released by EPA (2016a) was overly conservative, relied on poor quality data, and had a number of errors. A number of issues were highlighted in this report and include the following:

- Monitoring data were often misinterpreted and included erroneous values. We suggest that EPA work together with registrants for future assessments to ensure that data are correctly selected and interpreted.
- Aquatic and terrestrial exposure modeling relied on highly conservative assumptions that may not be representative of atrazine use areas.
- The best available effects data were not employed for fish, terrestrial plants, aquatic-phase amphibians, wildlife, and aquatic plants.
- Refined risk analyses were not performed for mammals, despite indications of potential risks in the screening-level assessment.
- Numerous issues were identified in EPA's refined avian assessment. When best available data are used as inputs to the exposure model, avian risks are negligible.
- Calculation of a level of concern for aquatic plants relied on poor quality data that have previously been rejected by multiple sources (SAP, 2009; 2012; Giddings, 2012; Moore et al., 2015; 2016).

Atrazine has an incredibly rich database of information with regard to exposure and effects to aquatic and terrestrial organisms. Numerous Scientific Advisory Panels have also given thoughtful recommendations and advice to the EPA that, if accepted, would have led to a far less conservatively biased and more scientifically defensible assessment. At almost every turn in the atrazine assessment, EPA ignored this advice and the availability of much high quality scientific information in favor of making decisions that significantly overestimated ecological risk.

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Appendix A
Study Evaluations

Study Evaluation for Panard et al. (2009) Study

Study Reference: Pannard, A., B.L. Rouzic and F. Binet. 2009. Response of phytoplankton community to low-dose atrazine exposure combined with phosphorus fluctuations. Archives of Environmental Contamination and Toxicology 57:50-59.

Classification for Relevance: Not relevant

Classification for Data Quality: Unacceptable

Abstract: The effects of atrazine on a controlled phytoplankton community derived from a natural freshwater wetland exposed to low doses of this photosynthesis-inhibiting herbicide were examined. The community was exposed for 7 weeks to doses of 0.1, 1, and 10 µg/L atrazine, combined with changes in nutrient concentration, and the photosynthetic activity, biomass, and community structure were noted during the experiment. Responses of the phytoplankton community were examined in terms of photosynthetic activity, biomass, and community structure. Significant effects of atrazine on the phytoplankton assemblage, in terms of primary production and community structure, were highlighted, even at doses as low as 1 and 0.1 µg/L, when associated with phosphorus fluctuations. The most abundant Chlorophyceae decreased in concentration with increasing atrazine dose, whereas cyanobacteria were more tolerant to atrazine, particularly with increased nutrient supply. The subinhibitory doses of atrazine used in the present study confirmed the higher sensitivity of long-term exposure of multispecies assemblages under resource competition. Our study supports the emerging hypothesis that the increasing prevalence of cyanobacterial blooms in European aquatic systems may result from a combination of unbalanced nutrient enrichment and selective pressures from multiple toxicants.

Table 1. Evaluation of data relevance[a]		
Question	***Yes or No***	***Comments***
Was the study relevant to communities of aquatic plants?	Yes	Phytoplankton community derived from a natural freshwater wetland; macrozooplankton filtered out; cultured semi-continuously until the start of the test; eight species, dominated by two Chlorophyceae at test stat.
Was atrazine the only active ingredient to which test organisms were exposed?	Yes	Commercial pure atrazine (Atrazine Pestanal, Riedel-de-Haen)
Were the test endpoints direct measures of community-level effects for aquatic plants (e.g., primary productivity, community structure, species richness, relative abundances of different guilds)?	Yes	Short-term – rate of ^{14}C incorporation Long-term - Photosynthetic activity, biomass and community structure
Was the exposure route in the study relevant to what is expected in the environment?	Yes	Water exposure
Was a recovery phase included?	No	Atrazine renewed weekly
CLASSIFICATION FOR RELEVANCE[b]		**NOT RELEVANT**

a. Only endpoints related to atrazine effects to aquatic plants were evaluated and presented in Tables 1, 2 and 3
b. Study is relevant if all answers are "yes", otherwise the study is not relevant

Table 2	Evaluation of data quality		
Assessment Factor	***Questions***	***Score***	***Comments***
Objectivity	Was the study conducted according to a recognized international standard (e.g., EFSA, OPPTS, OECD, SANCO)? If not, was a complete description of the test system and methods given, and were the methods used considered acceptable practices?	0/3	Study was not conducted according to a recognized international standard or laboratory guidance. Study score 0 for one Clarity and Transparency criterion
Clarity and Transparency	Were the identification, purity and source of test substance given and comparable to the current technical material?	1/3	**ID:** Commercial pure (Atrazine Pestanal) **Source:** Riedel-de-Haen **Batch #:** Not reported. **Purity/proportion a.i.:** Not reported. **Carrier solvent:** None
	Were appropriate controls included, reported and results adequate? Was a carrier solvent used? If a solvent was used, were positive and negative controls included? Did positive and negative controls have similar results?	1/3	Negative controls used, with results reported for species composition. No positive control used in the study and water quality parameters not reported. 40.0 to 92.4% decrease in species composition (one species increased) in controls over the course of the experiment
	Were statistical procedures reported and appropriate?[a]	2/3	**Short-term (48 hours)** **Method:** Non-parametric two-factor Friedman analysis for rate of carbon dioxide incorporation **# test concentrations:** 4 **# replicates:** 5 **Long-term (7 weeks)** **Method:** Non-parametric two-factor Friedman analysis for rate of carbon dioxide incorporation and evolution of biomass; chi-square analysis used to test homogeneity of community structures at different times and doses; single-factor (dose) or two-factor (time and dose) ANOVA used to evaluate species densities; Kruskall-Wallis analysis used to evaluate effects on Simpson's index of diversity **# test concentrations:** 3 **# replicates:** 4
	Were test concentrations provided and measured?	2/3	**Short-term (48 hours)** **Application method:** Applied

