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VIA FEDERAL EXPRESS

August 9, 2007

James H. Lecky, Ph.D.
Director, Office of Protected Resources
National Marine Fisheries Service
13th Floor, 1315 East-West Highway
Silver Spring, MD 20910

SUBJECT: SYNGENTA'S REVIEW OF THE NATIONAL MARINE FISHERIES SERVICES' LETTER OF MAY 29, 2007 (AND THE ACCOMPANYING TECHNICAL APPENDIX) TITLED "REQUEST FOR ENDANGERED SPECIES ACT SECTION 7 INFORMAL CONSULTATION ON THE ENVIRONMENTAL PROTECTION ACENGY'S RE-REGISTRATION AND USE OF ATRAZINE IN THE CHESAPEAKE BAY WATERSHED, SEPTEMBER 1, 2006"

Dear Dr. Lecky:

On behalf of Syngenta Crop Protection, Inc., a registrant for the pesticide atrazine, I am writing to express our concerns regarding the letter that you sent to the Environmental Protection Agency (EPA) on May 29, 2007, stating that the National Marine Fisheries Service (NMFS) did not concur with EPA's not likely to adversely affect determinations for atrazine and five listed species in the Chesapeake Bay. We believe that NMFS's conclusions are not supported by the science and, for the reasons summarized below and set forth in greater detail in the attached technical review, we respectfully urge you to reconsider your letter.

Background

On August 31, 2006, EPA issued an effects determination entitled the "Potential for Atrazine Use in the Chesapeake Bay Watershed to Affect Six Federally Listed Endangered Species" (USEPA 2006), pursuant to the settlement in *Natural Resources Defense Council v. United States Environmental Protection Agency, Civ. No: 03-CV-02444 RDB (filed March 28, 2006)*.

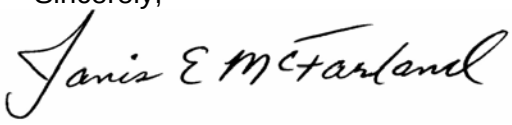
Using the procedures outlined in the "Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs" (Overview Document, U.S.EPA 2004), EPA concluded that atrazine will either have no effect (NE) or may effect but is not likely to adversely affect (NLAA), the listed shortnose sturgeon, or four listed turtle species (the Loggerhead turtle, Kemp's Ridley turtle, the Leatherback turtle and the Green turtle) evaluated in the Chesapeake Bay assessment. EPA initiated informal consultation with the National Marine Fisheries Service (NMFS).

On March 21, 2007, after informal consultation with NMFS, EPA issued an amended effects determination. The amended determination similarly concluded that atrazine use in the Chesapeake Bay is not likely to adversely affect the listed species evaluated. On May 29, 2007, NMFS issued a letter stating they did not concur with EPA's assessment concerning the finding of NLAA for the shortnose sturgeon and the four listed turtle species, and requested initiation of formal consultation under Section 7 of the Endangered Species Act[1]. NMFS included a technical appendix that detailed their response[2].

Syngenta has closely reviewed EPA's effects determinations as well as the response letter and technical appendix issued by NMFS. EPA conducted a thorough and conservative evaluation which resulted in "no effect" or "not likely to adversely affect" determinations for the species at issue. NMFS did not concur with EPA's findings, relying in part on a few studies that EPA scientists had discounted as unreliable or irrelevant. In addition, many of the conclusions reached by NMFS are based on erroneous toxicity values, studies with design flaws, an incorrect characterization of available monitoring data, and reliance on hypothetical effects that have not been shown to be likely to occur. For these reasons, we are concerned that the findings by NMFS do not reflect the best available scientific data.

To help facilitate further discussion, we have conducted our own technical review which we have enclosed for your consideration. We look forward to meeting with you in the near future to discuss our concerns in more detail.

Sincerely,



Janis McFarland
Head, Regulatory Affairs, NAFTA
Syngenta Crop Protection, Inc.

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[1] Letter from J. Lecky to A. Williams, Subject: "Request for Endangered Species Act Section 7 Informal Consultation on the Environmental Protection Agency's Re-Registration and Use of Atrazine in the Chesapeake Bay Watershed, September 1, 2006", May 29, 2007, http://www.epa.gov/oppfead1/endanger/effects/atrazine/2007/atrazine-ltr-may07.pdf

[2] "The National Marine Fisheries Service's Review of the Environmental Protection Agency's Pesticide Effects Determination for Atrazine on 6 Federally Listed Species in the Chesapeake Bay Watershed." Technical Appendix to NMFS 2007a, no author or date given, http://www.epa.gov/oppfead1/endanger/effects/atrazine/2007/atrazine-tech-appdx.pdf

cc: Steven Bradbury, OPP, EPA
James Gulliford, Assistant Administrator, OPPTS, EPA (Letter and Executive Summary)
Dale Hall, Director, FWS, DOI (Letter and Executive Summary)
Jane Luxton, General Counsel, NOAA
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David Bernhardt, Solicitor of the Interior, DOI
Jim Jones, Deputy Assistant Administrator, OPPTS, EPA (Letter and Executive Summary)
Bryan Arroyo, FWS, DOI
Debra Edwards, OPP, EPA (Letter and Executive Summary)

Technical Review

Syngenta Crop Protection's Technical Review of the National Marine Fisheries Service's Letter and Technical Appendix entitled "The National Marine Fisheries Service's Technical Review of the Environmental Protection Agency's Pesticides Effects Determination for Atrazine on 6 Federally listed Species in the Chesapeake Bay Watershed."

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1.0 EXECUTIVE SUMMARY

On August 31, 2006, the United States Environmental Protection Agency (EPA) issued an effects determination entitled the "Potential for Atrazine Use in the Chesapeake Bay Watershed to Affect Six Federally Listed Endangered Species" (USEPA 2006), pursuant to the settlement in *Natural Resources Defense Council v. United States Environmental Protection Agency, Civ. No: 03-CV-02444 RDB (filed March 28, 2006).*

Using the procedures outlined in the "Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs" (Overview Document, U.S.EPA 2004), EPA concluded that atrazine will either have no effect (NE) or may effect but is not likely to adversely affect (NLAA), the listed shortnose sturgeon, or four listed turtle species (the Loggerhead turtle, Kemp's Ridley turtle, the Leatherback turtle and the Green turtle) evaluated in the Chesapeake Bay assessment. EPA initiated informal consultation with the National Marine Fisheries Service (NMFS).

On March 21, 2007, after informal consultation with NMFS, EPA issued an amended effects determination. The amended determination similarly concluded that atrazine use in the Chesapeake Bay is not likely to adversely affect the listed species evaluated. On May 29, 2007, NMFS issued a letter (NMFS 2007a) stating they did not concur with EPA's assessment concerning the finding of NLAA for the shortnose sturgeon and the four listed turtle species, and requested initiation of formal consultation under Section 7 of the Endangered Species Act. NMFS included a technical appendix that detailed their response (NMFS 2007b).

Syngenta has reviewed the NMFS letter and technical appendix (NMFS review), and we provide our comments and findings in this document. In summary:

NMFS Used Incorrect Toxicity Values To Determine The Toxicity of Atrazine To Listed Species

- The toxicity values relied on in the NMFS review to support the asserted toxicity of atrazine to listed species are in many cases incorrect. For example, the NMFS review asserts that EPA failed to use the acute LC_{50} for brown trout of 27 ug/L in determining the toxicity of atrazine to the shortnose sturgeon. (NMFS review 2.2.2). The NMFS review claims that use of the brown trout value would result in a 74-fold lower Endangered Species LOC for the shortnose sturgeon. However, the NMFS review made a 1000-fold error: the correct LC_{50} for the brown trout is actually 27 mg/L not 27 ug/L. Thus, the LC_{50} value used by EPA for the sheepshead minnow is correct, and is the lowest reported valid fish LC_{50}. There are numerous other instances identified in Section 3 below where the NMFS review has used inappropriate toxicity values from the literature that are incorrect. The correct toxicity values do not support the NMFS review's claim that consideration of additional data would show that adverse effects to the shortnose sturgeon or other listed species are likely from environmental exposure to atrazine.

The NMFS Review's Criticism Of Avian Data As A Surrogate For The Sea Turtle Is Not Well Founded

- The NMFS review was critical of the use of avian data as a surrogate for the sea turtle assessment, and stated that effects on amphibians and fish are more appropriate. However, as discussed in detail below, it is well established that the phylogenetic relationship between birds and reptiles is closer than the relationship between reptiles and fish or amphibians, suggesting that the use of avian toxicity data as a surrogate for sea turtles is more relevant than the use of fish or amphibian data. Nevertheless, even if acute and chronic toxicity data for amphibians and fish are considered, they do not indicate a greater sensitivity compared to birds. Thus EPA's use of the avian surrogate data remains a conservative, upper bound assessment of the potential effect of atrazine on turtles.

The NMFS Review's Claims Of Sublethal Effects On The Assessed Species Are Based On Studies with Design Flaws That Render Them Unsuitable For Use As Assessment Endpoints

- The NMFS review cited literature studies that claim that sublethal effects of atrazine on fish result in the potential for atrazine effects on survival, growth and reproduction. However, the studies cited by the NMFS review concerning sublethal effects contained study design flaws, high data variability, and other factors that render the studies of limited scientific utility. Further the NMFS review did not demonstrate that studies provide "sufficient and reliable information establish[ing] a scientifically sound relationship between the proposed sublethal effect and the survival or reproductive capacity of an organism" (Overview Document). The majority of these studies were reviewed by EPA, and that Agency did not find them sufficiently complete or reliable to establish a likely cause and effect relationship on survival or reproduction.

The PRZM/EXAMS Models Represent Worst Case Exposure Scenarios Within The Chesapeake Bay Watershed

- To characterize the exposure of the listed species to atrazine in the Chesapeake Bay habitats, EPA used conservative modeling (PRZM/EXAMS) together with available monitoring data. The NMFS review was critical of EPA's use of standard PRZM/EXAMS scenarios due to concerns as to whether or not the "site-specific inputs used in the model are indicative of actual conditions" around the Chesapeake Bay. EPA's use of standard PRZM/EXAMS scenarios is well documented both within EPA's assessment and the Overview Document. Additionally, the EPA refined the standard scenarios to better focus on the habitats of the listed species within the Chesapeake Bay by utilizing local weather data, typical application dates for the region and maximum label rates for modeling exercises. EPA's modeled EECs are actually high-end estimates that do not reflect expected runoff reductions from label mandated set backs (66 ft for perennial/intermittent streams and 200 ft for

lakes/reservoirs). The modeled concentrations are also overestimates relative to available monitoring data in relevant habitats within the Chesapeake Bay.

- *The NMFS Review Incorrectly Relied On Monitoring Data From Areas Not Inhabited by The Listed Species*

In terms of monitoring data, the NMFS review suggested peak residue data from small, headwater streams indicated risk to the listed species. However, these data were collected at sites that are not representative of the listed species habitat. The NMFS review cited data associated with small freshwater non-tidal influenced streams. Due to the hydrology of small streams, these maximum concentrations are transient, and rapidly dissipate. For example, the cited value of 30 ppb came from a small headwater stream located in the Conestoga River watershed in Eastern Maryland that would be unsuitable habitat for the listed species: the 30 ppb value dissipated by approximately 50% within 6 hours. Further, data collected in relevant habitat indicates atrazine concentrations are orders of magnitude lower. As EPA indicates in their assessment, most of the detections for atrazine in the areas where the listed species occur are low. (Moreover, the data used in the NMFS review represent concentrations that occurred prior to full implementation of atrazine label changes that decreased application rates and required buffer set backs.) EPA's assessment followed the Overview Document and generated highly conservative, upper bound residue values that were used to determine NLAA determinations for the listed species.

EPA Properly Considered Available Data On Mixtures

The NMFS review concluded that the risk to listed species was likely underestimated because EPA did not consider potential exposure to co-occurring pesticides. However, in their evaluation of EPA's Overview Document (Williams and Hogarth, 2004), the Services' concluded that "*OPP will survey the open literature for any data addressing the effects of these types of mixtures, which it will use in its risk assessment process. The Services agree with this approach, and believe it will likely capture the best available scientific and commercial data."* EPA followed the Overview Document and conducted an exhaustive survey of the open literature for data addressing mixtures (see EPA Assessment Appendix A-Amended, J-1 and J-2) and included or considered the majority of the studies cited by the NMFS review in their assessment. Of the 14 studies that the NMFS review cited to support their conclusions regarding atrazine mixtures, four were not relevant as they either did not test atrazine or they did not examine synergistic/additive toxicity. Of the remaining studies, organic solvents were used as a vehicle for atrazine, and EPA properly concluded that claims of synergistic toxicity were questionable due to potential solvent-related effects on the study. For some studies EPA also concluded that the measured endpoints could not be linked to the listed species assessment endpoints or that the literature value was less sensitive than the endpoint used by EPA. Further, the NMFS review only characterized the potential hazard from exposure to multiple pesticides without providing the appropriate environmental context. Examination of available monitoring data from the Chesapeake Bay watershed demonstrates that atrazine does not co-occur with other pesticides at concentrations that

pose any risk to the listed species. As detailed below, the available information does not indicate that shortnose sturgeon or the listed sea turtle species will be adversely affected by exposure to multiple pesticides.

