Technical Appendix

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 Introduction

This attachment describes the technical findings of the National Marine Fisheries Service (NMFS) review of EPA's effects determination (referred to herein as Biological Evaluation [BE]) for the effects of atrazine on 5 listed species in the Chesapeake Bay watershed under NMFS' jurisdiction and concludes with recommendations for meeting the substantive requirements of section 7(a)(2) of the Endangered Species Act (16 U.S.C. 1536). As we discussed during the December 2006 and February 2007 meetings, before NMFS can concur with the conclusions presented in any biological evaluation (effects determination) developed by Environmental Protection Agency (EPA) or any other federal agency, NMFS must also agree the rationale and evidence for that determination are valid.

The ESA and its implementing regulations form the foundation for evaluating whether agency actions are not likely to jeopardize the continued existence of endangered or threatened species or destroy or adversely modify designated critical habitat. Additional guidance and interagency policy for meeting the procedural and substantive requirements of section 7 are established within a variety of sources including the Consultation Handbook (FWS and NMFS 1998), Interagency Policy on Information Standards of the ESA (59 FR 166, 34271-34274; July 1, 1994), Information Quality Act (Section 515 of the Treasury and General Government Appropriations Act for Fiscal Year 2001 [Public Law 106-554; H.R. 5658]), numerous judicial decisions resulting from litigation, and the Administrative Procedure Act (5 U.S.C. 706; hereafter APA).

1.1 The Evaluation Framework

1.1.1 The Principles, Practices and Protocols of Section 7 Determinations

Section 7 of the ESA requires federal agencies, in consultation with and with the assistance of the Secretaries of Commerce and Interior, insure that any action they authorize, fund, or carry out is not likely to jeopardize the continued existence of endangered or threatened species or destroy or adversely modify designated critical habitat (unless such agency has been granted an exemption for such action by the

Committee pursuant to section 7(h) of the ESA). Interagency consultations conducted pursuant to section 7 of the ESA were established to help fulfill the purposes of the ESA, which are: "...to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved, to provide a program for the conservation of such endangered species and threatened species..." and the policy that "...all Federal departments and agencies shall seek to conserve endangered species and threatened species and shall utilize their authorities in furtherance of the purposes of this Act (16 U.S.C. 1531 (b, c))." The procedural duty is to "consult" with the Secretary using procedures that have been codified in regulations found at 50 CFR Part 402. In so doing, federal agencies are required to "*use the best scientific and commercial data available* (16 U.S.C. 1536(a))."

To help agencies fulfill the statutory requirements of section 7 of the ESA, the Services first determine if actions are likely to adversely affect listed resources. When an action is likely to adversely affect listed resources, the Services conduct more detailed analyses that are designed to determine (a) if the action can be expected to reduce a listed species' reproduction, number, distribution; (b) if any reduction in reproduction, number, or distribution would appreciably reduce the species' likelihood of both surviving and recovering in the wild (given the importance of the action area, the species' base condition in the action area, and the species' overall extinction risk); (c) if the action can be expected to destroy or adversely modify constituent elements of critical habitat that has been designated for threatened or endangered species, and (d) if impacts to constituent elements effect the ability of critical habitat to fulfill its conservation role for the listed species.

Pending the outcome of NMFS' evaluation of the effects of the proposed action, the action may be modified to minimize or eliminate consequences to listed species and their designated critical habitat. The challenge in conducting these assessments is to characterize future environmental conditions resulting from the execution of specific federal activities, and making predictions of species responses to those future conditions in the face of uncertainty. The intent of section 7 consultations, when conducted using

the best available scientific and commercial data, is to make the best possible predictions of the likely outcome from exposing listed species and their habitat to proposed federal activities. Federal agencies then would consider this information in making their decision to take, or not take, or modify the action as it was originally proposed to minimize the risk of adverse consequences on listed species and their designated critical habitats. Through consultation the Services and the federal agency determine what, if any, changes to the federal action are necessary to insure listed species are not likely to be jeopardized or critical habitat adversely modified or destroyed.

1.1.2 The Standards of Review

Interagency consultations and the documents they produce (e.g., concurrence letters and biological opinions) generally must comply with the requirements of the ESA and the Administrative Procedure Act (5 U.S.C. 706). To comply with the role Congress established for us in section 7 consultations, the Services believe they have an obligation to provide federal agencies and applicants, if any, consultations and consultation documents that are legally-defensible. To insure the legal defensibility of our documents, the Services evaluate their consultations and consultation documents using the standards of review courts would use: the arbitrary and capricious standards of section 706 of the APA. Based on numerous opinions from federal courts, a section 7 consultation or consultation document would be arbitrary and capricious if we:

- Relied on factors that Congress did not intend us to consider;
- Failed to consider an important aspect of a problem;
- Offered an explanation for our conclusion that runs counter to the evidence before us;
- Or failed to articulate a rational connection between the facts that were found and the conclusions we reached[1].

[1] See *Bennett v Spear*, 520 U.S. 154 (117 S.Ct. 1154). See also, *Idaho Department of Fish and Game* v. *National Marine Fisheries Service et al.*, 850 F. Supp. 886 (D.Or 1994)] in which the court concluded that "judicial review is limited to an assessment of whether the agency 'conducted a reasoned evaluation of the relevant information and

Under the authority of the APA courts can hold unlawful and set aside any findings or conclusions that are found to be arbitrary and capricious. Therefore, our shared challenge in this consultation is to make certain that the conclusions we reach are not arbitrary and capricious. The Services endeavor to meet this standard by using strong arguments to demonstrate a reasoned reflection of the relevant evidence available, that the premises of our reasoning are acceptable and warranted, that the premises provide sufficient grounds for our conclusions, and that we consider and rebut obvious challenges to the reasoning we present. To comply with the requirements of section 7, our reasons and evidence must include the best scientific and commercial data available, the status of listed resources, the environmental baseline of an action area, the effects of the proposed action, and the cumulative effects of future state or private activities that are reasonably certain to occur within the action area.

We use the same four general criteria that we apply to our own arguments to determine if we can agree with the reasons, evidence, and conclusion presented to us by a federal action agency during consultation. When the argument presented to us by a federal agency during section 7 consultation does not meet these four general criteria we will come to the conclusion that has the strongest support from the evidence available. Pending the outcome of our review of any consultation documents, we will provide our own support for the conclusion of the federal action agency's argument (e.g., supplement the action agency's argument further demonstrating the reasons for our concurrence) or present our rebuttal to their argument (e.g., provide reasoning why the federal agency should request formal consultation or modify their action to eliminate potential adverse effects).

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reached a decision that, although perhaps disputable, was not arbitrary or capricious.'" In determining "whether an agency decision was 'arbitrary or capricious,' the reviewing court 'must consider whether the decision was based on a consideration of the relevant factors and whether there has been a clear error of judgment.'" *Marsh v Oregon Natural Resources Council*, 490 U.S. 360, 378 (1989). An agency action is also arbitrary and capricious when the agency fails "to articulate a satisfactory explanation for its action." *Northern Spotted Owl v Hodel*, 716 F.Supp. 479, 482 (W.D. Wash. 1988). "A biological opinion is arbitrary and capricious and will be set aside when it has failed to articulate a satisfactory explanation for its conclusions or when it has entirely failed to consider an important aspect of the problem. While courts must defer to an agency's reasonable interpretation of equivocal evidence, such deference is not unlimited. The presumption of agency expertise may be rebutted if its decisions, even though based on scientific expertise, are not reasoned." *Greenpeace et al. v NMFS*, 55 F.Supp. 2d 1248, 1259 (W.D. Wash. 1999), citing *Defenders of Wildlife v Babbitt*, 958 F.Supp. 670, 679 (D.D.C. 1997).

