Risks of Atrazine Use to Eight Federally Listed Endangered Freshwater Mussels:

Pink Mucket Pearly (*Lampsilis abrupta*),
Rough Pigtoe (*Pleurobema plenum*),
Shiny Pigtoe Pearly (*Fusconaia edgariana*),
Fine-rayed Pigtoe (*Fusconaia cuneolus*),
Heavy Pigtoe (*Pleurobema taitianum*),
Ovate Clubshell (*Pleurobema perovatum*),
Southern Clubshell (*Pleurobema decisum*), and
Stirrup Shell (*Quadrula stapes*)

Pesticide Effects Determination

Environmental Fate and Effects Division
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1. Executive Summary

The purpose of this assessment is to make an "effects determination" by evaluating the potential direct and indirect effects of the herbicide atrazine on the survival, growth, and reproduction of the following eight Federally listed species of freshwater mussels: pink mucket pearly mussel (*Lampsilis abrupta*), rough pigtoe mussel (*Pleurobema plenum*), shiny pigtoe pearly mussel (*Fusconaia edgariana*), fine-rayed pigtoe mussel (*F. cuneolus*), heavy pigtoe mussel (*P. taitianum*), ovate clubshell mussel (*P. perovatum*), southern clubshell mussel (*P. decisum*), and stirrup shell mussel (*Quadrula stapes*). In addition, this assessment evaluates the potential for atrazine use to result in the destruction or adverse modification of designated critical habitat for the ovate clubshell and southern clubshell mussels (the only two of the eight listed species for which critical habitat has been designated by the U.S. Fish and Wildlife Service). This assessment was completed in accordance with the U.S. Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS) *Endangered Species Consultation Handbook* (USFWS/NMFS, 1998) and procedures outlined in the Agency's Overview Document (U.S. EPA, 2004).

Atrazine is used throughout the United States on a number of agricultural commodities (primarily corn and sorghum) and on non-agricultural sites (including residential uses, forestry, and turf). Although the action area is likely to encompass a large area of the United States, given its use, the scope of this assessment limits consideration of the overall action area to those portions that are applicable to the protection of the eight listed mussels and their designated critical habitat. As such, the action area includes the current range of the species and designated critical habitat, which occur in streams and rivers from a point near the mouth of the Alabama and Tombigbee Rivers (near Mobile, Alabama) up into, and including, the Ohio River watershed. The action area also includes an isolated section in Arkansas, Missouri, and Nebraska.

Acute and chronic risk quotients (RQs) are compared to the Agency's Levels of Concern (LOCs) to identify instances where atrazine use within the action area has the potential to adversely affect the listed mussels or adversely modify designated critical habitat. When RQs for a particular type of effect are below LOCs, there is considered to be "no effect" to the listed species and their designated critical habitat. Where RQs exceed LOCs, a potential to cause adverse effects or habitat modification is identified, leading to a conclusion of "may affect". If atrazine use "may affect" the listed mussels, and/or cause adverse modification to designated critical habitat, the best available additional information is considered to refine the potential for exposure and effects, and distinguish actions that are NLAA from those that are LAA.

In accordance with the methodology specified in the Agency's Overview Document (U.S. EPA, 2004), screening-level EECs, based on the PRZM/EXAMS static water body scenario, were used to derive RQs for all relevant agricultural and non-agricultural atrazine uses within the action area. RQs based on screening-level EECs were used to distinguish "no effect" from "may effect" determinations for direct/indirect effects to the listed mussels and the critical habitat impact analysis. However, screening-level EECs based on the static water body are not considered to be representative of flowing waters where the assessed mussels and designated critical habitat occur. For "may affect" determinations, screening-level EECs were further refined and characterized, as follows, based on the location of the assessed mussels and designated critical habitat within or outside the boundary of vulnerable watersheds (defined as watersheds most vulnerable to atrazine runoff because they are located in high atrazine use areas):

- Flow-adjusted EECs and available non-targeted monitoring data (i.e., the study design is not specifically targeted to detect atrazine in high use areas) were used to refine the screening-level EECs for the shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussels, and designated critical habitat, that occur in watersheds outside of vulnerable areas. These refined EECs were used to distinguish "May Affect, But Not Likely to Adversely Affect (NLAA)" vs. "May Affect and Likely to Adversely Affect (LAA)" determinations for listed mussel species and designated critical habitat that occurs in watersheds not identified as highly vulnerable.

- Targeted monitoring data from the Ecological Monitoring Program was used to refine the screening-level EECs for the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels that occur in watersheds identified as highly vulnerable (based on factors such as atrazine use, runoff potential, and rainfall). Refined EECs based on the available targeted monitoring data were used to distinguish "NLAA" vs. "LAA" determinations for listed mussels that occur in watersheds identified as highly vulnerable.

Therefore, separate effects determinations were derived for direct/indirect endpoints based on the location of the assessed species within highly vulnerable and less vulnerable watersheds of the action area. Because all designated critical habitat for the ovate and southern clubshell mussels is located in watersheds outside of the vulnerable boundary, effects determinations for the critical habitat impact analysis are based on flow-adjusted EECs and available non-targeted monitoring data, as described above.

The assessment endpoints for the listed mussels include direct toxic effects on survival, reproduction, and growth of individual mussels, as well as indirect effects, such as reduction of the food source and/or modification of habitat. Acute toxicity data on freshwater mussels are available and were utilized for RQ calculations. However, chronic RQs were derived using data on the closest taxonomic group with available toxicity data (mollusks).

Given that the mussel's food source and habitat requirements are dependant on the availability of freshwater fish, aquatic plants, freshwater invertebrates, and terrestrial plants (i.e., riparian habitat), toxicity information for these taxonomic groups is also discussed. In addition to the registrant-submitted and open literature toxicity information, indirect effects, via impacts to aquatic plant community structure and function, are also evaluated based on time-weighted threshold concentrations that correspond to potential aquatic plant community-level effects.

Federally designated critical habitat has been established for the ovate and southern clubshell mussels. Adverse modifications to the primary constituent elements (PCEs) of designated critical habitat, as defined in 50 CFR 414.12(b), were also evaluated. PCEs evaluated as part of this assessment include the following:

- geomorphically stable stream and river channels and banks;
- water quality, including temperature, turbidity, oxygen content;
- sand, gravel, and/or cobble substrates with low to moderate amounts of attached filamentous algae and sedimentation;
- fish hosts with adequate living, foraging, and spawning areas; and
- chemical characteristics necessary for normal behavior, growth, and viability of all mussel life stages;

Effects determinations for direct/indirect effects to the eight assessed musels and the critical habitat impact analysis, by assessment endpoint, are presented in Tables 1.1 and 1.2. In addition, Table 1.3 provides a summary of the direct and indirect effects determinations for each of the eight assessed listed mussels. Effects determinations for this assessment are summarized below.

- The direct and indirect effects determination for the stirrupshell mussel is "no effect" because this species is presumed to be extinct (Hartfield, 2006).

- With the exception of "LAA" determinations for indirect effects to listed mussels via community level effects to aquatic plants in vulnerable watersheds and habitat impacts via atrazine-related alteration of grassy/herbaceous vegetation in all portions of the action area, all other effects determinations for direct/indirect assessment endpoints are "no effect" or "NLAA".

- An "LAA" determination was concluded for indirect prey and habitat effects to the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels that occur in highly vulnerable watersheds of the action area, based on potential direct aquatic plant community-level effects.

    - The "LAA" determination is based on the results of recently submitted atrazine monitoring data from vulnerable watersheds; however, the degree to which this targeted monitoring data represents exposures in occupied streams (for the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe) that co-occur with vulnerable watersheds is not available. For the purposes of this assessment, it is conservatively assumed that detected concentrations of atrazine from the monitoring data may be representative of exposures in vulnerable watersheds of the action area.
    - If further analysis reveals that the monitoring data are not representative of atrazine concentrations in vulnerable watersheds where the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels occur, the "LAA"

effects determination will be revisited and could be changed to “NLAA” for these species.

- An “LAA” determination was concluded for the seven listed mussels based on indirect effects to habitat and water quality via direct effects to herbaceous/grassy riparian vegetation. However, atrazine is not likely to adversely affect listed mussels in watersheds with predominantly forested riparian areas because woody shrubs and trees are generally not sensitive to environmentally-relevant concentrations of atrazine.

- In the critical habitat impact analysis, “LAA” effects determinations were concluded for the following PCEs associated with potential adverse modification to critical habitat via atrazine-related impacts to grassy/herbaceous riparian vegetation: alteration of host fish spawning habitat, increase in sedimentation and resulting impact on silt-free substrates and turbidity-related water quality parameters, and alteration of streambank stability. All other PCEs evaluated as part of the critical habitat impact analysis were determined to be either “no effect” or “NLAA”.