Table 2	Evaluation of data quality		
Assessment Factor	***Questions***	***Score***	***Comments***
			once to water **Nominal concentrations:** 0, 0.05, 0.5, 5.0, and 50 µg/L **Measured concentrations:** Not measured. **<u>Long-term (7 weeks)</u>** **Application method:** Discontinuous fresh input weekly and weekly renewal of atrazine concentrations **Nominal concentrations:** 0, 0.1, 1.0, and 10 µg/L **Measured concentrations:** Not measured.
	Was sampling sufficient for characterizing test system and responses?	2/3	**Sampling times:** **<u>Short-term</u>** **Rate of ^{14}C incorporation:** Measured after 1, 16, 22, 40, and 46 hours **<u>Long-term</u>** **Phytoplankton biomass:** Measured weekly by chlorophyll a concentration **Biological activity:** Measured weekly by carbon dioxide incorporation **Taxonomic incorporation:** Measured at test start then at 1, 4, and 7 weeks
	Was a concentration-response relationship clearly demonstrated?	0/3	Concentration-response observed for some species, but not others; usually no significant differences among treatments; large difference in community composition in controls between start and end of test is highly suspect. No results provided for individual concentrations, times, and endpoints.
	Were observed effects consistent with atrazine mode of action (i.e., effects apparent during or directly after treatment period)?	1/3	No negative effects observed for first few weeks of test and no significant effects observed for biomass
	Were appropriate test conditions (e.g., pH, conductivity, temperature, dissolved oxygen, water hardness, mesocosm size, etc.) reported and within	1/3	**Study location:** Natural phytoplankton community collected from freshwater wetland in Brittany, Pleine-

Table 2	Evaluation of data quality		
Assessment Factor	***Questions***	***Score***	***Comments***
	acceptable ranges?		Fougeres, France **General weather conditions:** Not reported. **pH:** Not reported. **Conductivity:** Not reported. **Air temperature:** 20°C **Water temperature:** Not reported. **Dissolved oxygen:** Not reported. **Water hardness:** Not reported. **Mesocosm size:** 240 mL water in 500 mL bottles
Integrity	Was the study conducted under GLP?	0/1	No GLP compliance statement
TOTAL SCORE OF STUDY		10/28	
CLASSIFICATION (ACCEPTABLE, SUPPLEMENTAL, UNACCEPTABLE) FOR DATA QUALITY[b]		**UNACCEPTABLE**	

[a] The appropriate statistical approach will depend on the type of data being analyzed (e.g. binomial, ordinal or continuous responses), and whether or not parametric or non-parametric approaches should be applied. For ECx estimation, appropriate statistical methods include probit, logit, Gompertz, Weibull, Gumbell and Burr, and appropriate Generalized Linear Models for parametric tests. Some non-parametric tests for ECx estimation included the Spearman-Karber and Trimmed Spearman-Karber methods. Graphical interpolation is not an acceptable method for calculating ECx values. For calculation of NOELs or LOELs, Dunnett's test is often used to compare treatment groups to controls when data are normally distributed and have homogeneity of variance. T-tests with Bonferroni correction can also be used when the number of replicates is not the same for all concentrations. When assumptions are not met, data can be transformed, or a non-parametric test such as Steel's Many-one Rank Test or Wilcoxon's Rank Sum Test may be used. For binomial data such as mortality, the Fisher's exact test should be used to establish the NOEL or LOEL.

[b] Acceptable studies have scores of 22-28, supplemental studies have scores of 12-21, and unacceptable studies have scores of 0-11.

Table 3. Study Results

Receptor Group (e.g., algae, periphyton, macrophytes)	*Exposure Duration (d)*	*Recovery Period (d)*	*Treatment Concentration (µg ai/L)*	*Effect Class*[a]	*Notes on Observed Responses*	*Study Classification (Table 2)*
Phytoplankton	7 weekly applications	0	0.1	1	Chlorophyll a increased progressively. No significant difference compared to controls. Large difference in community composition in controls between start and end of test.	Unacceptable
Phytoplankton	7 weekly applications	0	1.0	1	Chlorophyll a increased progressively. No significant difference compared to controls. Large difference in community composition in controls between start and end of test.	Unacceptable
Phytoplankton	7 weekly applications	0	10	1	Chlorophyll a increased progressively. No significant difference compared to controls. Large difference in community composition in controls between start and end of test.	Unacceptable

[a] Effect Class 1: No statistically significant effects from treatment or observed differences from controls show no clear causal relationship
Effect Class 2: Slight and transient effects (i.e., short-term, restricted to one or a few sensitive endpoints and/or sampling periods)
Effect Class 3A: Pronounced effects with recovery in <8 weeks of first application or period of effects <8 weeks; temporary effects on several species or endpoints; effects observed at some subsequent samples
Effect Class 3B: Pronounced effects with recovery in <8 weeks after last application; with repeated treatments, total duration of effects >8 weeks is possible; effects observed at some subsequent samples
Effect Class 4: Pronounced effects but study too short to demonstrate recovery within 8 weeks of last application
Effect Class 5A: Pronounced effects for >8 weeks and no recovery within 8 weeks of last application; full recovery by end of test
Effect Class 5B: Pronounced effects for >8 weeks and no recovery within 8 weeks of last application; no full recovery by end of test

Other Comments and Notes:

N/A