1.2 Interagency Identified Uncertainties in Pesticide Risk Assessments

In December 2002 EPA, NMFS and the Fish and Wildlife Service began an interagency dialogue aimed at assisting EPA to streamline section 7 consultation processes. In January 2003, the agencies jointly published an Advanced Notice of Proposed Rulemaking to address the consultation process for pesticides and to discuss potential joint counterpart regulations. In 2005, EPA, USFWS, and NMFS produced a draft document based on the Services' review of EPA Office of Pesticide Program's (OPP) Overview Document (OD) and the ongoing dialogue between EPA and the Services on the research planning process to address current uncertainties in listed species' effect determinations (EPA and NMFS 2005). The jointly developed document identified eight areas of risk assessment and research uncertainties. Three of the identified areas of uncertainty are of particular relevance to EPA's current assessment of atrazine's potential risk to listed sea turtles and shortnose sturgeon. NMFS disagrees with the manner in which EPA addressed these uncertainties and believes that EPA's approach likely underestimates the actual risks of adverse effects of atrazine to sea turtles and shortnose sturgeon.

The three uncertainties include:

- *"Toxicity of mixtures/formulated products, including environmental mixtures, tank mixtures and approaches for evaluating risks of chemical mixtures."* Information is presented in the assessment that demonstrates that atrazine in combination with other pesticides results in greater toxicity to primary producers and to aquatic invertebrates than from atrazine alone. However, this information was not used to support the effect determination. Rather, EPA risk quotients that formed the basis for the effect determination relied on toxicity and exposure data for the active ingredient alone.
- *"Development and use of ecologically relevant (sublethal) endpoints."* The BE did not incorporate studies on sublethal effects including data demonstrating that atrazine can adversely affect olfactory mediated behaviors such as male and female reproductive behaviors, and impair swimming, sheltering, and schooling behaviors at concentrations as low as 0.5 ug/L.

- *"Appropriate use of surrogate species and interspecies extrapolation, the potential to include additional test species (e.g., amphibians)."* Sea turtle responses to atrazine were predicted from bird dietary toxicity data although no information on the similarity in responses to atrazine or other contaminants between birds and sea turtles were presented. Data on other vertebrates e.g. fish and amphibians suggest sea turtles may be more sensitive to atrazine than birds, but these data were not utilized in the effect determination.

1.3 Background Information on Atrazine Use and Prevalence in Surface Waters

Atrazine is the active ingredient in several herbicides and is currently registered throughout the United States for use on a variety of crops as well as for uses on residential, industrial, and forested lands. Atrazine-containing products have been used in the United States for more than fifty years and atrazine is currently one of the most heavily used herbicides in the nation. As such, atrazine is a frequently sampled pesticide in local, state, academic, and federal monitoring programs. The United States Geological Survey's (USGS) National Water Quality Assessment (NAWQA) Program's monitoring data represent the most extensive monitoring efforts to date of current use pesticides in the United States. A recent analysis of pesticide monitoring data (1992-2001) indicate that of the top five detected pesticides in U.S. surface waters found in agricultural areas three were triazines: atrazine, deethylatrazine (an atrazine degradate), and cyanizine (Gilliom et al. 2006). Overall, atrazine was the most frequently detected pesticide in surface waters nationally, presumably due to its high use rates, persistence, and water solubility (Gilliom et al. 2006). Concentrations of atrazine range from below detectable levels to as much as 2300 ug/l, although the majority of detections are in the low ug/L range.

1.3.1 Atrazine in the Chesapeake Bay Watershed

Available atrazine use and monitoring data document year round applications to multiple crops. EPA estimated that annual use of atrazine on corn and sorghum was 500,000 lbs in Maryland, 600,000 lbs in Virginia, and 1,500,00 lbs in Pennsylvania [EPA 2006]) and surface water detections were upwards of 30 ug/L (EPA 2006). Atrazine has been

detected in streams, rivers, nearshore estuarine habitats, and in open portions of the bay throughout the year in the Chesapeake Bay watershed (EPA 2002). Atrazine concentrations as high as 98 ug/L have been detected in streams of the Chesapeake Bay watershed (Hall et al. 1999).

1.3.2 Commonly Detected Pesticides Co-Occurring With Atrazine

Atrazine is frequently applied within formulations and tank mixes that contain other pesticides (Table 1) and is also detected in surface water samples with multiple pesticides. This is particularly true of samples from watersheds, like the Chesapeake Bay, that have a high degree of urban and agricultural land uses. For example, a synthesis of national NAWQA monitoring data found that more than 80% of water samples from urban streams contained two or more pesticides, and about 15% of the samples contained 10 or more pesticides (Hoffman et al. 2000). Another study monitored 8 urban streams across the United States which resulted in detections of two or more herbicides and insecticides in 85 and 54% of the samples, respectively (Hoffman 2000). For herbicides, the co-occurrence of multiple compounds was common. Four or more herbicides were quantified in 61% of the water samples obtained from the eight urban streams. Atrazine was detected in 54% of the samples while simazine (another triazine) was detected in > 70% of samples. One finding from a USGS study that evaluated 10 years of pesticide monitoring data in surface waters (1989-1998) concluded that multiple samples containing herbicides had probable toxicity to duckweed and green algae based on a toxicity index (Battaglin and Fairchild 2002). Atrazine was present in each mixture and was highlighted as partially responsible for toxicity.

Table 1. Representative examples of registered atrazine containing formulations and tank mixes (Greenbook 2006).

Formulated product (% atrazine)	Crop	Other ingredients including active ingredients (% other ingredients) % active ingredient;	Recommended tank mixes (active ingredients)
ATREX 4L (42.6%)	Corn, sorghum, other crops	(56%)	s-metolachlor, glyphosate, alachlor, simazine
AATREX Nine-O (88.2%)	Corn, grain sorghum	(10%)	s-metolachlor, glyphosate, alachlor, simazine
Banvel-K-Atrazine (22.23%)	Corn, grain sorghum	Dicamba - 13.42% (64.35%)	cyanizine, simazine, paraquot, EPTC, acetochlor, 2,4-D, pendimethalin
Bullet (14.5%)	Corn, grain sorghum	Alachlor - 25.4% (59.3%)	pendimethalin, paraquat, linuron
Basis gold (82.44%)	Corn	Nicosulfuron - 1.34% Rimsulfuron - 1.34% (10.54%)	dicamba, esfenvalerate, methomyl
Cinch (33%)	Corn, grain sorghum	s-metolachlor - 26.1% (40.2%)	atrazine, paraquat, glyphosate, simazine,

2 Comments on the Atrazine Effects Determination

In this section NMFS provides technical comments on the atrazine effects determination. Comments are organized by key components of EPA's atrazine BE including discussion of exposure, effects, and risk characterization.

2.1 Exposure Assessment

2.1.1 PRZM/EXAMS Scenarios

EPA used PRZM-3 and EXAMS II (PRZM/EXAMS) exposure models based on several crop scenarios to estimate atrazine concentrations because GENEEC2, the initial screening level model, produced atrazine concentration estimates that exceeded Levels of Concern (LOC) (EPA 2004). According to EPA's process, PRZM/EXAMS was then used to refine atrazine exposure concentrations by incorporating site-specific conditions that likely influence runoff. However, it is unclear that the PRZM/EXAMS scenarios used in the BE provided reasonable exposure estimates for the registered uses of atrazine and that the site-specific inputs used in the model are indicative of actual conditions in treatment sites and aquatic habitats of the Chesapeake Bay watershed.

PRZM/EXAMS simulations were based on "typical" atrazine crop use scenarios which used inputs from sites outside the Chesapeake Bay watershed including locations in Pennsylvania (to represent corn and turf scenarios), Kansas (sorghum), and Oregon (forestry). The simulations for EPA's analysis included a single application of atrazine at a specified date despite the potential year-round use of atrazine-containing products. The resulting predictions for surface water concentrations are highly influenced by runoff characteristics assumed for the site (e.g. soil type, slope) and the timing and magnitude of corresponding rainstorm events. For example, predicted aquatic concentrations were greater for the Kansas sorghum scenario than for the Oregon forestry scenario despite a much lower atrazine application rate (50%) as a consequence of differences in site-specific assumptions. It is difficult to evaluate EPA's assertion that these PRZM-

EXAMS simulations provide "reasonable high-end estimates of exposure" because the input assumptions have not been put into perspective with regard to the range of site-specific conditions and uses (e.g. application dates and use rates) of atrazine within the Chesapeake Bay watershed.