Table 1.1 Effects Determination Summary for the Assessed Listed Mussels (by Assessment Endpoint)				
Direct and Indirect Effects to Listed Mussels[a]				
Assessment Endpoint	Effects Determination and Basis for Less Vulnerable Watersheds (applicable to shiny pigtoe [SP], heavy pigtoe [HP], ovate clubshell [OC], and southern clubshell [SC] listed mussels)		Effects Determination and Basis for Highly Vulnerable Watersheds (applicable to pink pearly mucket [PPM], rough pigtoe [RP], and fine-rayed pigtoe [FRP] listed mussels)	
	Effects Determination[b]	Basis	Effects Determination[b]	Basis
1. Survival, growth, and reproduction of assessed mussel individuals via direct acute or chronic effects	Acute direct effects: NE	No acute LOCs are exceeded.	Acute direct effects: NE	No acute LOCs are exceeded.
	Chronic direct effects: NLAA	Chronic LOCs are exceeded based on screening-level EECs; however, RQs based on flow-adjusted EECs and non-targeted monitoring data are less than concentrations shown to cause adverse effects in freshwater mollusks. This finding is based on insignificance of effects (i.e., chronic exposure to atrazine is not likely to result in "take" of a single SP, HP, OC, and SC mussel in less vulnerable watersheds).	Chronic direct effects: NLAA	Chronic LOCs are exceeded based on screening-level EECs; however detected concentrations of atrazine in monitoring data from vulnerable watersheds are less than those shown to cause adverse effects in freshwater mollusks. This finding is based on insignificance of effects (i.e., chronic exposure to atrazine is not likely to result in "take" of a single PPM, RP, and FRP mussel in vulnerable watersheds).
2. Indirect effects to assessed mussel individuals via reduction in food items (i.e., freshwater phytoplankton and zooplankton)	Phytoplankton: NLAA	Individual aquatic plant species within less vulnerable watersheds of the action area may be affected. However, refined 14-, 30-, 60- and 90-day EECs, which consider the impact of flow and non-targeted monitoring data, are less than the threshold concentrations representing community-level effects. This finding is based on insignificance of effects (i.e., community-level effects to aquatic plants are not likely to result in "take" of a single SP, HP, OC, and SC mussel in less vulnerable watersheds via a reduction in food items).	Phytoplankton: LAA[c]	Individual aquatic plant species within vulnerable watersheds of the action area may be affected. 14-, 30-, 60-, and 90- day rolling averages based on the ecological monitoring data exceed their respective threshold concentrations for 5 to 12.5% of the sampled vulnerable watersheds. Therefore, community-level effects are possible for phytoplankton, resulting in indirect effects to the food supply of the PPM, RP, and FRP mussels, within vulnerable watersheds of the action area.

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Assessment Endpoint	**Effects Determination and Basis for Less Vulnerable Watersheds (applicable to shiny pigtoe [SP], heavy pigtoe [HP], ovate clubshell [OC], and southern clubshell [SC] listed mussels)**		**Effects Determination and Basis for Highly Vulnerable Watersheds (applicable to pink pearly mucket [PPM], rough pigtoe [RP], and fine-rayed pigtoe [FRP] listed mussels)**	
	Effects Determination[b]	**Basis**	**Effects Determination[b]**	**Basis**
	Acute direct effects to zooplankton: NLAA	Acute LOCs are exceeded based on screening-level EECs and the most sensitive freshwater invertebrate toxicity data. Based on the refined analysis, which considered flow-adjusted EECs, non-targeted monitoring data, and effects data specific to zooplankton, acute effects to zooplankton are not likely to result in indirect effects to the SP, HP, OC, and SC mussels via reduction in food items because zooplankton are not the primary food source for these listed mussels, the probability of an individual effect to zooplankton is low (i.e., 0.2%), and the refined RQ based on peak NAWQA 2000-2004 monitoring data specific for a watershed within the action area is well below the acute LOC. This finding is based on insignificance of effects (i.e., effects to zooplankton in less vulnerable watersheds are not likely to be extensive over the suite of possible food items to result in "take" of a single listed SP, HP, OC, and SC mussel).	Acute direct effects to zooplankton: NLAA	Acute LOCs are exceeded based on the maximum peak atrazine concentration from the monitoring data. However, zooplankton are not the primary food source for listed mussels and there is a low probability of an individual effect to zooplankton. Therefore, direct acute effects to zooplankton are not likely to result in indirect effects to the listed mussels via a reduction in food items. This finding is based on insignificance of effects (i.e., effects to zooplankton in vulnerable watersheds are not likely to be extensive over the suite of possible food items to result in "take" of a single PPM, RP, and FRP mussel).
	Chronic direct effects to zooplankton: NLAA	Chronic LOCs are exceeded based on screening-level EECs and the most sensitive freshwater invertebrate toxicity data. However, all refined measures of	Chronic direct effects to zooplankton: NLAA	Chronic LOCs are exceeded based on screening-level EECs and the most sensitive freshwater invertebrate toxicity data. However, 21-day rolling averages based on

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	Effects Determination[b]	**Basis**	**Effects Determination[b]**	**Basis**
		exposure (21-day flow-adjusted EECs and non-targeted monitoring data) are well below levels of chronic effects in cladocerons. This finding is based on insignificance of effects (i.e., chronic exposure to atrazine in less vulnerable watersheds is not likely to result in a "take" of a single SP, HP, OC, and SC mussel via a reduction in zooplankton as food items).		the ecological monitoring data are well below levels of chronic effects in cladocerons. This finding is based on insignificance of effects (i.e., chronic exposure to atrazine in highly vulnerable watersheds is not likely to result in a "take" of a single PPM, RP, and RFP mussel via a reduction in zooplankton as food items).
3. Indirect effects to assessed mussel individuals via reduction in host fish for mussel glochidia	Acute direct effects to host fish: NE	No acute LOCs are exceeded.	Acute direct effects to host fish: NE	No acute LOCs are exceeded.
	Chronic direct effects to host fish: NLAA	Chronic LOCs are exceeded based on screening-level EECs; however RQs based on flow-adjusted EECs and non-targeted monitoring data are less than chronic LOCs. This finding is based on insignificance of effects (i.e., chronic exposure to atrazine is not likely to result in "take" of a single SP, HP, OC, and SC mussel via direct effects to host fish in less vulnerable watersheds).	Chronic direct effects to host fish: NLAA	Chronic LOCs are exceeded based on screening-level EECs; however, detected concentrations of atrazine in monitoring data from vulnerable watersheds are less than those that would result in LOC exceedances for freshwater fish. This finding is based on insignificance of effects (i.e., chronic exposure to atrazine is not likely to result in "take" of a single PPM, RP, and FRP mussel via direct effects to host fish in vulnerable watersheds).
4. Indirect effects to assessed mussel individuals via direct effects to aquatic plants (i.e.,	Direct effects to aquatic plants: NLAA	Individual aquatic plant species within less vulnerable watersheds may be affected. However, flow-adjusted 14-, 30-, 60-, and 90-day EECs and similar durations of exposure based on non-	Direct effects to aquatic plants: LAA[c]	Individual aquatic plant species within vulnerable watersheds of the action area may be affected. 14-, 30-, 60-, and 90- day rolling averages based on the ecological monitoring data from vulnerable watersheds exceed their

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Direct and Indirect Effects to Listed Mussels[a]

Assessment Endpoint	**Effects Determination and Basis for Less Vulnerable Watersheds (applicable to shiny pigtoe [SP], heavy pigtoe [HP], ovate clubshell [OC], and southern clubshell [SC] listed mussels)**		**Effects Determination and Basis for Highly Vulnerable Watersheds (applicable to pink pearly mucket [PPM], rough pigtoe [RP], and fine-rayed pigtoe [FRP] listed mussels)**	
	Effects Determination[b]	**Basis**	**Effects Determination[b]**	**Basis**
reduction of habitat and/or primary productivity)		targeted monitoring data, are less than the threshold concentrations representing community-level effects. This finding is based on insignificance of effects (i.e., community-level effects to aquatic plants are not likely to result in “take” of a single SP, HP, OC, and SC mussel via direct effects on habitat and primary productivity in less vulnerable watersheds).		respective threshold concentrations for a small percentage of the data set. Therefore, community-level effects are possible for phytoplankton, resulting in indirect effects to the PPM, RP, and FRP mussels, via direct effects on habitat and primary productivity, within vulnerable watersheds of the action area.
5. Indirect effects to assessed mussel individuals via reduction of terrestrial vegetation (i.e., riparian habitat) required to maintain acceptable water quality and habitat[d]	Direct effects to forested riparian vegetation: NLAA	Riparian vegetation may be affected because terrestrial plant RQs are above LOCs. However, woody shrubs and trees are generally not sensitive to atrazine; therefore, listed mussels in watersheds with predominantly forested riparian vegetation (i.e., woody shrubs and trees) are not likely to adversely affected. This finding is based on insignificance of effects (i.e., effects to forested riparian vegetation in the action area are not likely to result in “take” of a single listed mussel).	Direct effects grassy/herbaceous riparian vegetation: LAA	Riparian vegetation may be affected because terrestrial plant RQs are above LOCs. The LAA effects determination for listed mussels that are in close proximity to grassy/herbaceous riparian areas is based on the sensitivity of herbaceous vegetation to atrazine.

[a] The direct and indirect effects determination for the stirrupshell mussel is “no effect” because this species is presumed to be extinct (Hartfield, 2006). The following direct/indirect effects determinations apply to the other seven listed mussels included in this assessment.
[b] NE = “no effect”; NLAA ‘ “may affect, but not likely to adversely affect”; and LAA = “may affect and likely to adversely affect”.
[c] Further analysis of the ecological monitoring data is required to determine the representativeness of the data to other watersheds within vulnerable areas where the listed mussel species occur. If the analysis suggests that the monitoring data are representative of atrazine concentrations in vulnerable watersheds where the listed mussels occur, the effects determination will remain as “LAA.” However, if further analysis reveals that the monitoring data are not representative of atrazine concentrations in vulnerable watersheds where

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Direct and Indirect Effects to Listed Mussels[a]				
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	Effects Determination[b]	Basis	Effects Determination[b]	Basis
the listed mussels occur, the effects determination will be revised to "NLAA". [d] The effects determinations for indirect effects to the listed mussels based on direct impacts to riparian habitat is applicable to the entire action area including riparian areas adjacent to both vulnerable and less vulnerable watersheds. Separate effects determinations are based on the presence of forested or herbaceous/grassy riparian vegetation adjacent to the streams and rivers within the listed mussel's action area.				