The NMFS Review Used An Incorrect Legal Standard For Evaluating "Uncertainties"

- The NMFS review raises questions regarding three "areas of uncertainty" (toxicity of mixtures, sublethal endpoints, and extrapolation from surrogate species) for which it criticized EPA's risk assessment as incomplete. EPA's approach to each of these areas in the effects determination is consistent with the process outlined in the EPA Overview Document, which NMFS previously concurred with (Williams and Hogarth, 2004). "Likely to adversely affect" determinations cannot be reasonably based on mere speculation or possibilities. Limited evidence (particularly that which does not meet data quality requirements) which raises a mere possibility that an effect might occur does not satisfy the standard for showing that the effect is "likely" to occur. As NMFS notes, the objective is to make the best possible prediction "of the likely outcome," not of theoretical outcomes. *See also* 51 Fed. Reg. 19926, 19933 (June 3, 1986) ("there must exist more than a mere possibility" that an action will cause adverse effects in order to find that such effects are likely). An uncertainty is neither likely nor probable, and the NMFS review's conclusions that "uncertainties" in these areas warrant a finding of likely to adversely affect are not supported.

Based on these reasons, which are presented in detail below, the NMFS review's conclusions that atrazine is likely to adversely affect the shortnose sturgeon and four species of sea turtles in the Chesapeake Bay watershed are not supported by the best available data.

2.0 INTRODUCTION

Syngenta has reviewed the National Marine Fisheries Service's (NMFS) letter to Arthur – Jean Williams dated May 29, 2007 (NMFS, 2007a) and associated Technical Appendix (NMFS 2007b). We are providing comments on the May 29 letter and Technical Appendix (hereafter referred to as the NMFS review) and, where appropriate, additional technical details.

NMFS' ecological conclusion on the potential that atrazine is likely to adversely affect the listed sturgeon and turtle endangered species of the Chesapeake Bay was not supported by the best available scientific and commercial data. The NMFS review used incorrect acute and chronic values and has misinterpreted data from the ECOTOX data base and the open literature. The NMFS review discounted the use of refinements, as described in the Overview Document. The Overview Document was supported by NMFS in their joint letter with the U.S. Fish and Wildlife (Williams and Hogarth 2004). The NMFS review did not (1) use relevant available monitoring data, or (2) recognize the importance of using duration, frequency and magnitude of exposure data, as included in the Comprehensive Aquatic Systems Model for Atrazine (CASM_Atrazine), and which is critical to a science based risk assessment for atrazine. The NMFS review also incorrectly characterized the potential hazard and exposure of the listed species to mixtures of pesticides as a result of the use of atrazine. These aspects are all reviewed in detail in this document.

3.0 THE NMFS REVIEW USED INCORRECT TOXICITY VALUES TO DETERMINE THE TOXICITY OF ATRAZINE TO LISTED SPECIES

Our review of the acute and chronic effect values relied on in the NMFS review identifies a number of factual errors as detailed in Table 1. These errors in the atrazine hazard profile for acute and chronic effects led to invalid conclusions.

<u>Incorrect values cited by NMFS</u>

- The NMFS review reported a brown trout LC_{50} = 27 µg/L (NMFS Technical Appendix, page 16, starting on line 9). This value is incorrect. The correct LC_{50} for this brown trout study is 27 mg/L (i.e. 27,000 µg/L) as accurately evaluated by EPA in their Risk Assessment (EPA Appendix A, p 9). NMFS reported the value to be 1000-fold lower than was determined by the results. As a result, many statements made in the NMFS review to characterize the risk atrazine poses to sturgeon are incorrect. For example:
    - NMFS Technical Appendix, page 16, line 10: "…an LC50 of 27 ug/L would result in atrazine concentrations of 1.35 ug/L or greater triggering the Endangered Species LOC…." However, use of the correct LC_{50} value (27,000 µg/L) would result in atrazine concentrations greater than 1,350 µg/L triggering the LOC compared to EPA's use of 100 µg/L.

- NMFS Technical Appendix, page 16, line 12 to line 19: "…in the atrazine BE [EPA's Biological Evaluation] EPA selected 2000 ug/L from a sheepshead minnow study to represent the LC50 … If shortnose sturgeon are as sensitive as brown trout, the BE underestimated the acute lethality to sturgeon by more than 74 fold…" The accurate value of 27,000 µg/L indicates brown trout are less sensitive to atrazine compared to the sheepshead minnow (LC_{50} = 2,000 ug/L). The use of 2,000 ug/L by EPA represents the lowest valid fish LC_{50} (Table 1). Further, five other sheepshead LC_{50} values are listed in ECOTOX and range from 2,300 µg/L to 16,200 µg/L. These additional values indicate an LC_{50} = 2,000 µg/L may overestimate atrazine sensitivity for the sheepshead minnow thereby adding to the conservativism of EPA's assessment.

- The NMFS review reported a guppy LC_{50} = 50 µg/L (NMFS Technical Appendix, page 19, Table 2). This value is incorrect. The correct value as listed in ECOTOX is an LD_{100} = 50 ml/L. Since ml/L units are atypical for fish acute lethality studies, further evaluation of this study indicates the correct units are 50 mg/L based on the lowest concentration tested: "The LD100 for guppies was found to be at the 50 ml/liter level. The concentration of 0.5 mg/liter was harmless for fish" (Gzhetotskii et al., 1977). The NMFS review errantly reported the value to be 1000 fold lower than was determined by the authors of the report.
    - Data in ECOTOX (8 studies) indicate guppies are less sensitive to atrazine (LC50s range from 4,300 to 117,400 µg/L) compared to the lowest sheepshead minnow LC_{50} (Table 1).

- The NMFS review reported a loach LC_{50} = 147 µg/L (NMFS Technical Appendix, page 19, Table 2). This value is incorrect. ECOTOX indicates this value is an EC_{50} not an LC_{50}. Further evaluation of this study indicates this value is associated with the green algae, *Selenastrum capricornutum,* not the loach.

- The NMFS review failed to place the sheepshead minnow value (LC_{50} = 2,000 µg/L) used by EPA in appropriate context: this value is the lowest valid fish acute toxicity value for atrazine. The ECOTOX database indicates that there are approximately 90 acute toxicity values representing at least 20 species that have an LC_{50} value greater than the sheepshead minnow value used by EPA. Therefore these data do not support the NMFS review which states the EPA is underestimating risk.

- The NMFS review referenced two chronic NOEC values for channel catfish and rainbow trout (50 µg/L and 54 µg/L, respectively), (NMFS Technical Appendix, page 19, Table 2). Syngenta was not able to identify these values in ECOTOX nor were we able to verify these values with any of the known fish chronic toxicity values. Therefore, it is unclear what studies were relied on to support these values.

- Regardless, the chronic NOEC used by EPA (NOEC = 65 µg/L) is based on a study where fish were constantly exposed to atrazine for 44 weeks. There was no effect of atrazine on reproduction in this study.

- The NMFS review concluded that "[t]he dietary toxicity data for birds used in the EPA risk quotient analysis introduces an unquantifiable level of uncertainty to the BE given the distant taxonomic connection between birds and reptiles…[and] [s]ince only avian toxicity data were used as surrogates for sea turtles, atrazine's risk is potentially underestimated" (NMFS Technical Appendix, p 22, lines 20-23). However, it is well established that the phylogenetic relationship between birds and reptiles is closer than the relationship between reptiles and fish or amphibians (e.g., Feller and Hedges (1998), Hedges and Poling 1999), suggesting that the use of avian toxicity data as a surrogate for sea turtles is more relevant than the use of fish or amphibian data. Moreover, as agreed by the Services in their evaluation of the Overview Document, (Williams and Hogarth, 2004), "[w]here no other data is available, the Services agree that the toxicity tests on surrogate species constitutes the best available information to analyze the toxicological sensitivities of untested species (pg. 13)." Since sea turtle-specific data for atrazine are not available, EPA relied on avian toxicity data to conduct an extensive evaluation that included dietary, dermal and drinking water risk. Therefore, the EPA sea turtle effect determinations are appropriate. Further, it is our observation that contrary to the NMFS review's suggestion, the acute and chronic toxicity data for amphibians and fish do not indicate a greater sensitivity compared to birds and thus EPA's use of the avian surrogate data for reptiles is a conservative, upper bound assessment of the potential effect of atrazine on turtles. Additionally,
    - Available acute toxicity data for amphibians indicates that acute lethality is not expected at concentrations exceeding 20,000 µg/L. Further, toxicity data for reptilian species at atrazine concentrations up to 14,000 µg/L did not result in embryonic mortality to snapping turtles or alligators.
    - Available chronic toxicity data for amphibians indicates that exposure to atrazine for 2 years post-metamorphosis does not effect reproduction in amphibians at concentrations up to 25 µg/L and no lethality was observed (DuPreez et al. 2007 and Solomon et al. 2007).

Table 1. Evaluation of Acute and Chronic Effect Values Cited in the NMFS review.

Listed Species Assessment Endpoint	Measurement endpoint cited by EPA	Endpoint cited in the NMFS review (Tech. Appendix Reference)	EPA Evaluated Study	EPA[1] and/or Syngenta Evaluation
Sturgeon survival	2000 µg/L (LC50) sheepshead minnow	27 µg/L (LC50) brown trout (Section 2.2.2, p 16, Table 2, p 19, and p 22 lines 5-7)	Y	The NMFS review reported an incorrect value. EPA OPP considered this value in their assessment (EPA Appendix A, page 9, Table A-8, Grande et al. 1994) and correctly reported the LC_{50} as 27 mg/L (i.e. ppm) not µg/L (i.e. ppb). EPA OW also considered this value (EPA OW 2003, p. 8, 66, and others) and correctly reported the LC_{50} as 27 mg/L. This comparison indicates brown trout are less sensitive to atrazine compared to the sheepshead minnow.
		50 µg/L guppy (Table 2, p 19)	Y	The NMFS review reported an incorrect value. EPA included this study in Appendix J-1. ECOTOX indicates this value represents an LD_{100} = 50 ml/L not an LC_{50} = 50 µg/L. The concentration units for this study indicate the correct concentration is ppm not ppb (µg/L), which indicates this species is less sensitive to atrazine compared to the sheepshead minnow. The abstract for the report (available at http://toxnet.nlm.nih.gov/index.html) indicates ml/L is a translation error and that the correct LD_{100} units are 100 mg/L. Other ECOTOX guppy LC_{50} values are available and indicate the LC_{50} for this species is above the sheepshead minnow value (n = 8; LC_{50} range 4,300 to 117,400 µg/L).
		147 µg/L loach (Table 2, p 19)	Y	The NMFS review reported an incorrect value. EPA included this study in Appendix J-1. The ECOTOX endpoint is cited as an EC_{50} not an LC_{50}; EC_{50}'s are typically associated with aquatic plant studies. In fact the report associated with this value (Gaggi et al. 1995) clearly indicates that the endpoint is an EC_{50} = 147 µg/L for the green algae *Selenastrum capricornutum*, not the loach. EPA OW also considered this value (EPA OW 2003, p. 45 and 101) and correctly reported the species as *Selenastrum capricornutum*.
		220, 310, 340, 220 and 240 µg/L catfish (Table 2, p 19)	Y	EPA adequately considered these values in their assessment (see EPA Appendix A, Table A-11 with associated text, page 20, and page 90).
		660 µg/L atrazine 80 WP rainbow (Table 2, p 19)	Y	EPA adequately considered these values in their assessment (see EPA Appendix A, Table A-11 with associated text, page 20, Table A-15, and page 90). This value does not represent an acute lethality endpoint since the length of exposure was 27 days.
		LC_{50} values above 2000 µg/L	Y	The ECOTOX database also indicates that there are approximately 90 acute toxicity values representing at least 20 species that have an LC_{50} value greater than the sheepshead minnow value used by EPA (2,300 µg/L to 117,400 µg/L). Therefore these data do not support the argument that EPA is underestimating risk.

[1] USEPA 2006. Effect Determination for Atrazine. Potential for atrazine use in the Chesapeake Bay watershed to affect six federally listed species. September 1, 2006.