2.1.2 Modified PRZM/EXAMS Scenarios

When Levels of Concern (LOC) were exceeded using PRZM/EXAMS methods specified in the overview document (EPA 2004), the static pond model that EPA uses was modified by incorporating a variable flow model to account for potential dissipation of atrazine concentrations in running water habitats. This modification apparently resulted in atrazine concentrations that were significantly lower than the static pond model and likely do not represent realistic worst case exposure scenarios to shortnose sturgeon. For example, the resulting exposure estimates may not provide conservative estimates for individuals that utilize habitats < 2 meters deep or in locations where the watershed to surface water ratio is greater than 10:1. Consequently, risk to listed species that occupy such habitats will likely be underestimated.

2.1.3 Use of Monitoring Data to Replace Modeled Peak Estimated Environmental Concentrations (EECs)

The Chesapeake Bay monitoring data as a replacement for modeled-derived peak estimations likely results in an underestimation of exposure risk to listed species in the Chesapeake Bay. The monitoring data utilized in EPA's BE suggest that atrazine is commonly present in surface waters of the Chesapeake Bay watershed in the low part per billion range. However, EPA has previously recognized in the Overview Document that monitoring data are generally not representative of peak exposure (EPA 2004). Additionally, EPA previously concluded that the monitoring data utilized in this BE "underestimate the [atrazine] concentrations likely to be present in streams (EPA 2002)." EPA indicate atrazine was monitored in Chesapeake Bay's tidal rivers at concentrations as high as 30 ug/L. Hall *et al.* (1999) report concentrations up to 98 ug/L in surface waters of the Chesapeake Bay watershed following rainfall events. Recent atrazine monitoring data from other regions indicated atrazine concentrations in surface water

associated with corn and sorghum production may reach even greater concentrations (EPA 2007). For example, atrazine has been detected in streams at concentrations above 100 ug/L at monitoring sites in Nebraska, Indiana, and Missouri (EPA 2007). In some cases relatively high levels of atrazine were observed in surface water for extended periods. For example, in 2006 surface water concentrations averaged 30 ug/L for the month of May at one Missouri site (MO-01) and 24 ug/L at another (MO-02). EPA restrictions on atrazine use in corn and sorghum are identical between states. Differences observed in peak concentrations among the monitoring data discussed above may be due to site specific differences that contribute to runoff, different regional use patterns in atrazine, or inadequate replication to account for the variability in the data. Given EPA's recognition that the Chesapeake Bay monitoring data likely underestimate peak concentrations, and that monitoring data from other corn producing regions suggest peak short-term and longer term concentrations may be much greater than those observed in Chesapeake Bay monitoring data, NMFS does not concur with the use of the Chesapeake Bay monitoring data as a reliable indicator of either acute or chronic exposures in surface waters of the Chesapeake Bay watershed.

2.1.4 Concurrent Exposure to Multiple Pesticides

Estimated exposure concentrations were developed exclusively for atrazine (not for commonly occurring mixtures) in the BE and therefore likely underestimate the actual risk of atrazine-containing pesticides to threatened and endangered sea turtles and shortnose sturgeon under exposure conditions in the Chesapeake Bay watershed. As mentioned above, NAWQA monitoring data indicate that individual samples across the United States including the Chesapeake Bay watershed typically contain multiple pesticides (Gilliom et al. 2006). NAWQA data also indicate that three of the top five pesticides detected in surface waters of agricultural areas included the triazines: atrazine, deethylatrazine (a degradation product of atrazine), and cyanizine (Gilliom et al. 2006). Atrazine and other triazines share a common mode of action and are likely to cause additive toxicity to primary producers and to fish behavioral endpoints. Additive and synergistic responses have been observed in aquatic species when they are exposed to atrazine and other pesticides concurrently (see effects section below). The BE did not

produce exposure estimates for other active ingredients present in atrazine formulations or commonly detected in environmental mixtures known to result in additive and synergistic responses (see Table 1 and Table 3). Consequently, risk to listed species is likely underestimated because exposure to these co-occurring compounds, although likely, was not used in the risk quotient calculations.

2.1.5 NMFS' Exposure Conclusion

The reliability and accuracy of EPA's methods for estimating exposure to NMFS' listed species in the Chesapeake Bay watershed is difficult to ascertain from the description provided in the BE. Questions remain on the adequacy of the chosen PRZM/EXAMS scenarios to represent worst case use and exposure within the Chesapeake Bay watershed. Incorporation of a variable flow model into the static pond model is not described in sufficient detail to determine if atrazine exposure is accurately and reliably predicted. The best scientific and commercial data indicate that sea turtles and short nose sturgeon will be exposed to atrazine concentrations exceeding 1 ug/L throughout the Bay and may be infrequently exposed to atrazine concentrations exceeding 100 ug/L in fresh water areas. Additionally, atrazine is expected to co-occur with other pesticides including triazines and OP insecticides in Chesapeake Bay watershed which may result in increased risk to listed species and their habitats.

2.2 Effects Assessment

NMFS reviewed the BE on atrazine and compiled and reviewed other available toxicity information to ascertain if the best scientific and commercial data support the BE conclusions regarding atrazine's potential effects to listed species in Chesapeake Bay.

2.2.1 Toxicity Endpoints Used by EPA for Risk Quotient Calculations

The BE evaluated the potential direct toxic effects of atrazine on the survival, reproduction, and growth of sea turtles and shortnose sturgeon. The BE also addressed potential effects to listed species habitat by evaluating atrazine toxicity information from other aquatic species such as plants and invertebrates. For the most part toxicity information used in the BE to quantify risk to listed species was from standard laboratory

toxicity tests submitted by registrants and from studies acquired from ECOTOX. The toxicity data used in risk quotients to estimate effects to listed species and habitat included:

1) Avian acute oral, and subacute dietary toxicity to estimate direct effects on survival, growth and reproduction of listed turtles;
2) Median lethal concentration and NOEC from early-life stage study with sensitive fish surrogate to estimated direct effects to survival, growth, and reproduction in shortnose sturgeon;
3) Median effect concentrations in aquatic animal and plant studies to estimate effects to food and cover; and
4) Effect concentration in terrestrial plants to estimate potential water quality impacts associated with impacts to riparian habitat.

In addition to standard FIFRA guideline toxicity studies submitted to EPA by atrazine registrants, other data presented in this BE were obtained from the 2003 atrazine IRED and from the EPA's ECOTOX database on February 16, 2006. However, the ECOTOX database poses constraints that limit inclusion of ecologically relevant toxicity data. In order to be included in the ECOTOX database, papers must meet the following criteria (EPA 2006):

1) the toxic effects are related to single chemical exposure;
2) the toxic effects are on an aquatic or terrestrial plant or animal species (no microorganisms);
3) there is a biological effect on live, whole organisms;
4) a concurrent environmental chemical concentration/dose or application rate is reported; and
5) there is an explicit duration of exposure.

NMFS evaluation of procedures used in the atrazine BE to select toxicity concentrations for use in risk quotients suggest risk to listed species may be substantially underestimated because toxicity data from the following study categories were disregarded: (1) ecologically relevant studies not conducted by protocols that meet FIFRA registration

requirements (40 CFR, part 158), and (2) toxicity studies with atrazine-containing mixtures.

2.2.2 Toxicity Data Not Included in Risk Quotient Analysis

NMFS believes that the BE uses toxicity and endpoint values that do not consider available toxicity information, and therefore likely underestimates effects to shortnose sturgeon. Several studies suggest lethal and sublethal effects to sturgeon may occur at much lower atrazine concentrations than toxicity values used in EPA's risk quotient analysis (Table 2). For example, the lowest acute LC50 reported for fish was 27 ug/L (brown trout). Under EPA's methodology (EPA 2004) an LC50 of 27 ug/L would result in atrazine concentrations of 1.35 ug/L or greater triggering the Endangered Species LOC. However, in the atrazine BE EPA selected 2000 ug/L from a sheepshead minnow study to represent the LC50 which resulted in atrazine concentrations of 100 ug/L or greater triggering the Endangered Species LOC. As presented in the exposure section of this appendix, atrazine concentrations in the low ppb are common and concentrations above 100 ug/L have been reported in areas where atrazine is applied. Since the basis for a "no effect" call is predicated on exceeding an Endangered Species LOC, EPA concluded there are no direct, acute effects to sturgeon because acute LOCs were not triggered i.e., EPA's exposure concentration estimates did not exceed 100 ug/L. If shortnose sturgeon are as sensitive as brown trout, the BE underestimated the acute lethality to sturgeon by more than 74 fold (if sturgeon are more sensitive than brown trout the disparity is even larger). Furthermore, other acute LC50s for fish are less than 2000 ug/L (Table 2) which together with the brown trout data call into question the selection of 2000 ug/L as a conservative estimate for "no effect" to listed shortnose sturgeon.