Table 1.2 Effects Determination Summary for the Critical Habitat Impact Analysis[a]		
Assessment Endpoint	Effects Determination[b]	Basis
1. Fish hosts with adequate living, foraging, and spawning areas	Acute direct effects to host fish: NE	No acute LOCs are exceeded.
	Chronic direct effects to host fish: NLAA	Chronic LOCs are exceeded based on screening-level EECs; however, RQs based on flow-adjusted EECs and non-targeted monitoring data are less than chronic LOCs. This finding is based on insignificance of effects (i.e., chronic effects to the living areas for host fish are not likely to adversely modify designated critical habitat for the ovate and southern clubshell mussels).
	Acute direct effects to host fish food items: NLAA	Acute LOCs for freshwater invertebrates are exceeded based on screening-level EECs and the most sensitive ecotoxicity value for the midge. However, refined RQs based on flow-adjusted EECs, recent non-targeted monitoring data, and toxicity data for other freshwater invertebrate food items of host fish are less than LOCs. Based on the non-selective feeding nature of host fish and the low magnitude of anticipated individual effects to prey items, atrazine is not likely to affect host fish of the ovate and southern clubshell mussels via an acute reduction in freshwater invertebrate food items. This finding is based on an insignificance of effects (i.e., acute effects to freshwater invertebrates are not likely to adversely modify critical habitat foraging areas for host fish of the ovate and southern clubshell mussels).
	Chronic direct effects to host fish food items: NLAA	Chronic LOCs for freshwater invertebrates are exceeded based on screening-level EECs. However, chronic RQs based on flow-adjusted EECs and non-targeted monitoring data are less their respective chronic LOCs. This finding is based on an insignificance of effects (i.e., chronic effects to freshwater invertebrates are not likely to adversely modify critical habitat foraging areas for host fish of the ovate and southern clubshell mussels).

Table 1.2 Effects Determination Summary for the Critical Habitat Impact Analysis[a]		
Assessment Endpoint	**Effects Determination[b]**	**Basis**
	Direct effects to host fish spawning areas: LAA for herbaceous/grassy riparian areas and NLAA for forested riparian areas	LOCs are exceeded for aquatic and terrestrial plants based on screening-level EECs. Further analysis of potential impacts to host fish spawning habitat via community-level effects to aquatic plants was completed by comparing flow-adjusted 14-, 30-, 60-, and 90-day EECs and similar durations of exposure from non-targeted monitoring data to their respective threshold concentrations. All flow-adjusted EECs and non-targeted monitoring data are well below threshold concentrations; therefore, host fish spawning habitat is not likely to be adversely affected via community-level effects to aquatic plants. In addition, critical habitat spawning areas for host fish that are in close proximity to forested riparian vegetation are not expected to adversely modified because woody shrubs and treess are generally not sensitive to atrazine. However, critical habitat spawning areas for host fish that are in close proximity to grassy/herbaceous riparian areas may be adversely modified based on the sensitivity of herbaceous vegetation to atrazine.
2. Water quality necessary for normal behavior, growth and viability of all mussel life stages	Temperature: NLAA	Riparian vegetation may be affected because terrestrial plant RQs are above LOCs. However, water quality related to temperature within forested riparian areas is not likely to be impacted because mature woody shrubs and trees, which provide stream shading and thermal stability, are generally not sensitive to atrazine. This finding is based on insignificance of effects (i.e., atrazine is not likely to adversely modify temperature-related water quality within designated critical habitat for the ovate and southern clubshell mussels).
	Turbidity: LAA for herbaceous/grassy riparian areas and NLAA for forested riparian areas	Riparian vegetation may be affected because terrestrial plant RQs are above LOCs. Water quality related to turbidity via increased sedimentation may be impacted within designated critical habitats that are adjacent to grassy/herbaceous riparian vegetation. Therefore, atrazine may adversely modify critical habitat for the ovate and southern clubshell mussels in areas where grassy/herbaceous riparian vegetation is present. Adverse modification to designated critical habitat via turbidity-related water quality impact is not expected in areas where forested riparian vegetation is present.
	Oxygen content: NLAA	Individual aquatic plant species may be affected based on LOC exceedances. Oxygen levels may also be impacted if the atrazine negatively affects the aquatic plant community and primary productivity. However, flow-adjusted 14-, 30-, 60-, and 90-day EECs and similar durations of exposure from non-targeted monitoring data are less than their respective threshold concentrations representative of aquatic plant community-level effects. Therefore, atrazine may affect, but is not likely to adversely modify the oxygen content of the designated critical habitat for the ovate and southern clubshell mussels. This finding is based on insignificance of effects (i.e., atrazine is not likely to adversely modify oxygen content-related water quality via aquatic plant community-level impacts within designated critical habitat for the ovate and southern clubshell mussels).
3. Substrates with low to	Filamentous algae:	Atrazine is expected to reduce algal mass and the presence of filamentous algae on substrate necessary

Table 1.2 Effects Determination Summary for the Critical Habitat Impact Analysis[a]		
Assessment Endpoint	**Effects Determination[b]**	**Basis**
moderate amounts of filamentous algae and low sedimentation	NLAA	for normal growth and viability of listed mussels. Therefore, atrazine is not expected to adversely modify critical habitat of the ovate and southern clubshell mussels by increasing the amount of filamentous algae on substrate. This determination is based on insignificance of effects (i.e., adverse modification to critical habitat is not expected because atrazine use is likely to reduce the amount of filamentous algae).
	Sedimentation: LAA for herbaceous/grassy riparian areas and NLAA for forested riparian areas	Riparian vegetation may be affected because terrestrial plant RQs are above LOCs. Sedimentation may be impact silt-free substrates necessary for normal growth and viability of listed mussels within designated critical habitats that are adjacent to grassy/herbaceous riparian vegetation. Therefore, atrazine may adversely modify critical habitat for the ovate and southern clubshell mussels via sedimentation in areas where grassy/herbaceous riparian vegetation is present. Adverse modification to designated critical habitat via sedimentation is not expected in areas where forested riparian vegetation is present.
4. Stream and river bank stability	LAA for herbaceous/grassy riparian areas and NLAA for forested riparian areas	Riparian vegetation may be affected because terrestrial plant RQs are above LOCs. Streambank stability may be impacted within designated critical habitats that are adjacent to grassy/herbaceous riparian vegetation. Therefore, atrazine may adversely modify critical habitat for the ovate and southern clubshell mussels via reduction in streambank stability in areas where grassy/herbaceous riparian vegetation is present. Adverse modification to designated critical habitat via streambank stability is not expected in areas where forested riparian vegetation is present.
5. Chemical characteristics necessary for normal behavior, growth, and viability of all life stages of mussels	Acute direct effects: NE	No acute LOCs are exceeded.
	Chronic direct effects: NLAA	Chronic LOCs are exceeded based on screening-level EECs; however RQs based on flow-adjusted EECs and non-targeted monitoring data are less than concentrations shown cause adverse effects in freshwater mollusks. This finding is based on insignificant of effects (i.e., chronic exposure to atrazine is not likely to result in adverse modification of critical habitat for the ovate and southern clubshell mussels).
	Indirect food source of phytoplankton: NLAA	Individual aquatic plant species of the action area may be affected. However, refined 14-, 30-, 60- and 90-day EECs, which consider the impact of flow and the non-targeted monitoring data, are well below the threshold concentrations representing community-level effects. This finding is based on insignificance of effects (i.e., community-level effects to phytoplankton as a food source are not likely to adversely modify designated critical habitat for the ovate and southern clubshell mussels).
	Acute and chronic indirect food source of zooplankton: NLAA	Acute and chronic LOCs are exceeded based on screening-level EECs and the most sensitive freshwater invertebrate toxicity data. Based on the refined acute analysis for zooplankton (which consider flow-adjusted EECs, non-targeted monitoring data, and effects data specific for zooplankton).adverse modification to critical habitat is not likely because zooplankton are not the

Table 1.2 Effects Determination Summary for the Critical Habitat Impact Analysis[a]		
Assessment Endpoint	**Effects Determination[b]**	**Basis**
		primary food source, the probability of an individual effect to zooplankton is low (i.e., 0.2%), and the refined RQ based on peak NAWQA 2000-2004 monitoring data specific for a designated critical habitat watershed (i.e., Bogue Chitto Creek) is well below the acute LOC. Chronic effects to zooplankton and resulting adverse modification to critical habitat via reduction in food items is also not expected to occur because all refined measures of chronic exposure (i.e., 21-day flow-adjusted EECs and similar durations of exposure from non-targeted monitoring data) are well below chronic effect levels in zooplankton. Both NLAA effects determinations for adverse modification to critical habitat via reduction in zooplankton as a food source to the ovate and southern clubshell mussels are based on insignificance of effects (i.e., acute and chronic effects to zooplankton as food items are not likely to result in adverse modification of critical habitat for the ovate and southern clubshell mussels).