Table 1. Evaluation of Acute and Chronic Effect Values Cited in the NMFS review. (continued)

Listed Species Assessment Endpoint	Measurement endpoint cited by EPA	Endpoint cited in the NMFS review	EPA Evaluated Study	EPA and/or Syngenta Evaluation
Sturgeon growth, reproduction, and distribution	65 µg/L (NOEC brook trout)	50 µg/L (NOEC) channel catfish teratogenic effects, 54 µg/L rainbow embryo survival (Table 2, p 19)	Unknown	Syngenta was not able to identify these values in ECOTOX nor were we able to justify these values with any of the known fish chronic toxicity values. It is unclear where NMFS located these data. Regardless, the use of 65 µg/L as a chronic NOEC is extremely conservative as it represents atrazine exposure over a full life cycle (44 weeks) and the NOEC is based on functional endpoints (i.e. growth and reproduction). Additionally, the NOEC of 65 µg/L is 17 times lower than the lowest chronic NOAEC for estuarine fish. Further, four fish full life cycle studies have been conducted (fathead minnow, brook trout, bluegill sunfish) and none of these studies indicated atrazine affected functional reproduction endpoints. The NOECs from the full life cycle studies range from 65 µg/L – 210 µg/L.
Sea turtle survival	>5000 mg/kg diet (LC50) Mallard	Not available (Table 2, p 19)	Y	The NMFS review stated that the use of avian toxicity information to characterize potential effects on sea turtles potentially underestimated risk and further stated that sea turtles will be exposed via dietary, dermal and oral routes. EPA evaluated all of these potential routes of exposure to sea turtles. The NMFS review argued that sublethal amphibian data should have been used. EPA's Overview Document indicates avian data is a standard surrogate for these species and the Services concurrence letter agrees to the use of surrogate data. Syngenta also suggests that the weight of evidence for other vertebrates does not indicate the potential for acute risk. EPA reviewed numerous studies for aquatic and terrestrial vertebrates in their assessment. Specific to other amphibian data, EPA indicated that acute lethality is not expected at concentrations exceeding 20,000 µg/L (EPA Appendix A). Reptilian data are also available. In those studies, although the authors did not directly measure mortality as an endpoint, neither De Solla (2006), Crain (1997), nor Crain (1999) indicated mortality occurred to snapping turtles or alligators (atrazine exposed to eggs at concentrations of 8,100 or 14,000 ppb, respectively). Similarly Gross (2001a and b) did not observe any mortality when red-eared slider or alligator eggs were exposed to 500 ppb atrazine. Therefore, available reptilian data do not indicate the potential for acute toxicity of atrazine to developing reptile embryos.
Sea turtle growth, reproduction, and distribution	225 mg/kg diet (NOEC) mallard	Not available (Table 2, p 19)	Y	Similar to the comments above, the NMFS review indicated that the use of avian toxicity information to characterize potential chronic effects on sea turtles is inappropriate. EPA reviewed numerous studies for aquatic and terrestrial vertebrates (see comments for fish above). Specific to other amphibian data, EPA indicated that chronic lethality is not expected at concentrations exceeding 200 µg/L (EPA Appendix A). Further, data indicate that chronic exposure to atrazine for 2 years post-metamorphosis does not affect reproduction in amphibians at concentrations up to 25 µg/L and no lethality was observed (DuPreez et al. 2007 and Solomon et al. 2007).

EPA OPP's policy for assessing risk to any given species is to utilize the lowest effect value that meets certain data quality criteria. The values used by EPA in their assessment meet those criteria. EPA's Office of Water also relies on quality data and use a conservative approach to establish conservative Ambient Aquatic Life Water Quality

Criteria. The methods used to derive Aquatic Life Criteria are described elsewhere (http://www.epa.gov/waterscience/criteria/aqlife.html#guide). In the derivation of the Draft Ambient Aquatic Life Water Quality Criteria for Atrazine, OW utilizes species and genus mean values. Specific to saltwater fish, OW used an effect value of 4,208 µg/L based on LC_{50} data for sheepshead minnow compared to OPP's use of 2,000 µg/L.

In summary, EPA utilized the best available data in their effects determination. Due to the factual errors and misrepresentations in the NMFS review, many statements and conclusions are inaccurate and need to be corrected.

4.0 THE NMFS REVIEW'S CLAIMS OF SUBLETHAL EFFECTS ON THE ASSESSED SPECIES ARE BASED ON STUDIES WITH DESIGN FLAWS THAT RENDER THEM UNSUITABLE FOR USE AS ASSESSMENT ENDPOINTS

The NMFS review did not critically review the studies used for their arguments related to potential sublethal effects. The review stated that "a variety of relevant studies that indicate toxicity of atrazine at low environmentally realistic concentrations were not incorporated into EPA's risk quotient analysis (NMFS Technical Appendix, p 22, lines 10-12)." However, in their evaluation of EPA's Overview Document (Williams and Hogarth, 2004) , the Services' "have deemed appropriate the existing sublethal endpoints that are included by OPP in its risk assessment process, and the manner in which they are used for purposed of assessing potential sublethal effects." The Services also concluded that "OPP has the option of including additional sublethal data in its risk assessment, if sufficient and reliable information establish a scientifically sound relationship between the proposed sublethal effect and the survival or reproductive capacity of an organism" Id. at 20. The NMFS review of the open literature on potential sublethal effects does not demonstrate a "scientifically sound relationship between the proposed sublethal effect and the survival or reproductive capacity of an organism." Moreover, EPA's evaluation and in-depth analysis of the available literature consistent with the Overview Document did not establish this link. Therefore the data utilized by NMFS are not appropriate for calculating risk quotients.

The NMFS review also did not recognize study design flaws and high data variability in the studies it relies on – factors that render the studies of limited scientific utility. The majority of these studies have previously been addressed by EPA (one study had not been published and thus was not available when EPA completed its review), and Syngenta is providing additional detail for some studies (Table 2).

- The NMFS review cited an endpoint of 10 ug/L (Tierney et al., 2007) for rainbow trout and indicated that "altered olfactory-mediated behaviors in fish that {sic} may have implications for survival, growth, and reproduction" (NMFS Technical Appendix p. 19, Table 2). However, the NMFS review did not provide an evaluation of the study.
    - Tierney et al. (2007) actually reported that "none of the pesticides or vehicle controls evoked preference or avoidance responses at the highest concentrations tested" (pg. 58 in paper).

- The neurophysiological responses reported were potentially due to solvent-related effects since experiments were performed using only one 5-mg/ml stock of atrazine in acetone. Even though the authors tested a vehicle control using 200 µL acetone, it appears as though the 10-µg/L and 100-µg/L treatments contained ~272 µL and ~2720 µL acetone. Therefore, solvent effects on neurophysiological endpoints cannot be excluded.
- With regards to the neurophysiological response following exposure of 10 or 100 µg/l for 30 minutes, all fish recovered within 3 minutes when placed in clean water. Therefore, even if this response is not solvent-related, the response was not sustained and the fish fully recovered within minutes.
- Finally, a full life cycle (44 week continual exposure) study in a similar species (brook trout) indicates that there is no effect of atrazine on "survival, growth, and reproduction"; NOEC = 65 µg/L (Macek et al. 1976).

- The NMFS review cited an endpoint of 0.5 and 5.0 µg/L (Saglio and Triasse, 1998) and indicated alterations in swimming and social behaviors (NMFS Technical Appendix, p. 19, Table 2). However, EPA's previous evaluation (from their Chesapeake Bay assessment, provided in Table 2, indicated the results of Saglio and Triasse (1998) are subjective and can therefore not be used quantitatively. Further, the data associated with this study are highly variable (coefficients of variations as high as 100-200%) and Syngenta questions the statistical methodology used in the study. This study should not be used to select endpoints for risk assessment purposes. EPA's use of survival, growth, and reproduction endpoints from fish full life cycle studies in their risk characterization is scientifically valid compared to using subjective behavioral endpoints as suggested by the NMFS review. Additionally, the EPA's approach is consistent with the Overview Document.

- The NMFS review cited an endpoint of aromatase induction on a turtle-derived cell line (Keller and McClellan-Green, 2004) and suggested this could affect sea turtle growth, reproduction, and distribution (NMFS Technical Appendix, p. 19, Table 2). This study is not appropriate for endpoint selection for risk assessment:
  - The authors reported slight (and similar) induction in the 1.0 µM (=216 µg/L) and 10 µM (=2157 µg/L) treatments (but not 30 µM = 6471 µg/L) in cells pre-treated with atrazine for 24 hrs. However, this induction was not observed if cells were pre-treated with atrazine for 48 or 72 hrs. Therefore, there was no dose- or time-dependent responses.
  - In addition, the authors conceded that positive control responses were negative, demonstrating that these cell lines were not appropriate for evaluating chemically-mediated aromatase induction.
  - Finally, exposure concentrations are well above any modeled or monitored atrazine concentrations in the Bay, its tributaries, or other relevant habitats.

- The NMFS review cited two additional endpoints of aromatase induction (Vonier et al., 1996 and Crain et al. 1997) and correlated them with atrazine exposure and reproductive effects to sea turtles (NMFS Technical Appendix, p. 19, Table 2). However neither of these studies can be correlated to actual environmental atrazine concentrations in the Bay, its tributaries or other relevant habitats, nor can they be correlated to actual reproductive effects in sea turtles:
    - Vonier et al. 1996
        - The authors used an atrazine concentration of 20.7 µM or 4300 µg/L which is not environmentally relevant.
        - The authors reported that atrazine (IC_{50} = 20.7 µM = 4300 µg/L) exhibited >2500-fold lower affinity for the alligator estrogen receptor compared to 17β-estradiol – a circulating endogenous hormone. However, even the most estrogenic chemical tested (o,p'-DDD) was approximately 280-fold less potent than 17β-estradiol for binding the alligator estrogen receptor. Therefore, even under exposure concentrations and conditions that are not environmentally realistic, atrazine is not likely to activate the alligator estrogen receptor in the presence (or absence) of endogenous male or female levels of 17β-estradiol. These data suggest that atrazine will not affect reproduction in reptiles (i.e. sea turtles).

    - Crain et al., 1997
        - The NMFS review erroneously indicated that aromatase was induced. Crain et al. (1997) reported that atrazine did not significantly induce aromatase activity within alligator hatchlings, even at levels of atrazine significantly higher than environmentally realistic concentrations.
        - The authors used topical applications of atrazine (8,100 or 14,000 µg/L) to alligator eggs which are much higher than any concentrations expected in the environment.
        - Moreover, exposure of alligator eggs will not occur in the environment via a topical application in ethanol – as the authors note in the Materials and Methods, a topical ethanol application is frequently used to ensure that tested compounds are transported into the reptilian eggs after topical application."

Table 2. Evaluation of Sublethal Effect Values Cited in the NMFS review.

Listed Species Assessment Endpoint	Toxicity Value used by EPA	Sublethal Effect Cited in the NMFS review	Study Evaluated by EPA?	EPA Study Evaluation within USEPA 2006	Syngenta Study Evaluation
Sturgeon survival	LC_{50} = 2000 ug/L (sheepshead minnow)	LOEL = 2 ug/L. Effect on gill physiology suggest potential compromised ability of fish to survive in saltwater (Waring and Moore, 2004) (Table 2, p 19)	Yes	Main Document (pg. 104) and Appendix A (pgs. 20-23): EPA concluded that "[b]ased on distributional evidence, older juvenile and adult shortnose sturgeon are limited to oligohaline and low mesohaline regions of estuaries (<15 ‰)…[and] [t]he salinity used by Waring and Moore (2004) simulated full strength seawater (33 ‰). Therefore the relevance of findings from this study to the shortnose sturgeon is questionable." Overall, when considering this and other studies, EPA concluded that these papers do not demonstrate a "quantitative link between these sublethal effects and the selected assessment endpoints [survival, growth, and reproduction] for the assessed species (pg. 104 of Main Document)."	Consistent with EPA's conclusions.

Table 2. Evaluation of Sublethal Effect Values Cited in the NMFS review. (continued)

Listed Species Assessment Endpoint	Toxicity Value used by EPA	Sublethal Effect Cited in the NMFS review	Study Evaluated by EPA?	EPA Study Evaluation within USEPA 2006	Syngenta Study Evaluation
		10 ug/L. Altered olfactory-mediated behaviors in fish that may have implications for survival, growth, and reproduction (Tierney et al., 2007) (Table 2, p 19)	No	No EPA evaluation available – study published after EPA completed evaluation.	This study evaluated olfactory-based behavioral and neurophysiological responses in atrazine-exposed rainbow trout. While the NMFS review claimed that "altered olfactory-mediated behaviors in fish that may have implications for survival, growth, and reproduction," Tierney et al. actually reported that "none of the pesticides or vehicle controls evoked preference or avoidance responses at the highest concentrations tested (pg. 58 in paper)." Moreover, the neurophysiological responses reported were potentially due to solvent-related effects since experiments were performed using only one 5 mg/ml stock of atrazine in acetone. Given the reported solubility of atrazine in water, there is no apparent reason to use a solvent at the concentrations tested in this study. The authors apparently added larger volumes of stock for higher treatment concentrations. Even though the authors tested a vehicle control using 200 uL acetone, based on our calculations it appears as though the 10-ug/L and 100-ug/L treatments contained ~272 uL and ~2720 uL acetone. Given the acetone levels found in each of the study's treatments, solvent effects on neurophysiologic endpoints cannot be excluded (especially since a negative control was not tested). Another important point: Based on the electro-olfactogram response (neurophysiological endpoint), fish exposed to 10 or 100 ug/l for 30 min recovered within 3 min when placed in clean water -- therefore, even if this response is "real" (and not solvent-related), the response is not sustained and fish fully recover within minutes once atrazine is not present. Finally, a full life cycle (44 week continual exposure) study in a similar species (brook trout) indicates that there is no effect of atrazine on "survival, growth, and reproduction" at 10 µg/L. The NOEC established in this study was 65 µg/L.
		LC01= 29 ug/L for embryo-larval survival (Birge et al., 1979) (Table 2, p 19)	Yes	Appendix A (pg. 13): EPA concluded that the Birge et al. 1979 study was classified as "Supplemental" while the Macek et al. 1976 study was classified as "Acceptable". Therefore, the 65-ug/L NOEC for brook trout was used for chronic RQs.	This LC01 value is derived from a 27-day flow-through chronic exposure and does not represent an acute LC50 value. Regardless, the most conservative chronic fish endpoint was used by EPA (NOEC = 65 µg/L) which represents full life cycle atrazine exposure (44 weeks) and the NOEC is based on functional endpoints (i.e. growth and reproduction). Further, four fish full life cycle studies have been conducted (fathead minnow, brook trout, bluegill sunfish) and none of these studies indicated atrazine affected functional reproduction endpoints. The NOECs from the full life cycle studies range from 65 µg/L – 210 µg/L.