Direct, acute, sublethal effect data indicate that shortnose sturgeon are potentially adversely affected by low ug/L atrazine concentrations, however these ecologically relevant endpoints were not used in the BE. Atrazine adversely affected Atlantic salmon and goldfish at 0.5 ug/L (Moore and Lower 2001, Saglio and Trijasse 1998). Atrazine affected Atlantic salmon's olfactory-mediated reproductive behaviors evidenced by reduction in milt from males at 0.5 ug/L and reductions in female priming effects at 5

ug/L (Moore and Lower 2001). These gender-specific individual reproductive effects could result in reduced spawning success in atrazine exposed fish.

Imprinting of fish to natal waters may also be affected from short term, environmentally realistic atrazine concentrations, thereby affecting the distribution of fish. Moore and Lower (2001) inferred that imprinting and homing behaviors of salmon would be affected with acute exposure (30 minutes or longer) to atrazine at 0.5 ug/L given reductions in the ability of olfactory epithelium to detect the putative amino acid, L-serine. Shortnose sturgeon are also thought to rely upon imprinting and homing to locate natal spawning areas as evidenced by their high natal site fidelity (Kynard 1997). Therefore sturgeon distribution may be affected by atrazine. Additionally, Moore and Lower (2001) presented convincing evidence that simazine and atrazine mixtures (0.5 ug/L of each) resulted in additive toxicity to olfaction, male milt production, and female priming effect, which highlights the significance of the co-occurrence of other triazines in the Chesapeake Bay watershed.

In goldfish (a cyprinid), 24-h atrazine exposures resulted in impaired social and predator avoidance behaviors including burst swimming at 0.5 ug/L, and schooling, sheltering, and surfacing at 5 ug/L (Saglio and Trijasse 1998). Burst swimming reactions are a typical fish response to stressful situations including a predation event and toxicant exposure. Significant impairment of a predator avoidance response due to impaired olfaction may result in increased predation of shortnose sturgeon. Atrazine (5 ug/L; 24-h) also disrupted sheltering and swimming orientation behaviors of goldfish. These behaviors underlie the ability of fish to seek cover and avoid sources of stress and therefore impairment of these behaviors can increase the vulnerability of fish to predation in natural conditions. In aggregate, the available information on effects to behaviors indicates multiple fish species from several different families are affected by atrazine at low part per billion concentrations.

EPA concluded that acute exposure to atrazine at concentrations below 100 ug/L, and chronic exposure of less than 65 ug/L atrazine would result in no direct effects to

shortnose sturgeon. However, the acute threshold of 100 ug/L used in the BE was based on an LC50 study that was 74-fold less sensitive than an LC50 available from another species of fish. An array of other adverse effects to fishes were observed at atrazine concentrations (0.5 – 10 ug/L) well below the acute threshold presented in the BE (100 ug/L). Additionally, the chronic threshold of 65 ug/L used by EPA was 135-fold less sensitive than 0.5 ug/, the concentration of atrazine that impairs fish reproductive and behavioral endpoints. The available studies reviewed by NMFS above and presented in Table 2 do not support EPA's conclusion of no direct effects and suggest that adverse effects likely occur at concentrations of atrazine well below 65 and 100 ug/L. Consequently, the actual risk to listed species to atrazine use in the Chesapeake Bay watershed may be significantly underestimated in the current BE.

Table 2. Examples of assessment endpoints used in quantitative risk quotients versus a sample of measurement endpoints found in the ECOTOX database and the open literature that show adverse effects below EPA's selected toxicity values.

Listed Species Assessment Endpoint	Measurement Endpoint Used in Quantitative Assessment	Sensitive Measurement Endpoint in ECOTOX*	Other Pertinent Sensitive Measurement Endpoints
Sturgeon survival	**2000 ug/L** (LC50) sheepshead minnow	**27 ug/L** (LC50) brown trout, **50 ug/L** guppy, **147 ug/L** loach, **220, 310, 340, 220, and 240 ug/L** catfish, **660 ug/L** atrazine 80WP rainbow	**2 ug/L** (LOEL) effect on gill physiology suggest potential compromised ability of fish to survive in saltwater (Waring and Moore 2004), **10 ug/L** (Tierney et al. 2007) altered olfactory mediated behaviors in fish that may have implications for survival, growth, and reproduction, **29 ug/L** LC01 for embryo-larval survival (ECOTOX*, Birge et al. 1979)
Sturgeon growth, reproduction, and distribution	**65 ug/L** (NOEC) brook trout	**50 ug/L** (NOEC) channel catfish teratogenic effects, **54** ug/L rainbow embryo survival	**0.5 ug/L** reduced expressible milt in male fish and reduced detection of amino acids and **5.0 ug/L** reduced priming effect in female fish (Moore and Lower 2001), **0.5 ug/L** affected burst swimming speed and **5.0 ug/L** affected schooling, surface, and orientation behaviors (Saglio and Trijase 1998). **50 ug/L** altered plasma testosterone and vitellogenin levels in fish (EPA 2006,Wieser and Gross 2002).
Sea turtle survival	**>5000 mg/kg diet (LC50) Mallard**	**Not Available**	**Not Available**
Sea turtle growth, reproduction, and distribution	**225 mg/kg diet (NOEC) mallard**	**Not Available**	aromatase induction on turtle derived cell line (Keller and McClean-Green 2004), aromatase induction correlated with atrazine exposures and to reproductive effects (Vonier et al. 1996, Crain 1997). **0.1 ug/L** LOEL hermaphradism in leopard frog (Hayes et al. 2002 a/b), **1 ug/L** LOEL feminization in leopard frog (Hayes et al. 2002 a/b)

*ECOTOX, EPA Ecological Effects Database, http://cfpub.epa.gov/ecotox/

2.2.3 Potential Effects of Atrazine-Containing Mixtures

The exclusion of data that considers responses to more than a single active ingredient underestimates the potential risk of atrazine to aquatic organisms. Atrazine is frequently mixed with other active ingredients in pesticide formulations. EPA approved pesticide labels commonly recommend the use of atrazine containing pesticides in "tank mixes" with other pesticide formulations during application (Table 3, Greenbook 2006). Additionally, atrazine is commonly co-located with other pesticide ingredients in the aquatic environment (Gilliom et al. 2006, Battaglin et al. 2001, EPA 2002, EPA 1989). Several authors have investigated the response of aquatic organisms to pesticide mixtures

that contain atrazine and found a consistent pattern of additive and synergistic toxicity (Table 3). The mixtures represented an array of constituents, primarily in binary combinations, including other triazines, insecticides, and fungicides. These current-use pesticides co-occur in surface waters inhabited by shortnose sturgeon and sea turtles within the Chesapeake Bay watershed. However, existing monitoring and modeling data were not analyzed to address the potential for toxicity of mixtures occurring in these species' habitats.

2.2.3.1 Synergistic Toxicity

The available literature indicates that mixtures composed of atrazine (as well as some other triazines) and selected organophosphates (OP) result in synergistic toxicity in several aquatic invertebrates including *Chironomous elegans*, *Choronimous tentans*, and *Hyallela azteca* (Pape-Lindstrom and Lydy 1997, Belden and Lydy 2000, Miota et al. 2000, Belden and Lydy 2001, Anderson and Lydy 2002, Jin-Clark et al. 2002, Londono et al. 2004, Lydy and Austin 2005, Schuler et al. 2005, Belden and Lydy 2006, Trimble and Lydy 2006). Atrazine pontentiates the toxicity of an OP by inducing metabolic enzymes (cytochrome P450 monooxgenases) that are responsible for converting parent OP to much more toxic o-analog metabolites (Miota et al. 2000). The rapid metabolic activation to o-analog metabolites within invertebrates results in direct acute toxicity. Aquatic invertebrates are important prey items for rearing anadromous fish including shortnose sturgeon. Reduced populations of prey may affect growth and development at critical life stage transitions (e.g., alevin-fry).