[a] All designated critical habitat for the ovate and southern clubshell mussel occurs in watersheds that are outside of the vulnerable watershed boundary; therefore, the effects determination for the critical habitat impact analysis is conducted for less vulnerable watersheds only.
[b] NE = "no effect"; NLAA ' "may affect, but not likely to adversely affect"; and LAA = "may affect and likely to adversely affect".

Table 1.3 Effects Determination Summary for Each of the Eight Assessed Listed Mussels[a]									
Assessed Mussel Species	Direct Effects		Indirect Effects						
	Acute	Chronic	Food Items		Host Fish		Aquatic Habitat: community-level effects	Riparian Vegetation	
			Phytoplankton	Zooplankton	Acute	Chronic		Herbaceous/Grassy Vegetation	Forested Vegetation
Pink pearly mucket	NE	NLAA	**LAA**[b]	NLAA	NE	NLAA	**LAA**[b]	**LAA**	NLAA
Rough pigtoe	NE	NLAA	**LAA**[b]	NLAA	NE	NLAA	**LAA**[b]	**LAA**	NLAA
Shiny pigtoe	NE	NLAA	NLAA	NLAA	NE	NLAA	NLAA	**LAA**	NLAA
Fine-rayed pigtoe	NE	NLAA	**LAA**[b]	NLAA	NE	NLAA	**LAA**[b]	**LAA**	NLAA
Heavy pigtoe	NE	NLAA	NLAA	NLAA	NE	NLAA	NLAA	**LAA**	NLAA
Ovate clubshell	NE	NLAA	NLAA	NLAA	NE	NLAA	NLAA	**LAA**	NLAA
Southern clubshell	NE	NLAA	NLAA	NLAA	NE	NLAA	NLAA	**LAA**	NLAA
Stirrup shell	NE	NE	NE	NE	NE	NE	NE	NE	NE

[a] NE = "no effect"; NLAA ' "may affect, but not likely to adversely affect"; and LAA = "may affect and likely to adversely affect". See Table 1.1 for the basis of the effects determinations for each of the assessed mussel species.

[b] Further analysis of the ecological monitoring data is required to determine the representativeness of the data to other watersheds within vulnerable areas where the listed mussel species occur. If the analysis suggests that the monitoring data are representative of atrazine concentrations in vulnerable watersheds where the listed mussels occur, the effects determination will remain as "LAA." However, if further analysis reveals that the monitoring data are not representative of atrazine concentrations in vulnerable watersheds where the listed mussels occur, the effects determination will be revised to "NLAA".

2. Problem Formulation

Problem formulation provides a strategic framework for the risk assessment. By identifying the important components of the problem, it focuses the assessment on the most relevant life history stages, habitat components, chemical properties, exposure routes, and endpoints. The structure of this risk assessment is based on guidance contained in U.S. EPA's *Guidance for Ecological Risk Assessment* (U.S. EPA, 1998), the Services' *Endangered Species Consultation Handbook* (USFWS/NMFS, 1998) and procedures outlined in the Overview Document (U.S. EPA, 2004).

2.1 Purpose

The purpose of this endangered species risk assessment is to evaluate the potential direct and indirect effects resulting from the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) registered uses of the herbicide atrazine (6-chloro-N-ethyl-N-isopropyl-1, 3, 5-triazine-2, 4-diamine) on the survival, growth, and/or reproduction of individuals of the following eight federally listed species of freshwater mussels: (1) pink mucket pearly mussel (*Lampsilis orbiculata*); (2) rough pigtoe mussel (*Pleurobema plenum)*; (3) shiny pigtoe pearly mussel (*Fusconaia edgariana*); (4) fine-rayed pigtoe mussel (*Fusconaia cuneolus)*; (5) heavy pigtoe mussel (*Pleurobema taitianum)*; (6) ovate clubshell mussel (*Pleurobema perovatum*); (7) southern clubshell mussel (*Pleurobema decisum*); and (8) stirrup shell mussel (*Quadrula stapes*). In addition, this assessment evaluates whether FIFRA regulatory actions regarding atrazine use can be expected to result in the destruction or adverse modification of the species' critical habitat. A summary of the listing status for these species is provided in Table 2.1, and a brief summary of key biological and ecological components related to the assessment of these species is provided in Section 2.5. Critical habitat has been designated by the USFWS for two of the eight listed species including the ovate clubshell and southern clubshell mussels (USFWS, 2004: 69 FR 40084-40171) and is further described in Section 2.6. This ecological risk assessment is a component of the settlement for the *Natural Resources Defense Council, Civ. No: 03-CV-02444 RDB (filed March 28, 2006)*.

Table 2.1 Identification and Listing Status of Eight Listed Freshwater Mussel Species Included in This Assessment		
Species	**Status[1]**	**Date Listed**
Pink Mucket Pearly Mussel (*Lampsilis abrupta*)	Endangered 41 FR 24062-24067	June 14, 1976
Rough Pigtoe Mussel (*Pleurobema plenum*)	Endangered 41 FR 24062-24067	June 14, 1976
Shiny Pigtoe Pearly Mussel (*Fusconaia edgariana*)	Endangered 41 FR 24062-24067	June 14, 1976
Fine-rayed Pigtoe Mussel (*F. cuneolus*)	Endangered 41 FR 24062-24067	June 14, 1976
Heavy Pigtoe Mussel (*P. taitianum*)	Endangered 52 FR 11162-11168	April 7, 1987
Ovate Clubshell Mussel[2] (*P. perovatum*)	Endangered 58 FR 14339	March 17, 1993
Southern Clubshell Mussel[2] (*P. decisum*)	Endangered 58 FR 14339	March 17, 1993
Stirrup Shell Mussel (*Quadrula stapes*)	Endangered 52 FR 11162-11168	April 7, 1987
[1] All assessed species were listed by the U.S. Fish and Wildlife Service (USFWS). [2] Critical habitat was designated for these species on July 1, 2004 (USFWS, 2004).		

In this endangered species risk assessment, direct and indirect effects to the eight assessed mussels and potential adverse modification to critical habitat for the ovate and southern clubshell mussels are evaluated in accordance with the methods (both screening and species-specific refinements) described in the Agency's Overview Document (U.S. EPA, 2004). The indirect effects analysis in this assessment utilizes more refined data than is generally available for ecological risk assessment. Specifically, a robust set of microcosm and mesocosm data and aquatic ecosystem models are available for atrazine that allowed for a refinement of the indirect effects associated with potential aquatic community-level effects (via aquatic plant community structural change and subsequent habitat modification). Use of such information is consistent with the guidance provided in the Overview Document (U.S. EPA, 2004), which specifies that "the assessment process may, on a case-by-case basis, incorporate additional methods, models, and lines of evidence that EPA finds technically appropriate for risk management objectives" (Section V, page 31 of U.S. EPA, 2004).

In accordance with the Overview Document, provisions of the Endangered Species Act (ESA), and the Services' *Endangered Species Consultation Handbook*, the assessment of effects of the FIFRA regulatory action is based on a defined action area and the extent of association of this action area with locations of the assessed listed mussels and their designated critical habitat. It is acknowledged that the action area for a national-level FIFRA regulatory decision involving a potentially widely used pesticide may potentially involve numerous areas throughout the United States and its Territories. However, for the purposes of his assessment, attention will be focused on those parts of the action area with the potential to be associated with locations of the assessed listed mussels and their designated critical habitat.

As part of the "effects determination", the Agency will reach one of the following three conclusions regarding the potential for FIFRA regulatory actions regarding atrazine to directly or indirectly affect individuals of the eight listed freshwater mussels and/or result in the destruction or adverse modification of designated critical habitat for the ovate and southern clubshell mussels:

- "No effect";
- "May affect, but not likely to adversely affect" ("NLAA"); or
- "May affect and likely to adversely affect" ("LAA").

If the results of the initial screening-level assessment methods show no direct or indirect effects upon individual listed mussels or upon the PCEs of the ovate and southern clubshell mussel's designated critical habitat, a "no effect" determination is made for the FIFRA regulatory action regarding atrazine as it relates to these listed species and designated critical habitat. If, however, direct or indirect effects to individual listed mussels are anticipated and/or effects may impact the PCEs of the ovate and southern clubshell mussel's designated critical habitat, the Agency concludes a preliminary "may affect" determination for the FIFRA regulatory action regarding atrazine.

If a determination is made that use of atrazine within the action area(s) "may affect" the listed mussels and/or designated critical habitat, additional information is considered to refine the potential for exposure at the predicted levels and for effects to the listed mussels and other taxonomic groups upon which these species depend (i.e., freshwater fish and invertebrates, aquatic plants, riparian vegetation). Additional information including further evaluation of the potential impact of atrazine on the PCEs is also used to determine whether destruction or adverse modification to designated critical habitat may occur. Based on the refined information, the Agency uses the best available information to distinguish those actions that "may affect, but are not likely to adversely affect" ("NLAA") from those actions that are "likely to adversely affect" ("LAA") the eight listed mussels and/or PCEs of designated critical habitat. This information is presented as part of the Risk Characterization in Section 5.

The analysis for listed species' direct and indirect effects provides a basis for the evaluation of potential effects to the designated critical habitat of the ovate clubshell and southern clubshell mussels. Atrazine effects are limited to those that are linked to biologically-mediated processes. Therefore, the critical habitat analysis for atrazine is limited in a practical sense to those PCEs of the critical habitat that are biological or that can be reasonably linked to biologically mediated processes. PCEs have been identified by USFWS for the ovate and southern clubshell mussel's designated critical habitat; therefore, these attributes are used as part of the critical habitat impact analysis. Further discussion of the PCEs of the ovate and southern clubshell mussel's designated critical habitat is included in Section 2.6.