Table 2. Evaluation of Sublethal Effect Values Cited in the NMFS review. (continued)

Listed Species Assessment Endpoint	Toxicity Value used by EPA	Sublethal Effect Cited in the NMFS review	Study Evaluated by EPA?	EPA Study Evaluation within USEPA 2006	Syngenta Study Evaluation
Sturgeon growth, reproduction, and distribution	65 ug/L (NOEC) (brook trout)	0.5 ug/L reduced expressible milt in male fish and reduced detection of amino acids (Moore and Lower, 2001) (Table 2, p 19)	Yes	Main Document (pg. 104) and Appendix A (pgs. 20-23): EPA concluded that "a negative control was not included as part of the study design; therefore, potential solvent effect cannot be evaluated…[and] the study did not determine whether the decreased response of olfactory epithelium to specific chemical stimuli would result in similar responses in intact fish." Overall, when considering this and other studies, EPA concluded that these papers do not demonstrate a "quantitative link between these sublethal effects and the selected assessment endpoints [survival, growth, and reproduction] for the assessed species (pg. 104 of Main Document)."	Consistent with EPA conclusion.
		5.0 ug/L reduced priming effect in female fish (Table 2, p 19)			
		0.5 ug/L affected burst swimming speed (Saglio and Trijasse, 1998) (Table 2, p 19)	Yes	Appendix A (pg. 18): EPA concluded that "[t]his study shows a 24-hour exposure at 5 ug/L atrazine significantly affected aspects of swimming, positioning in water column, increased number of mouth openings at the surface, and social behaviors, although the results of this study appear to be rather subjective."	Data reported within this study are highly variable (some CVs as high as 100-200%). Moreover, data are categorical and not numerical, yet authors expressed data as mean/standard deviation and used parametric (and not non-parametric) statistical analysis. Combined with the highly variable data, this is inappropriate statistical methodology.
		5.0 ug/L affected schooling, surface, and orientation behaviors (Table 2, p 19)			
		50 ug/L altered plasma testosterone and vitellogenin levels in fish (Wieser and Gross, 2002) (Table 2, p 19)	Yes	Main Document (pg. 103) and Appendix A (pg. 17): EPA concluded "that there is a high degree of variability with the Vtg effects in these studies, which confounds the ability to resolve the effects of atrazine on plasma steroids and vitellogenesis."	Consistent with EPA conclusion.

Table 2. Evaluation of Sublethal Effect Values Cited in the NMFS review. (continued)

Listed Species Assessment Endpoint	Toxicity Value used by EPA	Sublethal Effect Cited in the NMFS review	Study Evaluated by EPA?	EPA Study Evaluation within USEPA 2006	Syngenta Study Evaluation
Sea turtle growth, reproduction, and distribution	225 mg/kg diet (NOEC) (mallard duck)	No concentration cited aromatase induction on turtle-derived cell line (Keller and McClellan-Green, 2004) (Table 2, p 19)	Yes	EPA rejected the use of this study due to an *in vitro* exposure scenario.	This study used testis-derived green turtle primary cell lines to evaluate whether atrazine pre-treatment resulted in aromatase induction (as measured by conversion of radioactive androstenedione). The authors reported slight (and similar) induction in the 1.0 uM (=216 ug/L) and 10 uM (=2157 ug/L) treatments (but not 30 uM = 6471 ug/L) in cells that were pre-treated with atrazine for 24 hrs. However, this induction was not observed if cells were pre-treated with atrazine for 48 or 72 hrs. Therefore, there was no dose- or time-dependent responses. A one-way ANOVA (tested within each time-point) was used instead of a 2-way ANOVA (dose by time), likely resulting in statistical errors with the significant 24-hr responses observed. In addition, the authors conceded that "[f]ailure of known inducers to significantly increase aromatase activity suggests that part of the pathway for induction may be missing in this cell line (pgs. 349-350 in paper)." In other words, since positive controls were negative, these cell lines are not appropriate for evaluating chemically-mediated aromatase induction.
		No concentration cited aromatase induction correlated with atrazine exposure and to reproductive effects (Vonier et al., 1996) (Table 2, p 19)	Yes	EPA rejected the use of this study due to an *in vitro* exposure scenario.	This study evaluated whether atrazine (and other chemicals) interacts with the alligator ER using a competitive binding assay (using radioactive estradiol). The authors reported that atrazine exhibited >2500-fold lower affinity (IC50 = 20.7 uM = 4300 ug/L) for the alligator estrogen receptor than 17β-estradiol (positive control) (IC50 = 0.0078 uM = 2.1 ug/L) – a circulating endogenous hormone. Indeed, even the most estrogenic chemical tested (o,p'-DDD) was approximately 280-fold less potent than 17β-estradiol for binding the alligator estrogen receptor. Therefore, under environmentally realistic levels, atrazine is not likely to activate the alligator estrogen receptor in the presence (or absence) of endogenous male or female levels of 17β-estradiol.

Table 2. Evaluation of Sublethal Effect Values Cited in the NMFS review. (continued)

Listed Species Assessment Endpoint	Toxicity Value used by EPA	Sublethal Effect Cited in the NMFS review	Study Evaluated by EPA?	EPA Study Evaluation within USEPA 2006	Syngenta Study Evaluation
Sea turtle growth, reproduction, and distribution	225 mg/kg diet (NOEC) (mallard duck)	No concentration cited aromatase induction correlated with atrazine exposure and to reproductive effects (Crain et al., 1997) (Table 2, p 19)	No	No EPA evaluation available.	This study evaluated whether topical application of atrazine onto alligator eggs resulted in alterations in steroidogenesis-related endpoints. While the NMFS review claimed otherwise, the authors reported that atrazine did not significantly induce aromatase activity within alligator hatchlings, even at levels of atrazine significantly higher (ppm levels) than environmentally realistic concentrations. Moreover, exposure of alligator eggs will not occur in the environment via a topical application in ethanol – as the authors note in the Materials and Methods, a topical ethanol application is frequently used to ensure that test compounds are transported into the reptilian eggs after topical application.
		0.1 ug/L (LOEL) hermaphrodism in leopard frog (Hayes et al., 2002a/b) (Table 2, p 19)	Yes	Appendix A (pg. 23): EPA cited 2003 white paper on amphibian data and concluded that "the weight-of-evidence does not show that atrazine produces consistent, reproducible effects across the range of exposure concentrations and amphibians tested."	Additionally, following the June 2003 FIFRA Scientific Advisory Pane, EPA required Syngenta to conduct a study that would allow EPA to evaluate if exposure to atrazine could affect amphibian gonadal development. Syngenta has completed this study and submitted it to the Agency. These data are the subject of the upcoming October SAP.
		1 ug/L (LOEL) feminization in leopard frog (Hayes et al., 2002a/b) (Table 2, p 19)	Yes	Appendix A (pg. 23): EPA cited 2003 white paper on amphibian data and concludes that "the weight-of-evidence does not show that atrazine produces consistent, reproducible effects across the range of exposure concentrations and amphibians tested."	See Preceding Study Evaluation.

Again EPA OPP's policy for assessing risk to any given species is to utilize the lowest effect value that meets certain data quality criteria. The values used by EPA in their assessment meet those criteria. Furthermore, EPA has already evaluated the sublethal amphibian data the NMFS review references using a Scientific Advisory Panel. Status of EPA's review of this information is summarized below,
(http://www.epa.gov/oppsrrd1/reregistration/atrazine/atrazine_update.htm)

> *"At present, there is no consistent evidence that atrazine exposure affects gonadal development in amphibians. Since the June 2003 FIFRA Scientific Advisory Panel (SAP) report, EPA has required the atrazine registrant to conduct a study that will enable the Agency to determine if exposure to atrazine can affect amphibian gonadal development. These data are expected to be completed in 2007, after which the Agency will discuss this issue again with the SAP.*
>
> *EPA is concerned about the potential for atrazine to affect amphibian gonadal development. After a careful assessment of 17 studies from the published literature and from the registrant, EPA concluded that none of them showed that atrazine produced consistent, reproducible effects across the range of exposure concentrations and amphibian species tested in the studies. No firm conclusion could be drawn about whether atrazine affects frogs' sexual development. The Agency also concluded that atrazine should be subjected to more definitive testing. The FIFRA SAP convened in June 2003 supported this conclusion, and agreed that additional studies are needed to determine if there is a relationship between atrazine exposure and gonadal developmental effects in amphibians.*
>
> *The SAP reviewed and approved EPA's proposed study approach to address uncertainties identified in the original studies. The protocol that the registrant Syngenta used to conduct the additional atrazine amphibian studies is consistent with the one recommended by EPA and concurred with by the FIFRA SAP. Results are expected to address uncertainties concerning the potential relationship between atrazine exposure and gonadal development in amphibians.*
>
> *Once data are received and reviewed by the Agency and after subsequent review by the SAP, if the studies show that environmentally relevant concentrations affect amphibian gonadal development, EPA will initiate the appropriate actions to address potential risks, consistent with FIFRA requirements."*

Syngenta has completed the required studies and submitted them to the EPA for evaluation. These data will be reviewed by another Scientific Advisory Panel in October 2007 (http://www.epa.gov/scipoly/sap/#october).

5.0 THE PRZM/EXAMS MODELS REPRESENT WORST CASE EXPOSURE SCENARIOS WITHIN THE CHESAPEAKE BAY WATERSHED

The NMFS review argued that EPA's exposure scenarios are inappropriate for assessing shortnose sturgeon and the listed sea turtle species. Specifically, the NMFS review argued that the standard PRZM/EXAMS scenarios were not calibrated to the Chesapeake Bay (Section 2.1.1 p 11 – 12) and modification of the PRZM/EXAMS models (Section 2.1.2 p 12), use of Chesapeake Bay monitoring data (Section 2.1.3 p 12-13), or the use of CASM_Atrazine (Sections 2.2.5.1 and 2.2.6 pp 23-29) were not conservative.

However, the conservative modeling techniques EPA used to refine the risk assessment were consistent with the Overview Document. The Overview Document (p 78) clearly indicates that PRZM/EXAMS scenarios have not been developed for all crops or all geographies and that "it is seldom possible to have a model to exactly fit a particular site." In their evaluation of EPA's Overview Document (Williams and Hogarth, 2004), the Services' concluded that "the information incorporated from these models [including PRZM-EXAMS] constitutes the best available data only if no alternative and superior data exists from a specific outside test or study (pg. 16)." The NMFS review did not indicate that there were available "superior or alternative data" for the Bay, its tributaries or other relevant habitats to use for modeling purposes. Further, EPA's exposure refinement techniques were consistent with the Services' recommendations that "[r]elevant data may include…analysis of practical application scenarios that do not meet existing models (pg. 16)." The refinements used by EPA in their assessment are consistent with the process outlined in the Overview Document stating that "… refinements of the screening-level risk assessment, which makes assumptions that the species or habitat will be exposed at levels estimated in the environment, focuses on refining the exposure information for listed species or critical habitat" (USEPA 2004, p 73).

5.1 Standard PRZM/EXAMS scenarios

The NMFS review was critical of EPA's use of standard PRZM/EXAMS scenarios due to concerns as to whether or not the "site-specific inputs used in the model are indicative of actual conditions" (p 11 line 17) around the Chesapeake Bay. As EPA's assessment indicates, the standard PRZM/EXAMS scenarios are designed to be conservative, upper bound exposure estimates that avoid underestimation of actual exposure. Documentation of the PRZM/EXAMS scenarios is extensive both within EPA's assessment, and in other guidance documents (Overview Document and http://www.epa.gov/oppefed1/models/water/index.htm#przm). Additionally, "the Services agree that the existing model necessarily represents the best available approach currently producing data for estimated aquatic exposure" (Williams and Hogarth 2004).

In terms of site-specific inputs, the NMFS review misinterpreted EPA's approach:

- The EPA used standard scenarios but refined the scenarios to better focus on the listed species by utilizing weather data from Wilmington DE, typical application dates and maximum label rates for modeling exercises.