Table 3. Examples of atrazine containing mixtures that adversely affect freshwater and marine aquatic species

Atrazine mixture constituents	Assessment endpoint	Assessment Measure	Chemical interaction	Reference
chlorothalonil chlorpyrifos	primary production	Algal population growth rate, marine phytoplankton	synergistic additive	(DeLorenzo and Serrano 2003)
Binary combinations chlorpyrifos, methyl parathion, diazinon	Amphipod mortality	Death of *Hyallela azteca*	synergistic	(Anderson and Lydy 2002).
Binary combinations chlorpyrifos, methyl parathion, malathion	Midge larvae nervous system	Acetylchloinesterase activity	synergistic	(Belden and Lydy 2000)
chlorpyrifos	Midge larvae nervous system	Acetylchloinesterase activity	synergistic	(Jin-Clark,et al.2002)
24 pesticides	Primary productivity	Algal reproduction, fresh water	additive	(Junghans et al 2006)
cyanazine, prometryn, propazine, sebuthylazine, simazine, terbuthylazine, terbutryn	Primary productivity	Algal reproduction, fresh water	additive	(Faust et al. 2001)

2.2.3.2 Additive Toxicity

Atrazine is a member of the triazine herbicide class. As such, it shares a common mode of action with the other triazines. The triazines adversely affect plants (and by extension aquatic communities) by interfering with photosynthetic processes. Due to the specific mechanism of action of atrazine, primary producers are expected to be the most susceptible part of the aquatic community. Indeed, phytoplankton and periphyton, both ecologically important primary producers, have been shown to be highly sensitive to triazines. EPA concluded in 2002 that the triazine-containing pesticides atrazine, simazine, and propazine and their three chlorinated degradates should be included in a common mechanism group and considered through a cumulative risk assessment. In the case of primary producer toxicity, a comprehensive experiment with seven triazines indicated strict additive toxicity in a freshwater alga which supported a concentration addition approach for addressing triazines containing mixtures (Faust et al. 2001). Therefore when aquatic habitats are exposed to several triazines simultaneously, resultant

toxicity is from the combination of triazines, not a single constituent. Additive toxicity to primary producers is the expected outcome, yet is not characterized in the current BE.

2.2.4 NMFS' Conclusions for Direct Toxicity to Listed Species

The acute exposure threshold of 100 ug/L used by EPA was based on an LC50 study that was 74-fold less sensitive than an LC50 available from another species of fish. ECOTOX excluded relevant toxicity information. For example, atrazine-containing mixtures result in enhanced toxicity to aquatic species. The effect to sea turtles, shortnose sturgeon, and their supporting habitats from exposure to atrazine containing mixtures was not assessed in the BE. Additionally, a variety of relevant studies that indicate toxicity of atrazine at low environmentally realistic concentrations were not incorporated into EPA's risk quotient analysis (Table 2). For example, atrazine caused adverse physiological and behavioral effects in fish at concentrations ranging from 0.5-50 ug/L. The adverse effects observed included damage to gill physiology, embryo-larval survival, reductions in expressible milt and priming effect, altered olfactory mediated behaviors, and others responses(Moore and Lower 2001, Tierney et al. 2007, Birge et al. 1979, Saglio and Trijase 1998, Wieser and Gross 2002). These adverse effects may impair survival, growth, reproduction, and distribution of shortnose sturgeon exposed to atrazine at low part per billion concentrations. Sea turtle toxicity data from atrazine exposures are not available. The 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. Since only avian toxicity data were used as surrogates for sea turtles, atrazine's risk is potentially underestimated. Toxicity data for other vertebrates including fish and amphibians suggest atrazine may cause adverse effects in sea turtles as well (Table 2, Keller and McClean-Green 2004, Hayes et al. 2002 a/b, Vonier et al. 1996, Crain 1997).

2.2.5 Effects to Habitat

Atrazine may cause effects to listed species by impacting aquatic communities due to its herbicidal action on aquatic primary producers. EPA analyzed the potential for adverse effects to listed species via associated habitat modifications in a number of ways.

Initially, LC50s of aquatic animal and EC50s of plant studies were used to estimate effects to listed species habitat according to standard EPA screening methods (EPA 2004). Some risk quotients exceeded LOCs by this method suggesting to EPA that potential habitat effects could impact listed species (EPA 2004). EPA modified PRZM/EXAMS assumptions to account for potential dissipation of atrazine due to flow in running water aquatic habitats. Even so, risk quotients values still exceeded LOCs for some uses of atrazine suggesting to EPA adverse affects to listed species may occur. The final step in EPA's analysis of habitat impacts and the basis for EPA's "may effect, not likely to adversely affect" determinations was a comparison of estimated environmental concentrations to "aquatic community Levels of Concern." Aquatic community LOCs were not assessed by NMFS in our evaluation of the EPA BE procedures for listed species effect determinations (EPA 2004). Therefore, EPA's application of aquatic community LOCs for making effect determinations is described and evaluated below.

2.2.5.1 Aquatic Community LOCs

NMFS' understands that aquatic community LOCs were developed specifically for atrazine under a MOA between EPA and the registrant and used for the atrazine registration eligibility decision (EPA 2003). The nominal LOC values utilized were recommended by the Atrazine MOA Ecological Subgroup which included EPA and registrant representatives (EPA 2003). The MOA subgroup was charged with reaching agreement on LOCs that "potentially adversely affect aquatic communities and/or ecosystems" (EPA 2003). The subgroup recommended the following "trigger values (ug/L atrazine) for monitoring atrazine in 2nd and 3rd order Midwestern streams:"

- 14-day average = 38 ug/l
- 30-day average = 27 ug/L
- 60-day average =18 ug/L
- 90-day average = 12 ug/L

EPA applied these thresholds to the current BE as a basis for the determination that atrazine is not likely to adversely affect listed species in the Chesapeake Bay watershed due to habitat degradation. These LOCs were formed using the Comprehensive Aquatic

Systems Model (CASM), which is described as a generic food chain model. NMFS does not agree with this approach for the reasons discussed below.

2.2.5.1.1 CASM Versus Empirical Data

Acute laboratory EC50 data on 26 species were used as CASM inputs to predict possible responses of the aquatic community to atrazine. CASM was originally developed to "investigate resilience in food-chain and food-web models as nutrient input and the trophic structure are varied..." (DeAngelis et al. 1989). The primary objective of the paper was therefore to study a single property of food webs limited by a nutrient i.e. modeled aquatic community resilience from biomass loss. The model is based on bioenergetic equations of modeled populations. Since the model incorporated biomass loss as a function of nutrient dynamics, hypothetically, other perturbations that directly affected community biomass such as reduction of primary producers from toxic chemicals could be incorporated. Bartell et al. 1999 applied this approach by adapting CASM to address potential effects of toxic chemicals (copper, mercury, diquat dibromide) in Quebec rivers, lakes, and reservoirs. Bartell et al. (1999) stressed that the role of ecological models such as CASM was to assist in estimating ecological risks especially when critical laboratory or field data are sparse. However, atrazine is a "data rich" compound with an abundant number of microcosm and mesocosm studies available. These empirical data frequently contradict the CASM model predictions. Many microcosm and mesocosm studies indicate adverse effects to aquatic communities occur at concentrations below EPA aquatic community LOCs, yet EPA relied on CASM-derived thresholds in place of existing data that directly measure community responses increasing the likelihood that risk to listed species within the Chesapeake Bay watershed was underestimated (Table 4).

Table 4. Examples of mesocosm and microcosm experimental results with statistically significant adverse effects to aquatic communities below EPA aquatic community LOCs (Adapted from EPA 2006, Appendix A/attachment 1).