2.2 Scope

Atrazine is currently registered as an herbicide in the U.S. to control annual broadleaf and grass weeds in corn, sorghum, sugarcane, and other crops. In addition to food crops, atrazine is also used on a variety of non-food crops, forests, residential/industrial uses, golf course turf, recreational areas, and rights-of-way. It is one of the most widely used herbicides in North America (U.S. EPA, 2003a).

The end result of the EPA pesticide registration process is an approved product label. The label is a legal document that stipulates how and where a given pesticide may be used. Product labels (also known as end-use labels) describe the formulation type, acceptable methods of application, approved use sites, and any restrictions on how applications may be conducted. Thus, the use or potential use of atrazine in accordance with the approved product labels is "the action" being assessed.

This ecological risk assessment is for currently registered uses of atrazine in portions of the action area reasonably assumed to be biologically relevant to the assessed mussel species and their designated critical habitat. Further discussion of the action area(s) for the eight listed mussels and their designated critical habitat is provided in Section 2.7.

Degradates of atrazine include hydroxyatrazine (HA), deethylatrazine (DEA), deisopropylatrazine (DIA), and diaminochloroatrazine (DACT). Comparison of available toxicity information for the degradates of atrazine indicates lesser aquatic toxicity than the parent for fish, aquatic invertebrates, and aquatic plants. Specifically, the available degradate toxicity data for HA indicate that it is not toxic to freshwater fish and invertebrates at the limit of its solubility in water. In addition, no adverse effects were observed in fish or daphnids at DACT concentrations up to 100 mg/L. Acute toxicity values for DIA are 8.5- and 36-fold less sensitive than acute toxicity values for atrazine in fish and daphnids, respectively. In addition, available aquatic plant degradate toxicity data for HA, DEA, DIA, and DACT report non-definitive EC_{50} values (i.e., 50% effect was not observed at the highest test concentrations) at concentrations that are at least 700 times higher than the lowest reported aquatic plant EC_{50} value for parent atrazine. Although degradate toxicity data are not available for terrestrial plants, lesser or equivalent toxicity is assumed, given the available ecotoxicological information for other taxonomic groups including aquatic plants and the likelihood that the degradates of atrazine may lose efficacy as an herbicide. Therefore, given the lesser toxicity of the degradates as compared to the parent, and the relatively small proportion of the degradates expected to be in the environment and available for exposure relative to atrazine, the focus of this assessment is parent atrazine. Additional details on available toxicity data for the degradates are provided in Section 4 and Appendix A.

The results of available toxicity data for mixtures of atrazine with other pesticides are presented in Section A.6 of Appendix A. According to the available data, other pesticides may combine with atrazine to produce synergistic, additive, and/or antagonistic toxic effects. Synergistic effects with atrazine have been demonstrated for a number of organophosphate insecticides including diazanon, chlorpyrifos, and methyl parathion, as well as herbicides including alachlor. If chemicals that show synergistic effects with atrazine are present in the environment in combination with atrazine, the toxicity of atrazine may be increased, offset by other environmental factors, or even reduced by the presence of antagonistic contaminants if they are also present in the mixture. The variety of chemical interactions presented in the available data set suggest that the toxic effect of atrazine, in combination with other pesticides used in the environment, can be a function of many factors including but not necessarily limited to: (1) the exposed species, (2) the co-contaminants in the mixture, (3) the ratio of atrazine and co-contaminant concentrations, (4) differences in the pattern and duration of exposure among contaminants, and (5) the differential effects of other physical/chemical characteristics of the receiving waters (e.g. organic matter present in sediment and suspended water). Quantitatively predicting the combined effects of all these variables on mixture toxicity to any given taxa with confidence is beyond the capabilities of the available data. However, a qualitative discussion of implications of the available pesticide mixture effects data involving atrazine on the confidence of risk assessment conclusions for the freshwater mussels is addressed as part of the uncertainty analysis for this effects determination.

2.3 Previous Assessments

The Agency completed a refined ecological risk assessment for aquatic impacts of atrazine use in January 2003 (U.S. EPA, 2003a). This assessment was based on laboratory ecotoxicological data as well as microcosm and mesocosm field studies found in publicly available literature, a substantial amount of monitoring data for freshwater streams, lakes, reservoirs, and estuarine areas, and incident reports of adverse effects on aquatic and terrestrial organisms associated with the use of atrazine. In the refined assessment, risk is described in terms of the likelihood that concentrations in water bodies (i.e., lakes/reservoirs, streams, and estuarine areas) equaled or exceeded concentrations shown to cause adverse effects to aquatic communities and populations of aquatic organisms. The results of the refined aquatic ecological assessment indicated that exposure to atrazine is likely to result in adverse community-level and population-level effects to aquatic communities at concentrations greater than or equal to 10-20 μg/L on a recurrent basis or over a prolonged period of time.

The results of the Agency's ecological assessments for atrazine are fully discussed in the January 31, 2003, Interim Reregistration Eligibility Decision (IRED)[1]. The assessment identified the need for the following information related to potential ecological risks was established: 1) a monitoring program to identify and evaluate potentially vulnerable waterbodies in corn, sorghum, and sugarcane use areas; and 2) further information on potential amphibian gonadal developmental responses to atrazine. On October 31, 2003, EPA issued an addendum that updated the IRED issued on January 31, 2003 (U.S. EPA, 2003b). This addendum described new scientific developments pertaining to monitoring of watersheds and potential effects of atrazine on endocrine-mediated pathways of amphibian gonadal development.

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[1] The 2003 Interim Reregistration Eligibility Decision for atrazine is available at the following Web site: http://www.epa.gov/oppsrrd1/REDs/0001.pdf.

As discussed in the October 2003 IRED, the Agency also conducted an evaluation of the submitted studies regarding the potential effects of atrazine on amphibian gonadal development and presented its assessment in the form of a white paper for external peer review to a FIFRA Scientific Advisory Panel (SAP) in June 2003[2]. In the white paper dated May 29, 2003, the Agency summarized seventeen studies consisting of both open literature and registrant-submitted laboratory and field studies involving both native and non-native species of frogs (U.S. EPA, 2003d). The Agency concluded that none of the studies fully accounted for environmental and animal husbandry factors capable of influencing endpoints that the studies were attempting to measure. The Agency also concluded that the current lines-of-evidence did not show that atrazine produced consistent effects across a range of exposure concentrations and amphibian species tested.

Based on this assessment, the Agency concluded and the SAP concurred that there was sufficient evidence to formulate a hypothesis that atrazine exposure may impact gonadal development in amphibians, but there were insufficient data to confirm or refute the hypothesis (http://www.epa.gov/oscpmont/sap/2003/June/junemeetingreport.pdf). Because of the inconsistency and lack of reproducibility across studies and an absence of a dose-response relationship in the currently available data, the Agency determined that the data did not alter the conclusions reached in the January 2003 IRED regarding uncertainties related to atrazine's potential effects on amphibians. The SAP supported EPA in seeking additional data to reduce uncertainties regarding potential risk to amphibians. Subsequent data collection has followed the multi-tiered process outlined in the Agency's white paper to the SAP (U.S. EPA, 2003d). In addition to addressing uncertainty regarding the potential use of atrazine to cause these effects, these studies are expected to characterize the nature of any potential dose-response relationship. A data call-in for the first tier of amphibian studies was issued in 2005 and studies are on-going; however, as of this writing, results are not available.

The Agency has completed three separate effects determinations for atrazine as it relates to eight of the listed species included in the Natural Resources Defense Counsel settlement agreement and one listed species included in a second settlement agreement with the Center for Biological Diversity and Save Our Springs Alliance. These effects determinations, which are available on the web at www.epa.gov/espp, review atrazine's potential direct and indirect effects to the following listed species: 1) Barton Springs salamander (*Eurycea sosorum*) (U.S. EPA, 2006c); 2) shortnose sturgeon (*Acipenser brevirostrum*), dwarf wedgemussel (*Alasmidonta heterodon*), loggerhead turtle (*Caretta caretta*), Kemp's ridley turtle (*Lepidochelys kempii*), leatherback turtle *(Dermochelys coriacea*), and green turtle (*Chelonia mydas*) in the Chesapeake Bay (U.S. EPA, 2006d); and the Alabama sturgeon (*Scaphirhynchus suttkusi*) (U.S. EPA, 2006e). Based on the results of these endangered species risk assessments, atrazine effects determinations for the eight aforementioned listed species are either "no effect" or "may affect, but not likely to adversely affect."

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[2] The Agency's May 2003 White Paper on Potential Developmental Effects of Atrazine on Amphibians is available at http://www.epa.gov/oscpmont/sap/2003/june/finaljune2002telconfreport.pdf.

2.4 Stressor Source and Distribution

2.4.1 Environmental Fate and Transport Assessment

The following fate and transport description for atrazine was summarized based on information contained in the 2003 IRED (U.S. EPA, 2003a). In general, atrazine is expected to be mobile and persistent in the environment. The main route of dissipation is microbial degradation under aerobic conditions. Because of its persistence and mobility, atrazine is expected to reach surface and ground water. This is confirmed by the widespread detections of atrazine in surface water and ground water. Atrazine is persistent in soil, with a half-life (time until 50% of the parent atrazine remains) exceeding 1 year under some conditions (Armstrong et al., 1967). Atrazine can contaminate nearby non-target plants, soil and surface water via spray drift during application. Atrazine is applied directly to target plants during foliar application, but pre-plant and pre-emergent applications are generally far more prevalent.