- The NMFS review's concerns around the "magnitude of corresponding rainstorm events," (p 11 lines 26-27) are not relevant since localized weather data were utilized.
- The NMFS review's concerns around application dates (p 12, line 3) are not founded since EPA's target application dates are based on typical crop emergence. Syngenta queried our local technical representative who indicated that corn planting starts around April 1 in Southern VA with the majority of pre-emergent atrazine applications on corn in the DelMarVa area (primary use in the Chesapeake Bay watershed) occur between April 15 and May 10. Therefore, EPA's use of April 1 as an application date across 30 years of weather data is appropriate.
    - Syngenta further evaluated potential sensitivity in application timing by varying the application date within the PRZM/EXAMS Graphical User Interface shell: application dates of April 1 and April 15 for the PA corn scenario were used (weather data associated with the standard scenario was used in our sensitivity analysis, not Wilmington, DE). This analysis indicated that the April 1 application date used by EPA yielded higher concentrations compared to the use of April 15 as an application date. Therefore, the date difference will not cause a change in EPA's risk conclusions.
    - Additionally, as EPA indicated in their assessment, the watersheds surrounding the Bay are in the lower predicted percentage of runoff vulnerability (40% to 50%) according to WARP (p 79). Further, the PA corn scenario is based on data for Lancaster County within Major Land Resource Area (MLRA) 148. Lancaster County is relevant to the Bay because it is in Southeastern PA and the Susquehanna River flows through the county. MLRA 148 is also relevant to the Bay because it includes a significant portion of Virginia
- The NMFS review's concerns around application rates (p 12, line 3) are not founded since EPA utilized maximum application rates. The use of maximum application rates is conservative because typical application rates in the Chesapeake Bay watershed are much lower than the maximum labeled rates (EPA 2006).

- Finally, EPA's modeled EECs are overly conservative because they do not reflect expected runoff reductions from label mandated set backs (66 ft for perennial/intermittent streams and 200 ft for lakes/reservoirs). Vegetated buffers have been shown to reduce atrazine runoff up to 100% (summarized in USDA 2000). In addition, the modeled concentrations are overestimates relative to available monitoring data in relevant habitats within the Chesapeake Bay.

5.2 Modified PRZM/EXAMS scenarios

The NMFS review questioned EPA's modification of the EXAMS model to account for different water volumes and flow rates. However, it is important to note that EPA did not rely on any refinements to the PRZM/EXAMS screening-level scenarios to assess direct

effects to the listed species or indirect effects on the prey of the listed species. Therefore, EPA utilized worst-case screening level exposure scenarios for their no-effect determinations with respect to direct effects on sturgeon and sea turtles and indirect effects due to prey items. As EPA indicated, "The standard water body was developed to provide an approximation of high-end exposures expected in water bodies, lakes, and perennial/intermittent streams …" (USEPA 2006, p 67). The EPA standard pond is extremely conservative: 1 ha X 2m deep pond; 10 ha watershed with 100% of the watershed cropped and treated at the maximum labeled application rate; and the pond captures all of the runoff and drift from the treated area with no inflow or outflow.

The only modified scenarios using higher tiered exposure modeling were for assessing potential indirect effects on the listed species habitat. The NMFS review's primary reason for questioning the modified scenarios appeared to be that the modifications resulted in lower EECs compared to the static pond and were therefore not worst case. However, the habitat description for the shortnose sturgeon indicates that they typically prefer rivers with swift current, the mouths of rivers, or the mainstem of the Bay – not static ponds (USEPA 2006). Sturgeon will primarily occur in larger water bodies in areas of higher flow and therefore have correspondingly larger water volumes compared to the standard pond (USEPA 2003b). Further, it is well understood that residue concentrations are reduced when moving to larger watersheds with faster flowing streams (Baker and Richards, 2000) and therefore any potential atrazine concentrations will be reduced compared to a static modeled water body, as the EPA modeling exercises indicate. The shallow static pond is even less relevant for the turtle species in question. The listed turtle species would not be found in the shallow tributaries or other shallow water bodies far from the Bay's mouth, as their preferred habitats are in deeper water near the mouth of the Bay or in the deep rivers emptying into the Bay. This preference for larger water bodies means that the turtles are exposed to correspondingly larger volumes of water relative to the small pond. Therefore, the foregoing data indicates it is unrealistic and overly conservative to use a 1 ha X 2m deep static pond to reflect sturgeon/turtle habitat or that modeled concentrations from a static body reflect concentrations likely to occur in the Bay or relevant sturgeon/turtle habitat.

The NMFS review specifically cited two examples for discounting EPA's approach.

- Sturgeon may utilize habitats less than 2 m deep (NMFS Technical Appendix, p 12, line 14).
    - Indeed sturgeon may inhabit water depths of 1-25 m or greater (USEPA 2003b). However, discounting a model due to water depth ignores the 3 dimensional aspects of a water body (length, width, and depth).
        - As described above, sturgeon may inhabit rivers with swift current. For example, sturgeon have been recorded in the Susquehanna River below the Conowingo Dam. The USGS gauge station at this location (Gauge ID 01578310) indicates that the average flow rate is 41,300 ft^3/s (annualized average using 38 years of data) and therefore fish located in this area for 20 seconds would be "exposed" to a water volume greater than the standard pond (20,000,000 L) regardless of water depth. Further, available monitoring data associated with this site (see Figure 4 below) indicate a peak concentration of only 0.9

µg/L compared to a peak modeled concentration of 55 µg/L used by EPA.

- Sturgeon may occur in watersheds where the surface water ratio is greater than 10:1 (NMFS Technical Appendix, p 12, line 15).
  - The standard pond scenario assumes 10 ha of cropped land is treated with the product and moves into an adjacent 1 ha pond. However, EPA also examined the Index Reservoir scenarios which utilizes 172 ha of treated land and a 5.3 ha reservoir. The resulting watershed to surface water ratio is 32:1. Therefore, EPA considered land to surface water ratios that are greater than 10:1.
  - Further, EPA's PRZM/EXAMS modeling assumes the entire watershed is cropped and treated with the target crop, which is rarely the case.

5.3 Monitoring Data

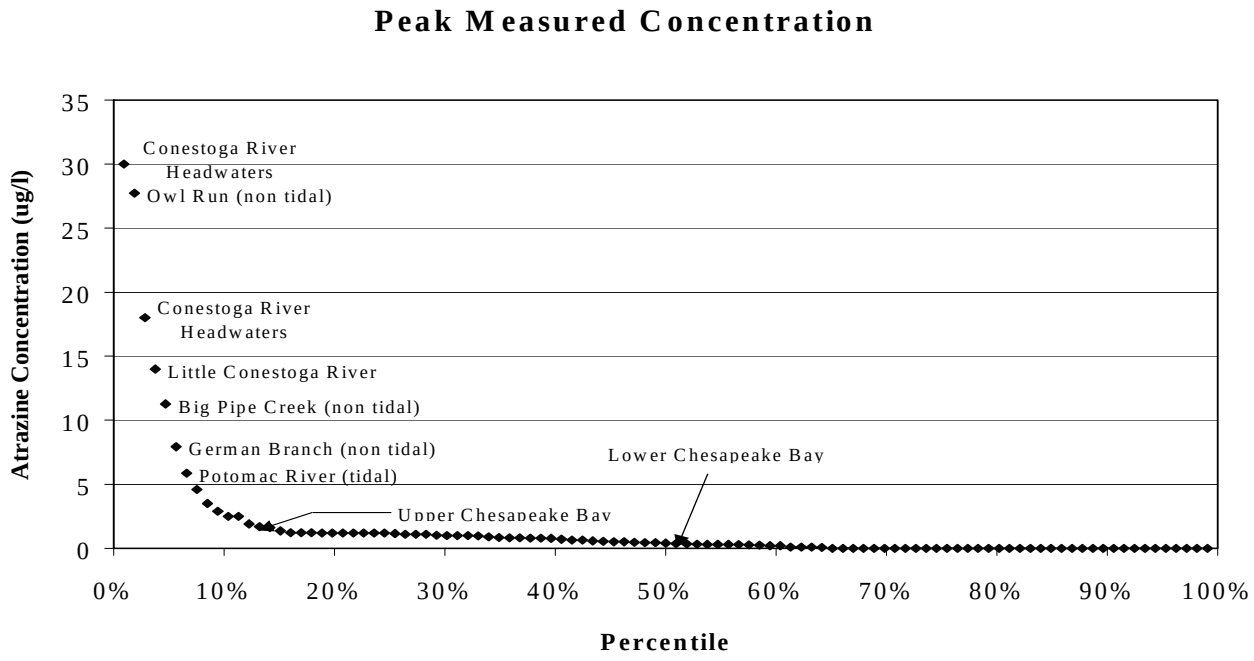
The NMFS review questioned the EPA's use of Chesapeake Bay monitoring data. The NMFS review mischaracterized the available data, did not include other relevant monitoring studies and misinterpreted the atrazine monitoring data submitted by Syngenta.

EPA conducted a review of the available monitoring data in their assessment that adequately characterized the data. However, the NMFS review misinterpreted EPA's guidance document when they stated that "monitoring data are generally not representative of peak exposure (NMFS p 12, line 25)." The Overview Document (p 42) also states:

"These [monitoring] data are evaluated on a case-by-case basis to determine the likelihood, extent, and nature of pesticide concentration in water under current use practices and actual field conditions. The risk assessment team considers such study aspects as the points and frequency of sample collection, the analyte suite, and detection limits when determining how such data will be incorporated into the risk assessment. When reliable surface water monitoring data are available, EPA uses it to help characterize the levels of pesticide that are being detected in the environment. Monitoring, though, does not necessarily target pesticide use areas or the time of year when pesticide concentrations may be at their peak, and for this reason may not provide a reliable estimate of acute exposure. If monitoring data shows higher confirmed detections than estimated by modeling, the higher monitoring values may be used in the risk assessment, and a re-evaluation of the model input parameters may be initiated to explore the impact of selected input values on the model output."

Syngenta has previously commented on the available Chesapeake Bay monitoring data (Syngenta 2001 and 2002). Briefly, the data presented by EPA represents water samples collected in upstream freshwater headwater streams, major tributaries, as well as in the Chesapeake estuary. Data from EPA 2002 is re-plotted in Figure 1to illustrate the difference in concentrations between the upstream sample locations and the estuary sample sites.

Figure 1. Summary of Chesapeake Bay Atrazine Monitoring Data from EPA 2002.



The higher peak atrazine concentrations (> 8 µg/L) are all associated with small freshwater non- tidal influenced streams (Figure 1). It must also be noted that these upstream data represent concentrations collected in the early to mid-1990's prior to the full implementation of changes to atrazine labels that decreased rates and mandated buffer set backs. Additionally, these maximum concentrations, due to the hydrology of small streams, are expected to be transient, and rapidly dissipate. As EPA indicates in their assessment, most of the detections for atrazine in the Bay where shortnose sturgeon and/or listed turtles might occur are low.

With regards to the value of 30 ppb included in the EPA assessment, this location (Conestoga River headwater) is in a small headwater stream located in Eastern MD that would be unsuitable habitat for the listed species (Figure 2). A further analysis of the 1991 data associated with this sampling location is provided in Table 3. These data indicate that the peak concentration was reduced by half within 6 hours and was further reduced at subsequent time points.

Table 3. Atrazine concentrations associated with station ID L0002488 from the Chesapeake Bay Monitoring Program

Date	Time (24 h)	Atrazine Concentration µg/L
3/5/91	1310	0.06
4/24/91	1050	0.05
4/24/91	1650	0.06
5/29/01	1130	0.05
6/18/91	1120	30
6/18/91	1730	16
6/26/91	0930	7.5
7/23/91	0900	2.7
8/19/91	1030	0.65
12/10/91	0930	0.9

Figure 2. **Sampling station L0002488 as identified from http://www.chesapeakebay.net**

The NMFS review also indicated that other data from Hall et al. (1999) (p 12, lines 29-30) showed higher concentrations in the Bay compared to those reported by EPA. However, similar to the peak value described above, the peak concentrations from Hall et al. were collected from small headwater streams that would not be suitable habitat for sturgeon or the listed turtle species (Figure 3: A = Morgan Creek; B= German Branch Creek). In fact, the

location where the highest concentration was collected in German Branch Creek is a small headwater stream that is dammed downstream from the sampling location. Figure 4 contains an analysis of the Hall et al. (1999) data in context to Bay geography and also includes data from other studies (McConnell et al., 2004, Kuang et al. 2003 and Liu et al., 2002) that were not included in the NMFS analysis.

- McConnell et al. (2004) conducted an intensive water monitoring study (156 samples collected over an approximate 4 month period) in the Patuxent River Estuary during 1996. The authors collected water samples 8 times in April, every day from May 1 to 11, every other day from May 13 to May 28 and 5 times in July 1996. Four sample sites were used ranging from the mouth of the Patuxent up to Jug Bay. The highest atrazine concentrations were associated with Jug Bay (minimum = 0.033 µg/L mean = 0.28 µg/L, maximum = 1.29 µg/L) and concentrations decreased with increasing river size (i.e., the river mouth sampling site concentrations were 0.026 – 0.036 – 0.084 µg/L, minimum - mean – maximum, respectively).

- Kuang et al. (2003) conducted a survey of the Choptank River at eight locations on five different events (May, June, July, August, and December) during 2000. The eight locations ranged from the mouth of the River to much smaller locations in terms of water volume (e.g., 0.56 m^3). Atrazine concentrations were low and ranged from 0.02 to 3.1 µg/L

- Liu et al (2002) conducted a similar study (41+ samples) in the Susquehanna River and Northern Chesapeake Bay in 1997 to 1998 and analyzed numerous herbicides and insecticides. The authors also used an intensive sampling schedule: every 9 days for 14 months (Feb 1997 to March 98). The average atrazine concentration was 0.067 µg/L, maximum = 0.5 µg/L.