Exposure		Ecosystem	Endpoint	Time to	
ug/ L	single pulse/ constant	Type	Observed Effects (Brock Score)	Recover	Ref *
20	Single	Ponds	Decreased biomass of tadpoles and abundance of a single fish species, cover by emerged, floating and submerged aquatic plants (5)	> 1 yr	1-7
10	Single	Lab microcosm	Change in species composition and density(4)	NA	8
15	Constant	Artificial stream	Decreased algal abundance(4)	>28 days	9
15	Constant	Artificial swamp	Decreased DO, metabolism or epiphyte/ increased nutrients(4)	NA	10
10	Constant	Artificial stream	Decreased gross primary productivity and abundance of periphyton (4)	>21 days	11-12
24	Constant	Artificial stream	Decreased chlorophyll-a and periphyton biomass (4)	NA	13
20	Single	Pond	Decreased biomass of phytoplankton/ change in species composition of phytoplankton (2)	7 days	15-17
10	Single	Lab microcosm	Decreased gross primary production(2)	7 days	18
22	Constant	Pond	Decreased DO, pH, conductivity (2)	NA	19
10	Constant	Pond	Decreased DO, pH, conductivity (2)	NA	19
1.89	NA	Stream	Decreased phytoplankton species richness and cell numbers(2)	150 days	20
1	NA	Lake	Decreased primary production(2)	14 days	21
32	Constant	Lab microcosm	Decreased number of protozoa species, DO, magnesium, calcium(2)	>21 days	22
20	Single	Lab microcosm	Decreased primary production (2)	1-10 days	23
5	Constant	Lab microcosm	Decreased photoactivity, higher conductivity and alkalinity, lower DO and pH(2)	NA	24

*[1]Carney 1983; [2]Kettle et al. 1987; [3]deNoyelles et al. 1989; [4]deNoyelles et al. 1989; [5]deNoyelles and Kettle 1983; [6]deNoyelles and Kettle 1980; [7]Dewey 1986; [8]Berard et al 1999; [9]Carder and Hoagland 1998; [10]Detenback et al 1996; [11]Kosinski 1984; [12]Kosinski and Merkle 1984; [13]Krieger et al. 1988; [14]Brockway et al. 1994; [15] deNoyelles et al 1982; [16]Kettle 1982; [17]DeNoyelles et al. 1989; [18]Johnson 1986; [19]Juttner et al. 1995; [20]Lakshminarayana et al 1992; [21]Lampert et al 1989; [22]Pratt et al. 1988; [23]Stay et al. 1989; [24]van den Brink et al. 1995.

The risk of atrazine to aquatic communities has been addressed in several, formal risk assessments. The conclusions from these suggest that EPA aquatic community LOCs may underestimate risk to listed species. For example, Huber (1993) and Draxal et al. (1994) predicted ecological effects to aquatic communities for atrazine exposure at 20 ug/L. In the latter study, concentrations below 20 ug/L were not tested indicating the actual effect threshold may be even lower. A risk assessment of atrazine in North American surface water concluded that aquatic ecosystem disturbance begins above 20 ug/L (Solomon et al. 1996). However, multiple studies show adverse effects to aquatic ecosystems below 20 ug/L (Table 4). Brock et al. (2000) evaluated results from 25 microcosm/mesocosm studies to assess the risk of atrazine to aquatic communities and concluded the $NOEC_{eco}$ of 5 ug/L is "a safer threshold value" than 20 ug/L (Brock et al. 2000). These evaluations of atrazine risk to aquatic communities indicate that adverse effects to aquatic habitats may occur at concentrations below aquatic community LOCs. Using the aquatic community LOCs to screen for potential adverse effects to aquatic habitat may therefore underestimate the risk of atrazine to listed species.

2.2.5.1.2 CASM as a Tool to Evaluate Dose-Response

EPA stated that "there was a need for methods to predict relative differences in effects for different types of exposure" because the effects observed in microcosm and mesocosm studies "varied with exposure duration and magnitude (EPA 2006, Appendix B)." EPA presented CASM as a tool for evaluating the relationships in various exposure regimes and responses observed in the microcosm and mesocosm studies. However, CASM was designed to predict ecological responses in the absence of empirical data and it is likely not well suited for interpreting dose-response relationships. The conclusion that CASM would be useful for predicting responses at variable exposure durations and concentrations is counterintuitive because the model is parameterized primarily with acute exposure data and lacks the "time-to-effect" analysis that is fundamental in ascertaining the relationship between exposure duration, concentration, and effect. CASM-derived aquatic community LOCs likely underestimated the effects of atrazine on aquatic communities in the Chesapeake Bay watershed because empirical data indicate adverse effects to aquatic habitats occur at concentrations less than CASM-derived

LOCs. In addition, the rationale provided in the effects determination as to why CASM is an appropriate tool to interpret ecological effects under variable exposure regimes remains unclear and unsubstantiated especially in the face of existing empirical data.

2.2.5.1.3 CASM Input from Midwestern Streams

CASM relies on site specific input parameters such as water temperature, dissolved nutrients, surface light intensity, species growth rates, species composition, and species interactions to predict changes in primary production and effects to aquatic communities. EPA used site-specific input parameters based on 2nd and 3rd order Midwest streams to predict effect to listed species in the Chesapeake Bay watershed. Presumably there are significant differences between the conditions of 2nd and 3rd order Midwestern streams and the habitats occupied by listed species in the Chesapeake Bay watershed (including streams, estuaries, and the bay). However, no information is provided in the effects determination to suggest that the Chesapeake Bay watershed shares similar environmental conditions as 2nd and 3rd order Midwestern streams. The BE does not address the predictive capability of the Midwestern stream simulations for aquatic communities in streams, estuaries, and marine habitats of the Chesapeake Bay watershed.

2.2.5.1.4 CASM Use for NLAA Determinations

EPA evaluated the use of a 5% change in Steinhaus Similarity Index (SSI), the endpoint used to derive aquatic community LOCs. This was done by comparing the severity of various community level responses to estimated changes in similarity index for the mesocosm results (EPA 2006, Appendix B, Figure B.5). According to EPA's analysis, this resulted in a false negative rate of 8%, meaning a 5% change in SSI would not be protective of community level impacts 8% of the time. EPA concluded the analysis represents a reasonable predictor of community level effects. However, NMFS asserts that a 5% change in SSI is not an appropriate threshold for a "may affect, not likely to adversely affect" determination given the relatively high frequency of false negatives and given the severity of effects observed to experimental aquatic communities when the threshold failed to be protective.

Severity of false negatives. EPA scored all mesocosm results based on the severity and persistence of adverse effects i.e., 1, 2, 3, 4, or 5; where one is the least severe and 5 is the most severe as described in Brock et al (2000). Scores of 4 and 5 are defined by reductions in functional endpoints and elimination of species that are either not reversed during the course of the evaluation (Brock score of 4), or persist for more than 8 weeks (Brock score of 5; Brock et al. 2000, EPA 2006). EPA assigned Brock scores of 4 and 5 to all of the false negative results suggesting that false negative errors associated with the aquatic community LOCs are likely to be severe and persistent. For example, studies that CASM predicted would not result in community level effects showed significant reductions in biomass of plants for more than a year (Table 4, Carney 1983, Kettle et al. 1987, deNoyelles et al. 1989, deNoyelles et al. 1994, deNoyelles and Kettle 1983, deNoyelles and Kettle 1980, Dewey 1986). Several other studies' results showed clear adverse effects in species composition and in abundance of primary producers which never recovered prior to study termination, were also erroneously judged by CASM to result in no adverse effects (Berard et al. 1999, Carder and Hoagland 1998, Detenback et al 1996, Kosinski 1984, Krieger et al. 1988, Kosinski and Merkle 1984).

Frequency of false negatives. The false negative error rate of 8% is an underestimate when all statistically significant adverse effects observed in the mesocosm studies are considered. Nineteen percent of the mesocosm results evaluated had statistically significant adverse effects (Brock scores of 2 or greater) at levels below the 5% change in similarity threshold (EPA 2006). Although statistically significant, Brock scores of 2 were results characterized as "slight" and/or "transient"and EPA did not consider these effects to be adverse. However, several studies with Brock scores of 2 reported statistically significant decreases in primary productivity (Johnson 1986, Lampert et al. 1989, Stay et al. 1989). Others reported statistically significant modifications in water chemistry parameters including reductions in dissolved oxygen and pH, indicators of reduced photosynthetic activity (Brockway et al. 1984, Juttner et al. 1995, Stay et al. 1989, van den Brink et al. 1995). Several studies reported significant reductions in plankton and changes in species composition that were significant (Lakshminarayana et

al. 1992, deNoyelles et al. 1982, Kettle 1982). The current analysis fails to demonstrate that these effects are insignificant, discountable or wholly beneficial. Additionally, other adverse effects would be overlooked by this process such as atrazine's effects on algal nutritional quality (Weiner et al. 2007). The results of this study suggested that atrazine as low as 25 ug/L may alter the ability of micro algae to incorporate carbon into macromolecular pools. Overall, the empirical data suggest a high likelihood of adverse effects to aquatic communities at exposure levels lower than and equivalent to CASM based LOCs.