The resistance of atrazine to abiotic hydrolysis (stable at pH 5, 7, and 9) and to direct aqueous photolysis (stable under sunlight at pH 7), and its only moderate susceptibility to degradation in soil (aerobic laboratory half-lives of 3-4 months) indicates that atrazine is unlikely to undergo rapid degradation on foliage. Likewise, a relatively low Henry's Law constant (2.6 X 10^{-9} atm-m^3/mol) indicates that atrazine is not likely to undergo rapid volatilization from foliage. However, its relatively low octanol/water partition coefficient (Log K_{ow} = 2 .7), and its relatively low soil/water partitioning (Freundlich K_{ads} values < 3 and often < 1) may somewhat offset the low Henry's Law constant value, thereby possibly resulting in some volatilization from foliage. In addition, its relatively low adsorption characteristics indicate that atrazine may undergo substantial washoff from foliage. It should also be noted that foliar dissipation rates for numerous pesticides have generally been somewhat greater than otherwise indicated by their physical chemical and other fate properties.

In terrestrial field dissipation studies performed in Georgia, California, and Minnesota, atrazine dissipated with half lives of 13, 58, and 261 days, respectively. The inconsistency in these reported half-lives could be attributed to the temperature variation between the studies in which atrazine was seen to be more persistent in colder climate. Long-term field dissipation studies also indicated that atrazine could persist over a year in such climatic conditions. A forestry field dissipation study in Oregon (aerial application of 4 lb ai/A) estimated an 87-day half-life for atrazine on exposed soil, a 13-day half-life in foliage, and a 66-day half-life on leaf litter.

Atrazine is applied directly to soil during pre-planting and/or pre-emergence applications. Atrazine is transported indirectly to soil due to incomplete interception during foliar application, and due to washoff subsequent to foliar application. The available laboratory and field data are reported above. For aquatic environments, reported half-lives were much longer. In an anaerobic aquatic study, atrazine overall (total system), water, and sediment half-lives were given as 608, 578, and 330 days, respectively.

A number of degradates of atrazine were detected in laboratory and field environmental fate studies. Deethyl-atrazine (DEA) and deisopropyl-atrazine (DIA) were detected in all studies, and hydroxy-atrazine (HA) and diaminochloro-atrazine (DACT) were detected in all but one of the listed studies. Deethylhydoxy-atrazine (DEHA) and deisopropylhydroxy-atrazine (DIHA) were also detected in one of the aerobic studies.

All of the chloro-triazine and hydroxy-triazine degradates detected in the laboratory metabolism studies were present at less than the 10% of applied that the Agency uses to classify degradates as "major degradates" (U.S. EPA, 2004); however, several of these degradates were detected at percentages greater than 10% in soil and aqueous photolysis studies. Insufficient data are available to estimate half-lives for these degradates from the available data. The dealkylated degradates are more mobile than parent atrazine, while HA is less mobile than atrazine and the dealkylated degradates.

2.4.2 Mechanism of Action

Atrazine inhibits photosynthesis by stopping electron flow in Photosystem II. Triazine herbicides associate with a protein complex of the Photosystem II in chloroplast photosynthetic membranes (Schulz et al., 1990). The result is an inhibition in the transfer of electrons that in turn inhibits the formation and release of oxygen.

2.4.3 Use Characterization

Atrazine has the second largest poundage of any herbicide in the U.S. and is widely used to control broadleaf and many other weeds, primarily in corn, sorghum and sugarcane (U.S. EPA, 2003a). As a selective herbicide, atrazine is applied pre-emergence and post-emergence**.** Figure 2.1 presents the national distribution of use of atrazine (Kaul and Jones, 2006).

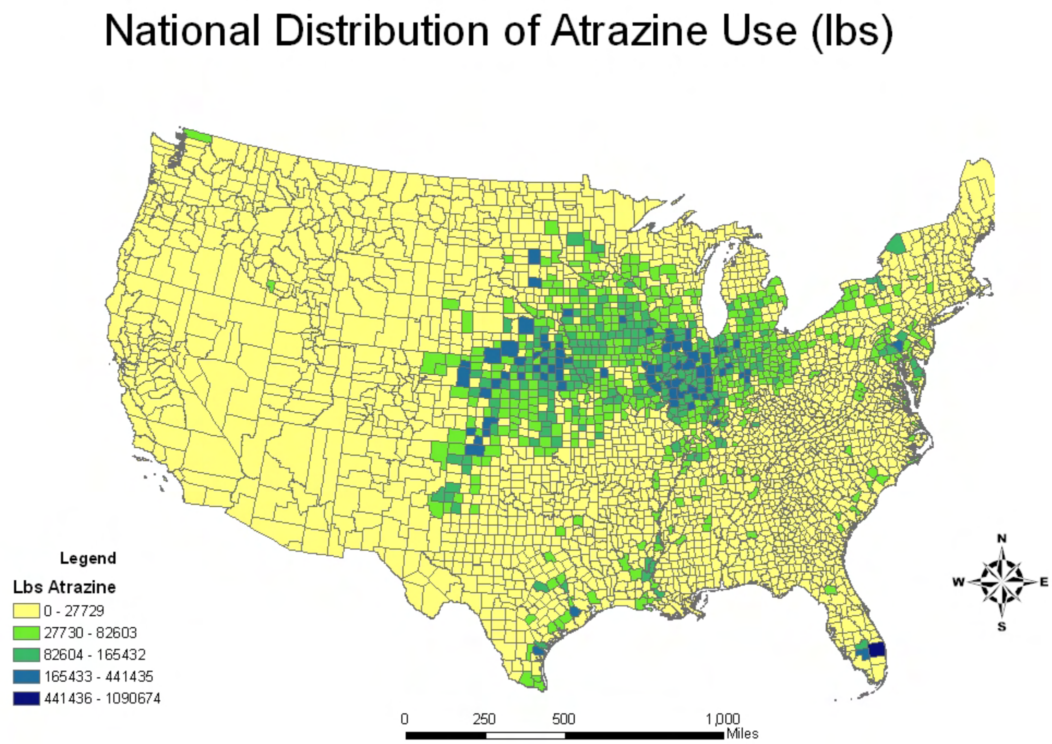

Figure 2.1 National Extent of Atrazine Use (lbs)

Atrazine is used on a variety of terrestrial food crops, non-food crops, forests, residential/industrial uses, golf course turf, recreational areas and rights-of-way. Atrazine yields season-long weed control in corn, sorghum and certain other crops. The major atrazine uses include: corn (83 percent of total ai produced per year - primarily applied pre-emergence), sorghum (11 percent of total ai produced), sugarcane (4 percent of total ai produced) and others (2 percent ai produced). Atrazine formulations include dry flowable, flowable liquid, liquid, water dispersible granule, wettable powder and coated fertilizer granule. The maximum registered use rate for atrazine is 4 lbs ai/acre; and 4 lbs ai/acre is the maximum, single application rate for the following uses: sugarcane, forest trees (softwoods, conifers), forest plantings, guava, macadamia nuts, ornamental sod (turf farms), and ornamental and/or shade trees.

Assessment of the use information is critical to the development of appropriate modeling scenarios and evaluation of the appropriate model inputs (Kaul and Jones, 2006). Information on the agricultural uses of atrazine in the states comprising the action area for the eight assessed mussels (Alabama, Georgia, Mississippi, Arkansas, Missouri, Nebraska, Illinois, Indiana, Ohio, Pennsylvania, West Virginia, Virginia, Kentucky, Tennessee, and North Carolina), as defined in Section 2.6 of this assessment, was gathered (Kaul and Jones, 2006). In addition, typical atrazine crop use information was considered (Kaul, et al, 2005). Use information within the action area is utilized to determine which uses should be modeled, while the application methods, intervals, and timing are critical model inputs. While the modeling described in Section 3.2 relies initially on maximum label application rates and numbers of applications, information on typical ranges of application rates and number of applications is also presented to characterize the modeling results. No state or county level usage information is available on non-agricultural uses (residential, rights-of-way, forestry, or turf) of atrazine.

Agricultural cropland and atrazine use relative to the mussel's action area are depicted in Figures 2.2 and 2.3, respectively. Non-agricultural uses associated with urban/suburban areas (residential, turf, and rights-of-way) are also likely to be co-located with the listed mussel's habitat ranges. Therefore, additional evaluation of urbanized areas and species' locations was completed to assess the relative importance of the residential, rights-of-way, and turf uses and to determine the location and percentages of impervious surface within the action area (Figure 2.4).