These data along with available NAWQA data (Figure 5) indicate that actual atrazine residue concentrations within the Chesapeake Bay and tributaries that could be considered as suitable habitat for sturgeon or turtles are low and do not pose a direct or indirect risk. They also indicate that the modeled EEC values used by EPA for the listed species assessment are highly conservative and overestimate actual exposure.

Figure 3. **Location of peak concentrations measured by Hall et al. (1999). Morgan Creek (A); German Branch (B).**

A

Figure 3. **Location of peak concentrations measured by Hall et al. (1999). Morgan Creek (A); German Branch (B). (continued).**

B

Figure 4. Spatial Distribution of Previous Monitoring Sites in the Chesapeake Bay.

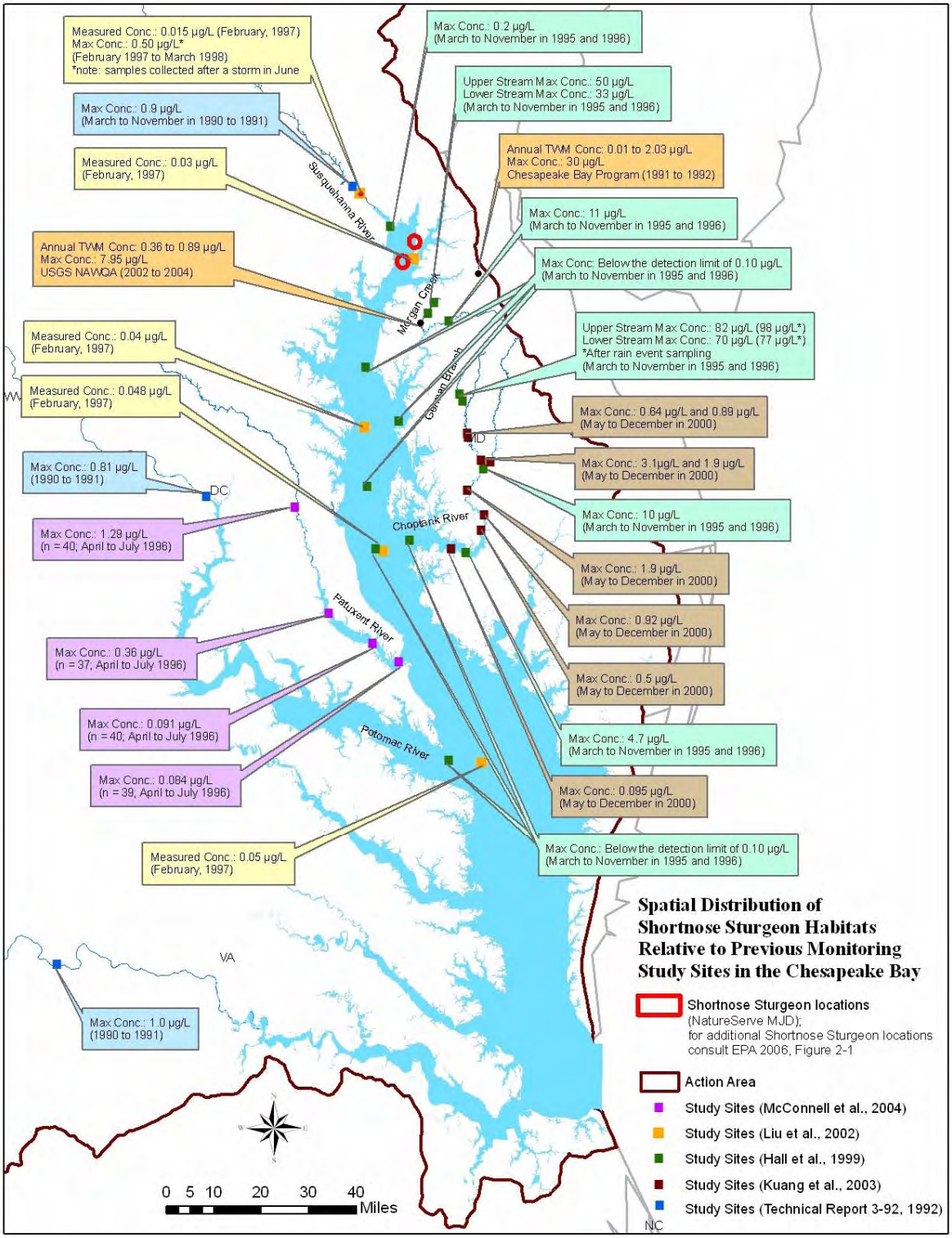
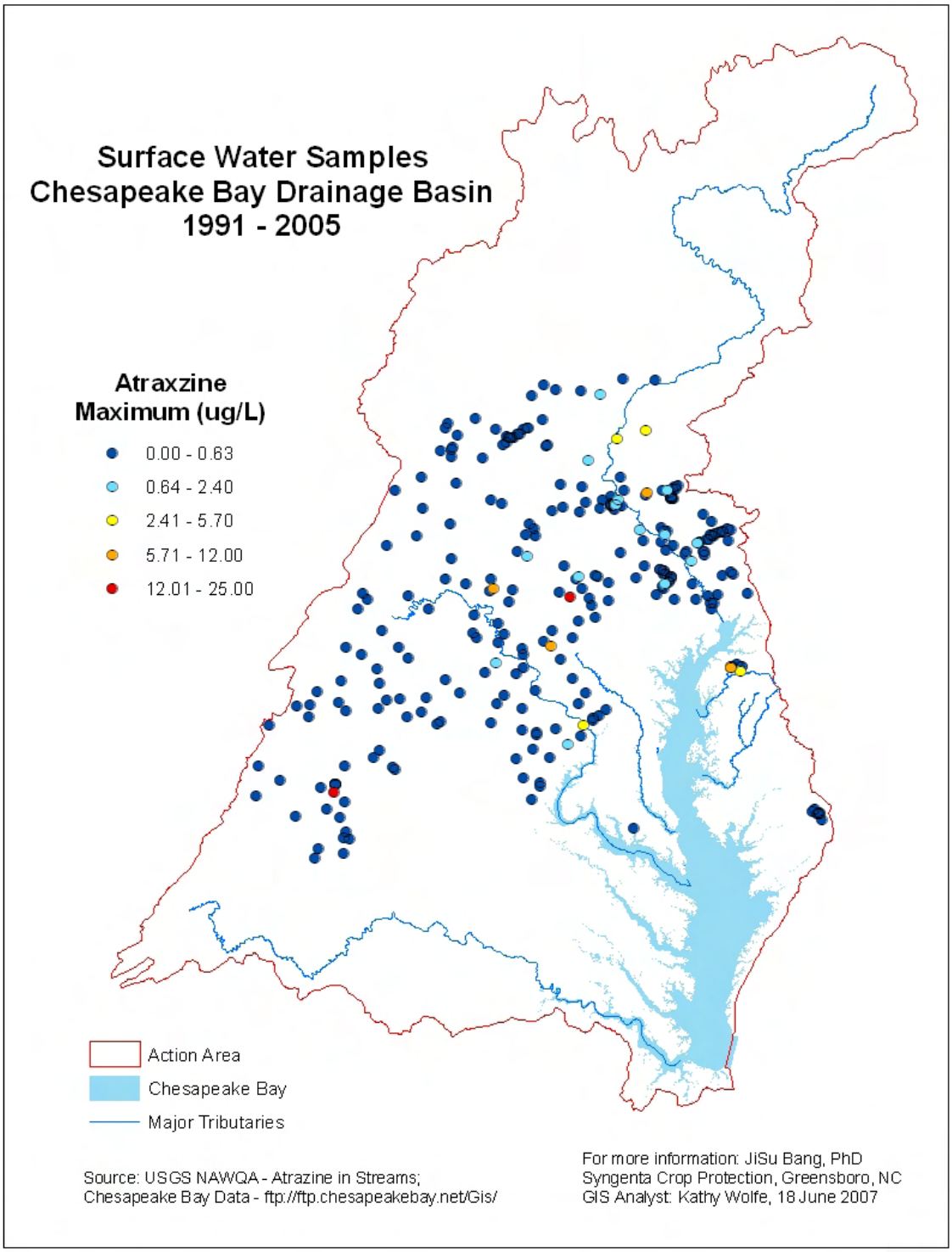


Figure 5. **Summary of available NAWQA atrazine monitoring data within or associated with the Chesapeake Bay.**



5.4 Monitoring data from the Atrazine Ecological Monitoring Program are not relevant to the Bay or it's tributaries

The NMFS review used Syngenta submitted atrazine monitoring data from the Atrazine Ecological Monitoring Program in small Midwestern subwatersheds to evaluate exposure to the listed species in the Chesapeake Bay and its tributaries. However, the NMFS review misinterpreted the data. The Atrazine Ecological Exposure Monitoring Program sub-watersheds are not representative of habitat within the Bay or its tributaries:

- The 40 representative corn/sorghum Midwestern sites involved in the Atrazine Ecological Exposure Monitoring Program were selected based on their vulnerability and atrazine use intensity based on a WARP assessment (described in EPA 2006). These 40 sites are in the upper 20^{th} centile of vulnerability and as EPA indicated are not relevant to watersheds in the Bay which rank in the upper $40^{th} – 50^{th}$ centile in vulnerability scores (EPA 2006).
- Also, the sampled sub-watersheds in Midwestern streams in Nebraska, Indiana, Illinois, Missouri, etc. are too small to be representative of streams that could sustain any of the listed species the NMFS review evaluated.
    - For example, two of the Nebraska sites NE-04 and NE-07 experienced complete dry down during significant portions of the monitoring season making these sites irrelevant for comparison to listed habitat. Numerous other sites experienced dry conditions at times during the monitoring season.

Monitoring data in suitable sturgeon/turtle habitat clearly indicate that atrazine concentrations do not approach the concentrations measured in small Midwestern watersheds (NAWQA, Kuang et al. 2003, Liu et al., 2002, and McConnell et al., 2004).

6.0 EPA PROPERLY CONSIDERED AVAILABLE DATA ON MIXTURES

The NMFS review indicated that the risk to listed species was likely underestimated since EPA did not consider concurrent exposure from multiple pesticides. However, in their evaluation of EPA's Overview Document (Williams and Hogarth, 2004), the Services' concluded that:

> "At the present time, OPP's screening level assessment does not consider effects to non-target species caused by such mixtures. Moreover, general agreement was reached between OPP and the Services that it is unlikely that OPP can develop specific testing methods to measure the effects of such mixtures in a quantifiable manner. However, OPP will survey the open literature for any data addressing the effects of these types of mixtures, which it will use in its risk assessment process. The Services agree with this approach, and believe it will likely capture the best available scientific and commercial data (pg. 19)."

EPA followed the agreed upon procedures to capture the best available scientific and commercial data and surveyed the open literature for data addressing mixtures (EPA Assessment Appendix A Amended, J-1 and J-2) and evaluated the majority (10) of the studies cited in the NMFS review (including Belden and Lydy, 2000; Pape-Lindstrom and Lydy, 1997) as well as several others (see Table 3). In these studies EPA concluded that claims of synergistic toxicity were questionable and could not be demonstrated because the use of solvents in combination with the pesticides prevented any reasonable cause and effect conclusions or that the measured enzymatic endpoints could not be linked to or otherwise demonstrate any adverse effects on the assessment endpoints (i.e. survival, growth, or reproduction).

Additionally, the NMFS review did not provide an adequate review of the literature. Unlike EPA, who conducted an exhaustive literature review, the NMFS review selectively characterized the potential hazard by only choosing select studies and, consequently, did not adequately describe the available studies. For example, while Lydy and others have identified potential synergistic and/or additive toxicity of atrazine and organophosphate chemicals in aquatic invertebrates in the presence of solvents (principally the aquatic midge). This same research group has conducted research on potential synergistic and/or additive toxicity of atrazine and chlorpyrifos (an organophosphate) in animals with far more relevance to the listed species: fish and amphibians, which did not demonstrate any synergistic effects (Wacksman et al., 2006). The NMFS review, however, did not include this study in their analysis nor did they include relevant literature that indicates potential antagonistic (as opposed to synergistic) effects of atrazine in combination with other pesticides. EPA has followed the agreed procedures to obtain the best available scientific and commercial data and critically evaluated the available information. EPA has therefore adequately addressed the topic of atrazine and potential co-occurrence of other compounds in their assessment. Table 3 provides a review of the publications cited in the NMFS review.

Briefly, the NMFS review cited a total of 14 studies regarding mixtures. As detailed below and in Table 3, four out of the 14 studies cited in the NMFS review did not evaluate atrazine and/or synergistic/additive toxicity between pesticides, and are therefore not relevant. In the remaining ten studies, solvents were used in the mixtures (Table 3) and the results from these studies are therefore questionable given that the presence of the solvents in the mixtures potentially affected the results.