2.2.6 NMFS' Habitat Effect Data Conclusions

A large number of studies were reviewed that evaluate aquatic ecosystem responses to atrazine. Atrazine is highly toxic to primary producers. Experimental ecosystems indicate statistically significant adverse impacts to primary producers can occur at concentrations as low as 1 ug/L. Adverse impacts to primary production were observed at concentrations below EPA aquatic community LOCs (12-38 ug/L) under a variety of exposure scenarios (single pulse to constant) and in a variety of experimental ecosystems including stagnant and running water, laboratory microcosms, and artificial streams, ponds and wetlands. The severity and frequency of adverse effects to aquatic organisms at aquatic community LOCs indicate that CASM derived LOCs are not an effective screen for making NLAA determinations. Atrazine impacts to primary producers are expected to result in a cascade of adverse ecological impacts including effects to abundance of aquatic animals. Adverse impacts to aquatic vertebrates were seen at concentrations as low as 20 ug/L. The degree to which sea turtles and shortnose sturgeon will be impacted is dependent on exposure, and site- and species-specific relationships which will be further discussed in the risk characterization section below.

2.3 Risk Characterization

2.3.1 Exposure and Direct Toxicity to Listed Species

The BE evaluated the potential direct toxicity of atrazine to listed species using a risk quotient analysis to support the effect determinations. Risk quotients were calculated by dividing estimates of exposure by toxicity values and comparing the result to EPA established Levels of Concern (LOC) (EPA 2004). The resulting risk quotients did not exceed EPA LOCs for direct effects, therefore EPA concluded that atrazine use in the Chesapeake Bay watershed would have "no effect" on shortnose sturgeon or listed sea turtles via direct toxicity (EPA 2006). However, NMFS does not agree with EPA's conclusions (see sections 2.1 and 2.2 above). A convincing case was not provided in the current BE as to why these data should not be incorporated into the risk quotient analysis. NMFS concludes that these data indicate that these effects are relevant to listed shortnose sturgeon and sea turtles and are not insignificant, discountable, or wholly beneficial.

2.3.1.1 Shortnose Sturgeon (A. brevirostrum)

NMFS expects that early life stage, juvenile, and adult shortnose sturgeon are likely exposed to atrazine throughout their geographic range within the Chesapeake Bay watershed. The frequency and severity of a shortnose sturgeon's response to atrazine will depend on exposure duration, concentration, and exposure as well as co-occurrence of chemicals that interact with atrazine (other triazines and certain insecticides). Available atrazine use and monitoring data document year round applications to multiple crops and surface water detections exceeding 30 ug/L (EPA 2006, Hall et al. 1999). Concentrations near 30 ug/L of atrazine would likely reduce a sturgeon's ability to migrate from freshwater to saltwater (e.g. impairment of gill physiology), impair olfactory mediated behaviors important to survival, growth, and reproduction, and in some sensitive individuals potentially lead to acute lethality. Sturgeon feed primarily by olfaction and taste given their use of poor visibility habitats (Atema 1977, Buddington et al. 1995). It is unlikely the maximum 30 ug/L detected in the Chesapeake Bay monitoring data is representative of peak atrazine concentrations in the watershed given the sampling design

(EPA 2002). Other researches have reported concentrations as high as 98 ug/L in streams of the Chesapeake Bay watershed (Hall et al. 1999). Recent monitoring data from other corn producing regions have detected concentrations as high as 230 ug/L in streams, with average concentrations exceeding 30 ug/L for several weeks at some sites (EPA 2007). Other studies have detected maximum concentrations of atrazine ranging from 20 ug/L to 2300 ug/L in surface waters (Selim 2003, Battaglin et al. 2001, Perry 1990, EPA 1989). Atrazine concentrations in the Chesapeake Bay watershed are expected to frequently affect sublethal endpoints, and at times are expected to result in acute lethality to sensitive individuals. The data do not support EPA's conclusion regarding the direct effects of atrazine on shortnose sturgeon because estimates of atrazine exposure and measured atrazine concentration in the Chesapeake Bay exceed concentrations that likely impair the survival, growth, reproduction, and distribution of individual shortnose sturgeon.

2.3.1.2 Sea Turtles

NMFS expects that individuals from each of the four species will likely be exposed to atrazine and its degradates while foraging in the Chesapeake Bay. NMFS does not expect that sea turtle eggs will be exposed to atrazine because nesting within the watershed is infrequent and atrazine is not likely to be applied to habitat utilized by nesting turtles. However, juvenile and adult sea turtles are likely to be exposed to atrazine through dermal, oral, and dietary routes. Direct effects remain uncertain. Direct exposure could potentially result in physiological and biochemical changes to an individual's endocrine system; however, there remains significant uncertainty on the frequency, magnitude, and probability of such a response. Atrazine induced aromatase in a green turtle cell line suggesting the potential to disrupt endocrine processes in sea turtles. However, it is difficult to translate the measured effects to environmentally relevant routes of exposure. Toxicity data indicated birds are relatively tolerant to dietary atrazine exposure but birds may not be representative of sea turtle sensitivity. Other vertebrates such as fish and amphibians appear more sensitive than birds, but exposure by routes that caused adverse effects in fish and amphibians may or may not be pertinent in turtles. For example, the presumed route of exposure in toxicity studies with fish was absorption of atrazine through the gills. Sea turtles do not have gills and presumably

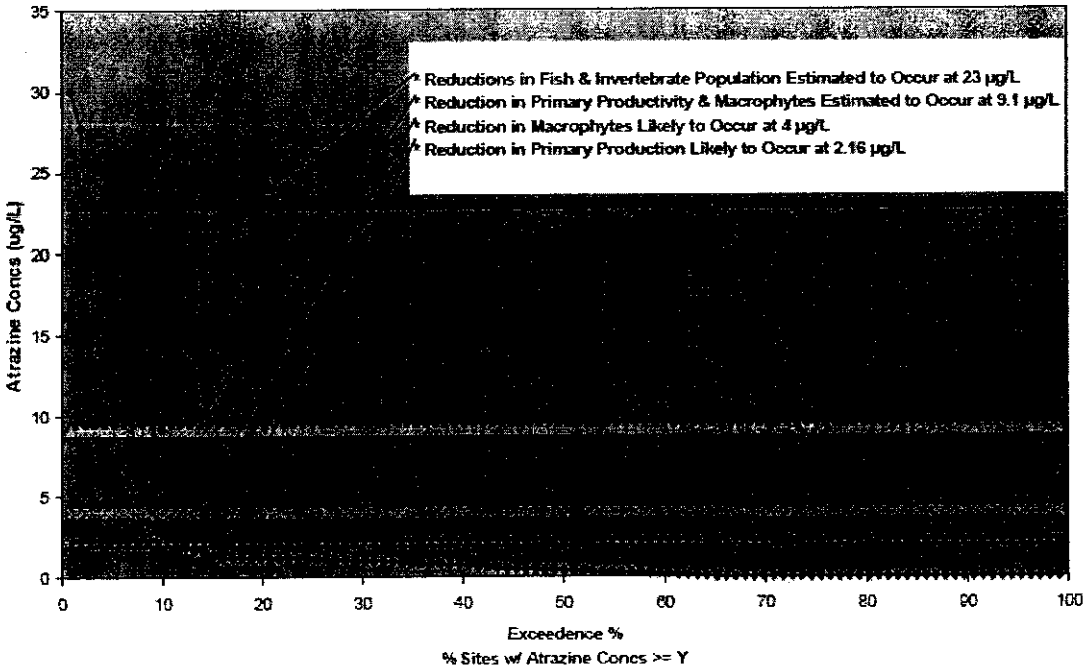
would absorb less atrazine through analogous dermal absorption pathways. However, water continually flows through the nasal passage and out the mouths of sea turtles as they swim. Olfactory senses are important for sea turtle location of food. The degree to which atrazine affects olfactory processes in turtles has not been evaluated. If comparable to fish, atrazine will impact sea turtle olfaction at 0.5-10 ug/L (Moore and Lower 2001, Tierney et al. 2007). Concentrations greater than 10 ug/L of atrazine have been detected in surface water habitats of the Chesapeake Bay watershed. Adverse endocrine mediated effects to amphibians have been observed at concentrations comparable to those commonly detected in the Chesapeake Bay watershed (i.e. ≥0.1 ug/L caused hermaphradism and ≥1 ug/L caused feminization, Hayes et al. 2002a/b). Exposure to sea turtles during organogenesis is not expected to be comparable given the habitat sea turtles occupy prior to hatching. However, sea turtles do not fully mature for many years (e.g. Loggerhead turtles take approximately 20 years to reach sexual maturity) and sea turtles will be repeatedly exposed to atrazine over multiple seasons as juveniles. Assessing the potential risk of atrazine to sea turtles is severely hindered by the paucity of data on atrazine effects in sea turtles or closely related species. However, the information does not support EPA's determination that atrazine use in the Chesapeake Bay watershed will cause "no effect" to the listed sea turtles from direct toxicity because data suggest a potential for adverse sublethal effects, including endocrine mediated effects and effects associated with disruption of olfaction that are not insignificant, nor discountable or wholly beneficial.