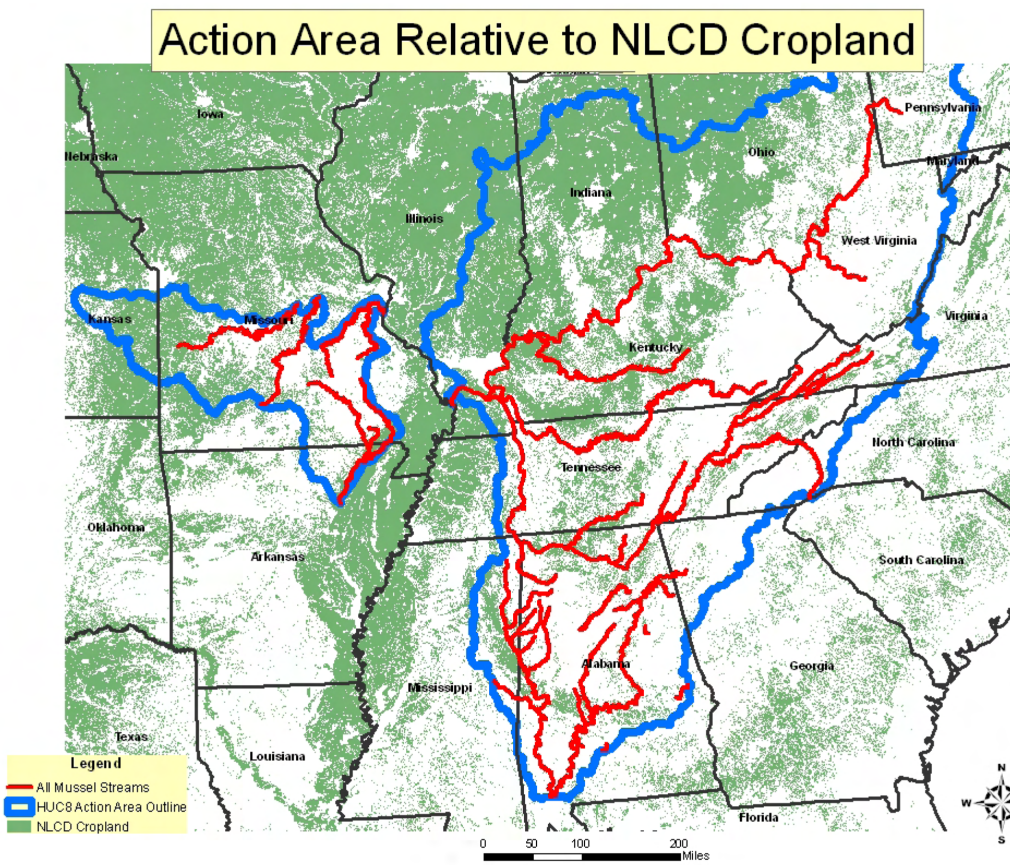

Figure 2.2 Agricultural Cropland Relative to Mussel Action Area

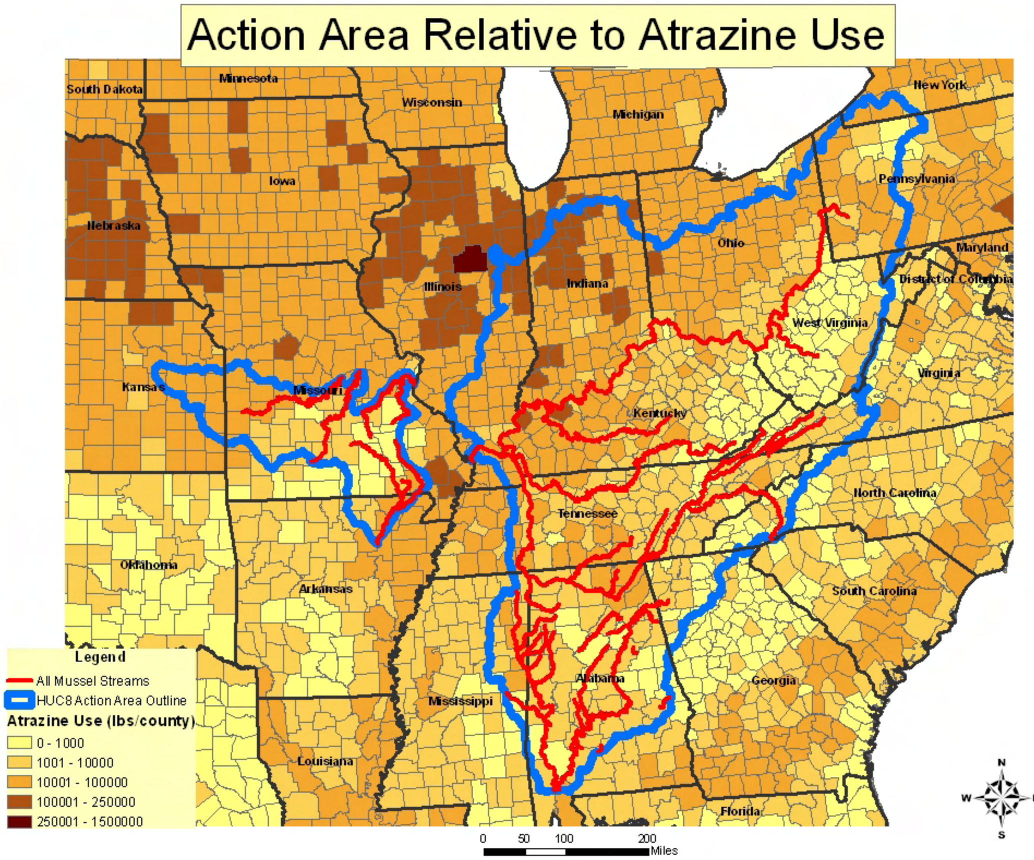

Figure 2.3 Atrazine Use Relative to Action Area

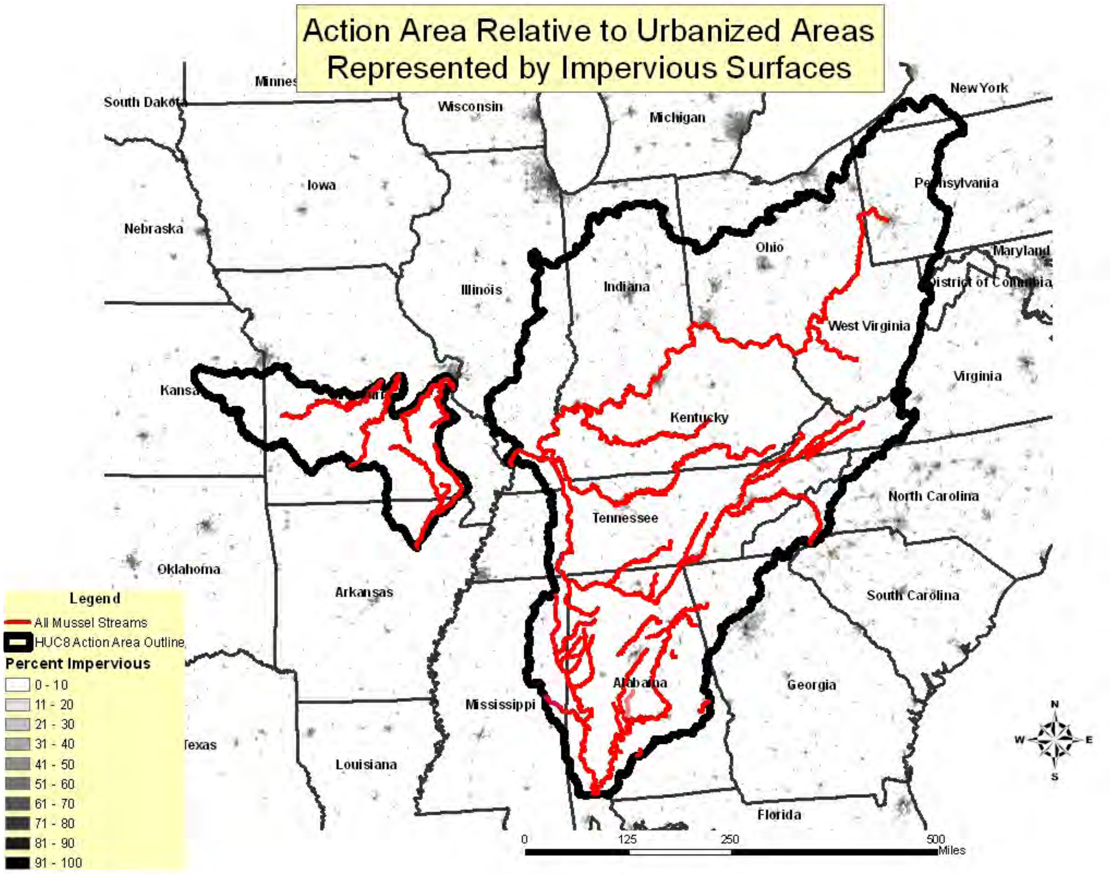

Figure 2.4 Atrazine Use in Action Area Relative to Urbanized (Impervious) Areas

All agricultural use information for atrazine was considered in order to determine which uses occur within the action area for the listed mussels and their designated critical habitat (discussed further in Section 2.7). As noted above, information is not available for non-agricultural uses; therefore, they are presumed assumed to occur within the action area and are included in this assessment. Agricultural uses of atrazine within the action area include corn, sweet corn, sorghum, and fallow/pasture. Specifically, county level data for the areas within and immediately surrounding the action area were used (Kaul and Jones, 2006). County level estimates of atrazine use were derived using state level estimates from USDA-NASS and data obtained from Doane (www.doane.com; the full dataset is not provided due to its proprietary nature). State level data from 1998 to 2004 were averaged together and extrapolated down to the county level based on apportioned county level crop acreage data from the 2002 USDA Agriculture of Census (AgCensus).

In general, this information suggests that the northern portion of the action area is located within the highest atrazine use area in Illinois, Iowa and Nebraska. In general, atrazine use decreases in intensity further south and east of Illinois, with the lowest use in the eastern portion of the action area defined by a line extending from western portions of West Virginia to northeastern Alabama. The atrazine use pattern within the action area is graphically presented in Figure 2.3. It should be noted, however, that information on non-agricultural use of atrazine is not available and, therefore, was not included in Figure 2.3.