- Belden and Lydy (2006) did not examine synergistic toxicity of atrazine with other chemicals, but rather the synergistic toxicity of esfenvalerate and chlorpyrifos.
- Londono et al. (2004) did not examine synergistic toxicity of atrazine and other chemicals, but rather evaluated the potential for atrazine to induce CYP4 mRNA in the aquatic midge.
- Lydy and Austin (2005) did not examine synergistic toxicity of atrazine with other chemicals.
- Miota et al. (2000) did not examine synergistic toxicity of atrazine and other chemicals, but rather evaluated the potential for atrazine to induce P450 enzyme activity in the aquatic midge.

Table 3. Evaluation of Mixtures Effect Values Cited in the NMFS review.

Paper Cited in the NMFS review	Study Evaluated by EPA?	EPA Study Evaluation within USEPA 2006	Syngenta Study Evaluation	Solvent used for atrazine?
Anderson and Lydy 2002 (p 20, line 13)	Yes, Appendix J-1	No effect at single test concentration. Biochemical enzymatic endpoints cannot be quantitatively linked to the selected assessment endpoints.	This study examined the joint toxicity of atrazine and three OPs (chlorpyrifos, methyl parathion, and diazinon) to *Hyallela azteca* (aquatic amphipod) and *Musca domestica* (common housefly) and drew conclusions on increased toxicity at atrazine concentrations (>40 ug/L) in combination with each of the OPs. Similar to EPA's conclusions of Pape-Lindstrom and Lydy 1997, acetone was used as a solvent with atrazine in the mixture, raising potential solvent-related effects on synergism.	Yes (acetone)
Battaglin and Fairchild (2002) (p 19, line 12)	No	No EPA evaluation available.	This study reviewed pesticide toxicity data for duckweed, green algae, bluegill sunfish, and bull frogs within the open literature, and related these data to USGS water samples from 71 streams (two samples per stream) in the Upper Mississippi, Missouri, and Ohio River basins. The data were related using a toxicity index (concentration of pesticide in sample divided by EC50 or LC50 value = risk quotient), where a toxicity index >1.0 indicate probably toxicity; >0.5 indicates potential toxicity; and >0.1 indicates limited toxicity. The NMFS review stated (pg. 9) that "a USGS study…concluded that multiple samples containing herbicides had probable toxicity to duckweed and green algae based on a toxicity index." In fact, this review found that only <1% (1 sample) and 3% (4 samples) of the total samples collected (142 samples) exceeded the average triazine EC50 for duckweed and green algae respectively. The authors did not indicate	N/A

			where these exceeding samples were derived from. Importantly, the authors noted that "this study does not agree with Fairchild et al. (1999) [a study from the same lab], who indicated that herbicides were unlikely to affect aquatic plants in the lower Missouri River." Therefore, the low-tier RQ approach presented in this study did not agree with a higher-tier risk assessment conducted by the same group.	
Belden and Lydy 2000 (p 20, line 12)	Yes, Appendix A (pg. 97).	Based on data presented within this study, EPA concluded that "[t]he variety of chemical interactions produced by atrazine mixtures indicates that the effect of atrazine on an organism is dependent on the species, co contaminant, and the concentration of atrazine." Therefore, all three of these factors are necessary for synergistic toxicity to occur.	This study examined the joint toxicity of atrazine and three OPs (chlorpyrifos, methyl parathion, diazinon, and malathion) to *Chironomus tentans* (aquatic midge) and drew conclusions on increased toxicity at atrazine concentrations >40 ug/L in combination with chlorpyrifos, methyl parathion, or diazinon.. Similar to EPA's conclusions of Pape-Lindstrom and Lydy 1997, acetone was used as a solvent in the mixture with atrazine, raising potential solvent-related effects on synergism. In any event, no synergistic toxicity was observed with malathion.	Yes (acetone)
Belden and Lydy 2001 (p 20, line 13)	Yes, Appendix J-1.	No effect at single test concentration. Biochemical enzymatic endpoints cannot be quantitatively linked to the selected assessment endpoints	The toxicity data presented in this paper are from Belden and Lydy 2000. This paper simply examined acetylcholinesterase activity in aquatic midges following exposure to mixtures of atrazine with chlorpyrifos or methyl parathion. However, similar to EPA's conclusions of Pape-Lindstrom and Lydy 1997, acetone was used as a solvent with atrazine, raising potential solvent-related effects on synergism.	Yes (acetone)
Belden and Lydy 2006 (p 20, line 14)	No	No EPA evaluation available since it is not related to atrazine	This study did not examine synergistic toxicity of atrazine and OPs, but rather the synergistic toxicity of esfenvalerate and chlorpyrifos. Therefore, this study has no relevance to atrazine.	N/A

Paper Cited in the NMFS review	Study Evaluated by EPA?	EPA Study Evaluation within USEPA 2006	Syngenta Study Evaluation	Solvent used for atrazine?
Jin-Clark et al. 2002 (p 20, line 13)	Yes, Appendix J-1	Study reports toxicity of mixtures of atrazine and chlorpyrifos. The biochemical enzymatic endpoints cannot be quantitatively linked to the selected assessment endpoints.	This study examined the joint toxicity of atrazine or cyanazine and chlorpyrifos to *Chironomus tentans (aquatic midge)* and drew conclusions on increased toxicity at atrazine concentrations >10 ug/L in combination with chlorpyrifos. Similar to EPA's conclusions of Pape-Lindstrom and Lydy 1997, acetone was used as a solvent with atrazine, raising potential solvent-related effects on synergism. and rendering the study unreliable.	Yes (acetone)
Londono et al. 2004 (p 20, line 13)	Yes, Appendix J-1	Biochemical enzymatic endpoints cannot be quantitatively linked to the selected assessment endpoints.	This study did not examine synergistic toxicity of atrazine and other chemicals, but rather evaluated the potential for atrazine to induce CYP4 mRNA in the aquatic midge. Therefore, this study has no relevance to potential synergistic toxicity of atrazine.	N/A
Lydy and Austin 2005	No	No EPA evaluation available since it is not related to atrazine	This study did not examine synergistic toxicity of atrazine with other chemicals. Therefore, this study has no relevance to atrazine.	N/A
Miota et al. 2000 (p 20, line 12)	Yes, Appendix J-1	Rejected due to “no endpoint.”	This study did not examine synergistic toxicity of atrazine and other chemicals, but rather evaluated the potential for atrazine to induce P450 enzyme activity in the aquatic midge. Therefore, this study has no relevance to potential synergistic toxicity of atrazine.	N/A

Paper Cited in the NMFS review	Study Evaluated by EPA?	EPA Study Evaluation within USEPA 2006	Syngenta Study Evaluation	Solvent used for atrazine?
Pape-Lindstrom and Lydy 1997 (p 20, line 12)	Yes, Appendix A (pg. 97).	EPA concluded that "results from these tests are questionable, since DMSO was used as a solvent with atrazine."	This study examined the joint toxicity of atrazine and chlorpyrifos to the aquatic midge and drew conclusions on increased toxicity at atrazine concentrations (10,000 ug/L) in combination with chlorpyrifos. Atrazine concentrations used in this study are not environmentally realistic. Moreover, as EPA noted, DMSO was used as a solvent with atrazine, raising potential solvent-related effects on synergism. The authors similarly conceded that "the carrier solvents acetone and DMSO are also known to affect cellular permeability, which complicates this hypothesis [that atrazine may increasing the penetration of the insecticides through the midge cuticle or increasing the cellular permeability of the organophosphates."	Yes (DMSO)
Schuler et al. 2005 (p 20, line 14)	Yes, Appendix J-1.	Rejected	This study examined the joint toxicity of atrazine and chlorpyrifos to the aquatic midge and drew conclusions on increased toxicity at atrazine concentrations (>50 ug/L for diazinon and >100 ug/L for chlorpyrifos) in combination with chlorpyrifos or diazinon. Similar to EPA's conclusions of Pape-Lindstrom and Lydy 1997, acetone was used as a solvent with atrazine, raising potential solvent-related effects on synergism.	Yes (acetone)

Trimble and Lydy 2006 (p 20, line 14)	No	No EPA evaluation available.	This study examined the joint toxicity of atrazine and chlorpyrifos to *Hyalella azteca* (aquatic amphipod) and drew conclusions on increased at atrazine concentrations (200 ug/L) in combination with chlorpyrifos. Similar to EPA's conclusions of Pape-Lindstrom and Lydy 1997, acetone was used as a solvent with atrazine, raising potential solvent-related effects on synergism.	Yes (acetone)

Paper Cited in the NMFS review	Study Evaluated by EPA?	**EPA Study Evaluation within USEPA 2006**	Syngenta Study Evaluation	Solvent used for atrazine?
DeLorenzo and Serrano 2003 (p 3, Cover Letter)	Yes, Appendix J-1	EPA accepted this study for ECOTOX but did not use this study in the assessment since the study endpoint was less sensitive than the assessment endpoint.	This study examined the joint toxicity of atrazine and chlorpyrifos or chlorothalonil to *Dunaliella tertiolecta* (phytoplankton) and drew conclusions on increased toxicity at atrazine concentrations (>25 ug/L) in combination with chlorothalonil. Atrazine in combination with chlorpyrifos did not result in increased toxicity at environmentally realistic chlorpyrifos concentrations (only observed an effect at >400 ug/L chlorpyrifos). These data for chlorpyrifos are inconsistent with effects observed in the aquatic midge. Similar to EPA's conclusions of Pape-Lindstrom and Lydy 1997, acetone was used as a solvent with atrazine, raising potential solvent-related effects on synergistic or additive toxicity.	Yes (acetone)
Faust et al. 2001 (p 21, line 16)	Yes, Appendix J-1	EPA accepted this study for ECOTOX but did not use this study in the assessment since the study endpoint was less sensitive than the assessment endpoint.	This study examined the toxicity of 18 different s-triazines to the freshwater algae *Scenedesmus vacuolatus* either alone or in mixtures containing all 18 chemicals at 1/18XEC50 or EC01 concentrations. For mixture toxicity, observed effects were compared to predicted effects. While the NMFS review stated that "a comprehensive experiment with seven triazines indicated strict toxicity," the study actually evaluated 18 different triazines. Similar to EPA's conclusions of Pape-Lindstrom and Lydy 1997, methanol was used as a solvent for all chemicals, confounding potential solvent-related effects on additive toxicity.	Yes (methanol)

The study below was not cited in the NMFS review, but is important since (a) it was published from the same lab, and (b) the authors reported no synergistic or additive toxicity of atrazine and chlorpyrifos to four different aquatic vertebrates (all their previous studies were done with aquatic invertebrates).				
Wacksman et al. 2006	No	No EPA evaluation available.	This study examined the joint toxicity of atrazine and chlorpyrifos to four different aquatic vertebrates (fathead minnow, bluegill, green frog, and African clawed frog). Atrazine (tested up to 1000 ug/L) in combination with chlorpyrifos resulted in no significant increase in toxicity compared with chlorpyrifos alone.	Yes (acetone)

6.1 Lack of potential for concurrent exposure to multiple pesticides

Although none of the multiple pesticide effects data cited in the NMFS review warranted inclusion in EPA's quantitative risk assessment, exposure data alone would not support the NMFS review's conclusions. The NMFS review only discussed the potential hazard and postulated that concurrent exposure to multiple pesticides would "produce greater risk than exposure to atrazine alone" (NMFS p 35, lines 30-31). However, available data do not support this claim. McConnell et al. (2004), Kuang et al. (2003) and Liu et al. (2002) monitored a variety of compounds in the Bay and it's tributaries. These studies indicated that atrazine and other pesticide products would not be present in the Bay or it's tributaries at concentrations cited in the NMFS review for synergistic effects.

- McConnell et al. (2004) monitored numerous pesticides in the Patuxent River Estuary during 1996. The mean reported atrazine concentrations were well below any alleged "mixture" effects cited as a concern in the NMFS review. Further, concentrations of other products were also below any alleged mixture effects

Concentration (µg/L)	Atrazine	DEA	DIA	Simazine
Average	0.28	0.43	0.26	0.14
Maximum	1.3	1.1	0.76	0.49

- Kuang et al. (2003) conducted a similar study in the Choptank River and found similar results.

Concentration (µg/L)	Atrazine	DEA	DIA	Cyanazine	Simazine	Chlorpyrifos	Diazinon
Minimum	0.02	0.03	0.04	<LOD	<LOD	<LOD	<LOD
Maximum	3.1	0.73	0.75	0.23	2.4	0.005	0.02

- Liu et al (2002) conducted a similar study in the Susquehanna River and Northern Chesapeake Bay and found similar results.

Concentration (µg/L)	Atrazine	DEA	DIA	Cyanazine	Simazine	Chlorpyrifos
Average	0.067	0.029	0.064	0.003	0.037	0.0005
Maximum	0.5	0.15	0.15	0.14	0.13	0.002

- Additionally, Liu summarized other available monitoring data from the Chesapeake Bay Tributaries (Patuxent and Choptank rivers) and these data support a similar conclusion; atrazine does not co-occur with other pesticides in the Bay or it's tributaries at concentrations cited as a potential concern in the NMFS review.

Finally, the NMFS review relied on a recent USGS publication (Gilliom et al. 2006) to indicate the probability of mixture effects containing atrazine. However, even the results in Gilliom et al. (2006) indicated that neither atrazine nor chlorpyrifos were present at the same time in concentrations that have allegedly had effects in the laboratory (Gilliom et al. p14). Further, the result in Gilliom et al. indicate that atrazine degradates are at least 10 times less toxic than parent; EPA's evaluation indicates the degradates are >1000 times less toxic to aquatic organisms than parent.