2.3.2 Exposure and Effects to Listed Species Habitat

Surface waters of the Chesapeake Bay watershed (streams, rivers, estuary, and seawater) are currently exposed to atrazine, its degradates, other triazines, and other pesticides that interact with atrazine in an additive and synergistic manner. Atrazine effects to aquatic primary producers including periphyton, algae, and macrophytes result in cascading ecological responses of exposed aquatic habitats. For example, consumer species feeding on fewer, smaller, primary producers may have reduced feeding efficiency and therefore reduced growth. Experimental ecosystems indicate statistically significant adverse impacts to primary producers can occur at concentrations as low as 1 ug/L. Atrazine use

in the Chesapeake Bay results in concentrations that impact primary producers. Atrazine has been monitored in Chesapeake Bay surface waters at concentrations EPA previously concluded would reduce primary production and fish and invertebrate populations (EPA 2002, Figure 1). NMFS concurs with EPA's previous conclusion that these data are "likely to underestimate the concentrations likely to be present in streams" because sampling was not designed correspond with atrazine treatment areas, timing of atrazine applications, or runoff events (EPA 2002). More rigorous monitoring data from other corn producing regions indicate atrazine can occur at much greater concentrations (maximum 238 ug/L) and may remain elevated > 30 ug/L for several weeks in streams (EPA 2007). These observations do not support EPA's effect determinations and suggest that atrazine is likely to adversely impact aquatic communities, thereby adversely affecting listed species that utilize those habitats. The degree to which each listed species is affected will be dependent on life stage and life history habitat requirements coupled with the frequency and magnitude of encounters with atrazine induced degraded habitat.

Figure 1. Surface Water Monitoring Results for Atrazine in the Chesapeake Bay's Tidal Rivers. Maximum Concentrations by Site and Year (1977-1993). Figure from EPA (2002, page 59).



2.3.2.1 Shortnose Sturgeon (A. brevirostrum)

Shortnose sturgeon utilize primarily riverine and estuarine habitats and are omnivorous consuming a variety of benthic and epibenthic invertebrates including mollusks, crustaceans, amphipods, insect larvae, and oligochaete worms. Juvenile prey availability may be affected by atrazine use in the Chesapeake Bay via direct impacts to primary production. Particularly, during vulnerable life-stage transitions such as when shortnose sturgeon transition from utilizing the yolk-sac to active feeding. This critical period is referred to as time to first feeding and is a foundation of early life stage fish ecology. Fry must begin active feeding during the first week of this critical period to avoid the onset of starvation (Ware et al. 2006). Hatching of shortnose sturgeon in the Chesapeake Bay occurs throughout spring corresponding with the periods of high atrazine use and peak runoff. Subsequent atrazine reductions of primary production are likely to occur throughout surface water in the Chesapeake Bay, particularly in freshwater habitats utilized by early life-stages during the critical time to first feeding and during the 1-2 year freshwater residence time. The information on the ecology of shortnose sturgeon and their use of aquatic habitats in the Chesapeake Bay watershed does not support EPA's effect determination that atrazine is not likely to adversely affect sturgeon via habitat effects. Atrazine use within the watershed likely degrades freshwater habitats utilized by sturgeon and likely results in cascading ecological effects that can extend to early life stages of limited mobility.

2.3.2.2 Green Turtle (C. mydas)

Juvenile and adult green turtles forage in Chesapeake Bay feeding on benthic macroalgae and sea grasses that grow in waters ≤ 10 meters in depth (Witherington et al. 2006a). These same habitats are expected to be exposed to atrazine at varying concentrations throughout the year. Adverse impacts to primary producers can occur at concentrations as low as 1 ug/L. It is likely that seagrassess and micro algae will be affected by atrazine throughout the Chesapeake Bay. Individual turtles reliant on affected sea grass habitats are likely to be adversely affected. NMFS does not agree with EPA's determination that atrazine use in the Chesapeake Bay watershed is not likely to adversely affect green turtle

through impacts to habitat because green turtles rely on vegetation in shallow water habitats that are likely directly impaired by atrazine.

2.3.2.3 Leatherback Turtle (D.coriacea), Kemp's Ridley Turtle (L. kempii) and Loggerhead Turtle (C. caretta)

These three species of sea turtles are primarily carnivorous with varying degrees of omnivory (Witherington et al. 2006b). They feed principally on slow moving or sessile macroinvertebrates (large crustaceans and hard-shelled mollusks). Atrazine degradation of primary producers will affect invertebrate prey to varying degrees depending on atrazine exposure and prey to primary producer relationships. Given the opportunistic feeding and utilization of different habitats by Leatherback, Kemp's Ridley, and Loggerhead sea turtles, NMFS expects it is unlikely that individuals of these three species will be adversely affected by atrazine-induced habitat degradation in the Chesapeake Bay.

2.3.3 Ecological Relevance of Mixture Toxicity

Risk to ESA-listed species' habitat from mixtures containing atrazine will likely be underestimated if only atrazine is evaluated. Exposure to atrazine and other pesticides present in the Chesapeake Bay watershed will likely result in greater toxicity than would be predicted by atrazine exposure alone. Atrazine-containing pesticide mixtures known to cause additive (triazines) and synergistic (acetyl-cholinesterase inhibiting insecticides) responses are present throughout the action area and exposure to these mixtures is expected to occur in listed sea turtles, shortnose sturgeon, and their habitats. Although EPA discussed mixture interactions, neither the likely exposure of listed species to multiple pesticides nor the potential additive or synergistic responses were factored into the effect determination contributing to the likelihood that risk to listed species was underestimated. EPA indicated that exposure to environmental mixtures could also reduce risk to listed species through antagonist interactions. However, data suggesting exposure to environmental mixtures may reduce risk through antagonist interactions is lacking. Exposure to real world environmental mixtures containing atrazine is likely to produce greater risk than exposure to atrazine alone. By not incorporating the risk posed

to listed species from atrazine containing mixtures, the atrazine effects determinations likely underestimated the risk posed to sea turtles and shortnose sturgeon.

3 Summary

NMFS reviewed EPA's effect determination using the substantive requirements of section 7 and cannot concur with EPA's determination that the registered uses of atrazine are not likely to adversely affect the threatened loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*), the endangered shortnose sturgeon (*Acipenser brevironstrum*), Kemp's ridley turtle (*Lepidochelys kempii*), and leatherback turtle (*Dermochelys coriacea*). NMFS expects atrazine may affect, and is likely to adversely affect the endangered shortnose sturgeon, and threatened and endangered sea turtles that utilize the Chesapeake Bay watershed. Measured and predicted environmental concentrations of atrazine in surface waters of the Chesapeake Bay watershed are likely to be directly toxic to shortnose sturgeon, listed sea turtles, and their habitats.

NMFS recommends that EPA initiate formal section 7 consultation on the effects of atrazine on threatened loggerhead turtle and green turtle, the endangered shortnose sturgeon, Kemp's ridley turtle, and leatherback turtle.

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