In conclusion, the NMFS review mischaracterized the available literature associated with potential effects of atrazine and other pesticides, omitted data showing contrary effects, and did not place this alleged hazard in the context of potential exposure based on monitoring data in the Chesapeake Bay watershed. The best available information therefore indicates that neither the shortnose sturgeon nor the listed sea turtle species are likely to be adversely affected by mixtures of atrazine or other pesticide products.

7.0 CASM_ATRAZINE

The "Comprehensive Aquatic Systems Model for Atrazine" (CASM_Atrazine) ecosystem model simulates complex ecological production dynamics of a generic 2nd-to-3rd-order Midwestern stream with or without atrazine exposure. CASM_Atrazine predicts daily biomass (carbon) production of modeled populations of aquatic plants and animals as complex, nonlinear functions of fluctuating environmental conditions (e.g., light, temperature, nutrients), time-varying ecological conditions (e.g., competition within trophic guilds, grazing, predator-prey interactions), and producer- and consumer-specific sensitivities to atrazine exposure. Using atrazine monitoring data from the Atrazine Ecological Monitoring Program conducted in Midwestern streams, the primary objective of this model is to predict the potential for realistic time-varying atrazine concentrations to influence total biomass production of primary producer and consumer populations. The development, application, and utility of CASM_Atrazine for risk assessment will be fully evaluated by an EPA Science Advisory Panel (SAP) in November of 2007.

The NMFS review concluded that the Comprehensive Aquatic Systems Model for Atrazine (CASM_Atrazine) is not an appropriate tool for EPA's effects determinations, and that EPA's refined assessment should not rely on CASM_Atrazine data. The NMFS review appeared to discount CASM_Atrazine since NMFS did not previously review CASM_Atrazine in its evaluation of the Overview Document. Based on a number of statements made within the NMFS review (e.g., CASM should be used when data are sparse pg. 24, lines 16-19; pg. 26, lines 22-28), it is apparent that there is not a complete understanding of the methodology and utility of CASM_Atrazine for evaluating time-variable atrazine chemographs. The NMFS review indicated that experimental micro- and mesocosm data should be directly used in lieu of CASM_Atrazine-based modeling. Syngenta has provided a response to the studies cited by the NMFS review in Table 4. When evaluating the use of these data and/or modeled results, it is important to consider that (a) atrazine concentrations vary with time during the growing season, (b) atrazine effects on primary producers are dependent on both atrazine concentration and exposure duration, and (c) adverse atrazine-related effects on primary producers are recoverable. Consequently, a simple comparison of peak atrazine concentrations to effects observed within experimental studies without consideration of exposure duration (in both monitored water bodies and micro/mesocosm studies) does not capture the likelihood of adverse effects on primary producer communities. This is precisely why CASM_Atrazine was developed by EPA, and why a level-of-concern for CASM_Atrazine was developed via correlation of CASM_Atrazine predictions to effects observed within experimental micro/mesocosm studies.

Table 4. Evaluation of the Mesocosm Studies Cited in the NMFS review.

Mesocosm Value Cited in the NMFS review	Effect Stated by in the NMFS review	Paper Cited by in the NMFS review **	Study Evaluated by EPA?	EPA Study Evaluation within USEPA 2006	Syngenta Study Evaluation
1.89 ug/L	Adverse impacts on primary productivity, resulting in adverse cascading ecological responses of exposed aquatic habitats	Lakshminarayana et al., 1992 (p 3, Cover Letter; p 25, Table 4)	Yes	Appendix A (pg. 57): EPA summarized the study and qualitatively considered the data. However, for all microcosm/mesocosm tests in general, EPA concluded that "[d]ata from non-guideline microcosm/mesocosm tests are not typically used quantitatively to derive RQs in the Agency's ecological risk assessments, but rather to provide qualitative information regarding potential aquatic community-level effects of atrazine (pg 44)."	This study evaluated potential atrazine effects on phytoplankton (mainly diatoms) and zooplankton in a small Canadian 1st-order stream adjacent to a tiled-drained corn field. The field was divided into 4 plots and each drained separately into a small canal and into the stream. Water, phytoplankton, and zooplankton were sampled at 15-day intervals at 11 sampling sites during the growing season. The authors reported that total phytoplankton numbers in downstream samples (downstream of atrazine entry in stream) were less than those from upstream samples (upstream of atrazine entry in stream). However, the experimental design included no replication, no controls, and no pre-treatment sampling, and the effects of atrazine, if any, were confounded by many other factors (e.g., water chemistry, flow, etc.). Because of these shortcomings, this study was previously discounted by Giddings et al. (2005).
1 ug/L	Adverse impacts on primary productivity, resulting in adverse cascading ecological responses of exposed aquatic habitats	Lampert et al., 1989 (p 3, Cover Letter; p 25, Table 4)	Yes	Appendix A (pg. 49): EPA summarized the study and qualitatively considered the data. However, for all microcosm/mesocosm tests in general, EPA concluded that "[d]ata from non-guideline microcosm/mesocosm tests are not typically used quantitatively to derive RQs in the Agency's ecological risk assessments, but rather to provide qualitative information regarding potential aquatic community-level effects of atrazine (pg 44)."	This study evaluated potential atrazine effects on lake phytoplankton communities. Samples of lake phytoplankton communities were enclosed in plastic tubes and treated with various atrazine concentrations. Using dissolved oxygen measurements, the authors reported reductions in phytoplankton photosynthesis after exposure to as low as 0.1 ug/L. However, the effects in these enclosures did not appear until at least a week after atrazine treatment. This is inconsistent with other studies where effects on productivity, if any, occur immediately after atrazine exposure. As Giddings *et al.* 2005 concluded (pg. 173): "These discrepancies were probably caused by the ethanol used as a solvent for the atrazine treatments but not added to the controls (Brock et al. 2000). Although Lampert et al. (1989) stated that no solvent was used, the original report on this study (Fleckner 1988) indicated otherwise. Enough ethanol was added to each atrazine microcosm to more than triple the dissolved organic carbon concentration of the controls. The reduced dissolved oxygen concentrations in these microcosms were most likely caused by microbial consumption during ethanol degradation, not by [atrazine-induced] photosynthetic inhibition."

** A review of only these two studies is provided since the NMFS review generally relies heavily on these data for their arguments.

Paper Cited in the NMFS review	Effect Stated in the NMFS review	Study Evaluated by EPA	EPA Study Evaluation within USEPA 2006	Syngenta Study Evaluation
Weiner et al. 2007 (p 29, line 4)	Other adverse effects would be overlooked by this process such as atrazine's effects on algal nutritional quality.	No	No EPA evaluation available.	This study evaluated the potential effects of atrazine on carbon allocation within five algal species using a 96-hr toxicity test. Algae were exposed to 0.5X EC50, 1X EC50, and 2X EC50 for 96 hr, then 14C uptake and distribution into different macromolecular compartments (proteins, polysaccharides, lipids, and low molecular weight molecules) were assessed for 24 hr after exposure. The authors reported significant differences in carbon uptake and assimilation in 4 out of 5 species. The authors also suggested that "[a]lterations in the macromolecular composition of microalgal species may negatively affect higher trophic levels, as nutritionally altered algal cells may have a reduced energy mass per uptake for consumers." This is an over-interpretation of the data for three reasons. (1) Atrazine was tested at concentrations that inhibit growth of all species; thus, impacts on carbon uptake and assimilation were likely directly due to growth inhibition and not, as the authors suggest, due to direct atrazine interference with carbon uptake or assimilation. This is an important distinction. (2) The authors conceded that "these responses may not occur at environmentally relevant concentrations of atrazine." To address this issue, the authors will need to examine these same endpoints following short-term atrazine exposure and recovery (e.g., 24-hr exposure with 72-hr recovery; 48-hr exposure with 48-hr recovery; etc.). (3) The authors did not test whether effects on algal nutritional quality impact higher trophic levels.[2] This study simply tested the effects of a 96-hr exposure to unrepresentative atrazine concentrations on carbon assimilation in five different species of phytoplankton.

[2] To test this hypothesis, exposed algae would be fed to organisms (e.g., zooplankton) that use phytoplankton as a food source.

8.0 THE NMFS REVIEW USED AN INCORRECT LEGAL STANDARD FOR EVALUATING "UNCERTAINTIES"

The NMFS review identified three "areas of uncertainty" (toxicity of mixtures, sublethal endpoints, and extrapolation from surrogate species) for which it criticized EPA's risk assessment as incomplete. However, EPA's assessment did use data pertaining to these "areas of uncertainty". Limited evidence which raises a mere possibility that an effect might occur does not satisfy the standard for showing that the effect is "likely" to occur. "Likely" means probable, or "seeming like truth, fact, or certainty" (Randomhouse Unabridged Dictionary 2006). To find that a pesticide is "likely" to adversely affect a species, or that it is "likely" to cause jeopardy, it is necessary that the weight of the evidence supports the proposition that such an outcome is more probable than not. As NMFS notes, the objective is to make "the best possible predictions of the likely outcome," not of theoretical, possible outcomes.

The meaning of "likely" is further explained in the Services regulations. The Services are charged with evaluating the "effects of the action" during a consultation (50 C.F.R. § 402.14(g)(3)), and then formulating a biological opinion as to whether the action is "likely to jeopardize," (§ 402.14(g)(4)). The regulations incorporate the concept that "effects of the action," whether direct or indirect, are those that "are caused by" the proposed action and "are reasonably certain to occur." 50 C.F.R. § 402.02. In the preamble to the 1986 regulations, the Service stated that it rejects the suggestion that "future, speculative effects" might be said to jeopardize a species: "Congress did not intend that Federal actions be precluded by such speculative actions." 51 Fed. Reg. 19926, 19933 (June 3, 1986). Such an interpretation "would open the door for speculative actions to be factored into the" analysis. *Id*. Thus, "there must exist more than a mere possibility" that an action will cause adverse effects in order to find that such effects are likely. *Id*.

Applying these principles to the "areas of uncertainty" identified by NMFS, the very fact that predicting the effects of mixtures, or the consequences of sublethal effects, are recognized to be "areas of uncertainty" means, almost by definition, that such theories will not satisfy the standard for establishing that adverse effects are "likely." An uncertainty is an event whose occurrence is, by definition, uncertain. An uncertainty is neither likely nor probable.

Available data do not allow for a reliable quantitative assessment of the combined effects of multiple pesticides. The ESA requires decisions to be made on the basis of the best "available" data, not on the basis of speculation as to what data might show if they were available. Limited sporadic data (particularly data which does not meet data quality standards or requirements) that might support a hypothesis as to possible effects from mixtures cannot be said to show a likelihood of adverse effects to listed species, and are not a sound or acceptable basis for ESA regulation.

Similarly, with respect to sublethal effects, the NMFS review cited several studies with atrazine. However these studies cannot reasonably be viewed as showing that environmental

exposures to atrazine would be *likely* to cause adverse effects on growth, survival or reproduction.

Finally, with respect to the use of surrogates, the NMFS review asserts that reliance on avian data as a surrogate for sea turtle data introduces an unquantifiable level of uncertainty in the risk assessment. However, the use of surrogate data is a basic tenet of EPA's risk assessment process. EPA requires testing on the most appropriate surrogate species, and then uses the lowest toxicity values as the basis for the risk assessment. This methodology has been approved by the Services: "toxicity tests on surrogate species constitutes the best available information to analyze the toxicological sensitivities of untested species", where no other data is available (Williams and Hogarth, 2004 at 13). Section 7(a)(2) of the ESA requires that the best *available* scientific and commercial data be utilized in evaluating the potential impacts of an action to listed species. Since sea turtle-specific data for atrazine were not available, EPA properly relied on surrogate avian toxicity data to conduct an extensive evaluation that included dietary, dermal and drinking water risk. Thus, while every risk assessment has some level of uncertainty, the mere fact that there may be some unquantifiable uncertainty does not constitute evidence that exposure is *likely* to cause adverse effects. A mere unquantifiable possibility is not a likelihood.

In summary, the NMFS review's analysis is in large part based on an incorrect legal standard: the asserted existence of mere hypotheses, possibilities and uncertainty. The proper legal standard under the ESA and the Services' regulations turns on whether the best available data demonstrate that atrazine is "likely" to adversely affect, or jeopardize, listed species. As shown in EPA's assessment and in this paper, the weight of the scientifically valid and credible data does not demonstrate any such likelihood of adverse effects.

9.0 CONCLUSIONS

The data do not support the NMFS review's ecological conclusion on the potential for atrazine to adversely affect the listed endangered species. The NMFS review's use of incorrect toxicity values, misinterpretation of the literature associated with potential effects of atrazine and other pesticides, mischaracterization of available monitoring data, and an incorrect reliance on hypothetical effects from "uncertainties" led to invalid risk conclusions. EPA has conducted a thorough and conservative evaluation which resulted in "no effect" or "not likely to adversely affect" determinations for the species at issue, and the NMFS review did not cite any new reliable data that support a change in EPA's original effects determinations.

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