Typical use information from the 15 states within the action area is summarized in Table 2.2. The total average atrazine use per year from 1998 to 2004 was roughly 41,600,000 lbs within these states. Of this, roughly 40,000,000 lbs are used on corn or approximately 97% of total atrazine use. Of the remainder, only sweet corn and sorghum are used at amounts at or above 100,000 lbs. For all uses, the typical application rate and number of applications are fairly consistent across all states and all uses. For all uses, the average application rate is 1.1 lbs per acre, while the average number of applications is also 1.1. For corn, the average application rate is 1.2 lbs per acre, and the number of applications is also 1.1.

Table 2.2 Summary of Typical Atrazine Use Information Collected Between 1998 and 2004 for all States in the Mussel Action Area			
Crop	**Total Pounds by Crop**	**Average Number of Applications by Crop**	**Average Application Rate (lbs/acre) by Crop**
corn	40,200,000	1.1	1.2
sorghum	1,329,000	1.1	1.3
sweet corn	95,000	1.1	1.2
wheat	7,000	1.0	0.8

2.5 Assessed Species

General information on the following eight listed freshwater mussels, including a summary of habitat requirements, designated critical habitat, food habits, and reproduction data relevant to this endangered species risk assessment is provided below:

- Pink mucket pearly mussel;
- Rough pigtoe mussel;
- Shiny pigtoe pearly mussel;
- Fine-rayed pigtoe mussel;
- Heavy pigtoe mussel;
- Ovate clubshell mussel;
- Southern clubshell mussel; and
- Stirrupshell mussel.

All eight of the assessed listed mussels are freshwater species that share similar general habitat requirements and reproductive cycles. In general, they live embedded in the bottom sand, gravel, and/or cobble substrates of rivers and streams. They also have a unique life cycle that involves a parasitic stage on host fish. Juvenile mussels require stable substrates with low to moderate amounts of sediment, low amounts of filamentous algae, and correct flow and water quality to continue to develop (USFWS, 2004). During the spawning period, males discharge sperm into the water column, and the sperm are taken in by females through their siphons during feeding and respiration. The females retain the fertilized eggs in their gills, until the larvae (glochidia) fully develop. The mussel glochidia are released into the water where they must attach to the gills and fins of appropriate host fishes, which they parasitize for a short time until they develop into juvenile mussels. The presence of suitable host fish is considered an essential element in the mussels' life cycles. Once the glochidia metamorphose to the juvenile stage, they drop to the substrate. If the environmental conditions are favorable, the juvenile mussel will survive and develop. Freshwater mussels are long lived, up to 50 years or more. They usually reach sexual maturity in 3-9 years.

All eight listed species are members of the Unionidae family, which exhibit two reproductive cycles based on the length of time glochidia are retained in the gills of females. Fertilization occurs in the spring in tachytictic mussels (short-term brooders) and glochidia are released during spring and summer. In bradytictic species (long-term brooders), fertilization occurs in mid-summer and fall, and glochidia are released the following spring and summer (USFWS, 1976).

All adult freshwater mussels are filter-feeders, orienting themselves in the substrate to facilitate siphoning of the water column for oxygen and food (Kraemer, 1979). Phytoplankton is the principal food of bivalves, although mussels have also been reported to consume detritus, diatoms, zooplankton (microscopic animals that live suspended in the water), and other microorganisms (Ukeles, 1971; Coker et al., 1921; Churchill and Lewis, 1924; Fuller, 1974). Specific percentages of these food items within the mussel's diet are not known, although the available information indicates that adult mussels can clear and assimilate fine particulate organic matter (FPOM) particles ranging in size from 0.9 to 250 µm (Silverman et al., 1997; Wissing, 1997; and Nichols and Garling, 2000). This size range includes bacteria and algal cells, detritus, and soil particles (Allan, 1995). Juveniles up to two weeks old feed on bacteria, algae, and diatoms with small amounts of detrital and inorganic colloidal particles (Yeager et al., 1994). The diet of the glochidia comprises water (until encysted on a fish host) and fish body fluids (once encysted).

According to the USFWS (1985), the greatest single factor contributing to the decline of freshwater mussels is the alteration and destruction of stream habitat due to impoundments for flood control, navigation, hydroelectric power, and recreation. These dams and their impounded waters present physical barriers to the natural dispersal of mussels, including emigration (dispersal) of host fishes, and effectively isolate surviving mussel populations causing fragmentation in limited portions of their habitat range. Mussels are also susceptible to adverse effects caused by siltation in waterways. Specific biological impacts on mussels from excessive sediments include reduced feeding and respiratory efficiency from clogged gills, disrupted metabolic processes, reduced growth rates, increased substrata instability, limited burrowing activity and physical smothering (Ellis, 1936; Stansbery, 1971; Markings and Bills, 1979; Kat, 1982; Vannote and Minshall, 1982; Aldridge et al., 1987; and Waters, 1995).

A summary of the current range, habitat type, designated critical habitat, reproductive cycle, and glochidial hosts for each of the eight assessed species is provided in Table 2.3. As shown in Table 2.3, the current range of the eight assessed species spans various watersheds within ten states, including Tennessee, Kentucky, Alabama, Missouri, Ohio, West Virginia, Virginia, Arkansas, Mississippi, and Georgia. Information on the current habitat ranges of the listed mussels was obtained from USFWS recovery plans, which exist for all eight assessed species (USFWS 1984a, 1984b, 1984c, 1985, 1989, and 2000), species-specific information available on the USFWS website (http://www.fws.gov/endangered/; accessed in November 2006), the 5-year review for the ovate and southern clubshell mussel (USFWS, 2006), and personal communications with several known freshwater mussel experts (personal communications with Paul Hartfield [USFWS] 2006, Jeff Powell [USFWS] 2006, James Williams [USGS] 2006, Bob Butler

[USFWS] 2006, and Paul Johnson [Alabama Aquatic Biodiversity Center] 2006). It should be noted that the stirrupshell mussel is presumed to be extinct, given that the species has not been observed for over 20 years (Hartfield, 2006). Therefore, registered uses of atrazine are presumed to have "no effect" on the stirrupshell mussel. Further detail on the general and specific status and life history information for the assessed mussels, including species-specific maps depicting known occurrences, are provided in Appendix C.

Table 2.3 Summary of Current Distribution, Habitat Requirements, and Life History Information for the Eight Assessed Mussels					
Assessed Species	**Current Range**	**Habitat Type**	**Designated Critical Habitat?[a]**	**Reproductive Cycle[b]**	**Known Glochidial Hosts**
Pink mucket pearly mussel	(TN, KY, AL, MO, OH, WV, AR): Tennessee, Cumberland, Osage, and Meramec Rivers; a small portion of the Kanawha River (below the Kanawha Falls); Clinch River upstream from Norris Dam and downstream from Melton Hill Dam; lower Ohio River; Green River; Current River, Big River; Black and Little Black Rivers; Gasconade River; French Broad River; and Bear Creek	Medium to large rivers with strong currents and sand, gravel, and mud substrates	No	Long-term breeder (bradytictic)	Largemouth bass, spotted bass, smallmouth bass, walleye, sauger, and freshwater drum
Rough pigtoe mussel	(TN, KY, AL): Downstream of three Tennessee River mainstem dams (Pickwick, Wilson, and Guntersville); Clinch River (between river mi. 323 and 154); middle reaches of the Cumberland River; Green River (below Lock & Dam No. 5 near Gilmore, Warren County to Lock 4 near Woodbury, KY); and Barren River (from Lock 1 near Greencastle to mouth of the river)	Medium to large rivers with sand, gravel, and cobble substrates; intolerant of impoundments	No	Short-term breeder (tachytictic)	Unknown
Shiny pigtoe mussel	(VA, TN, AL): Clinch River and its tributary Copper Creek (from VA-TN border upstream to Lee County, VA), Powell River (from VA-TN border upstream to Lee County, VA), North Fork Holston River (VA), and Paint Rock River (AL – rare)	Riffle species; moderate to swiftly flowing streams and rivers with stable substrates of sand/gravel; intolerant of impoundments, deep pools, or lentic waters	No	Short-term breeder (tachytictic)	Whitetail shiner, common shiner, warpaint shiner, and telescope shiner
Fine-rayed pigtoe mussel	(VA, TN, AL): Portions of the Clinch and Powell Rivers (Buchanan Ford, McDowell Shoal, and Fletcher Ford), North Fork Holston River (Cloud Ford), Paint Rock River, Elk River, Sequatchie River (near Dunlap, TN below Euton Bridge), and Little River (Blount County, TN)	Riffle species; moderate to high gradient streams with firm cobble or gravel substrates; intolerant of impoundments of lentic waters	No	Short-term breeder (tachytictic)	Fathead minnow, river chub, stoneroller, telescope chub, Tennessee shiner, white shiner, whitetail shiner, and mottled sculpin

Heavy pigtoe mussel	(AL): Only remaining extant population is in Alabama River near Selma (Dallas and Lowndes Counties, AL)	Moderate to large rivers with moderate to swift current; preferred habitat is riffle-run or shoal areas with stable sandy gravel to gravel-cobble substrates	No	Unknown	Unknown
Ovate clubshell mussel	(AL, MS): Tombigbee River tributaries including the Buttahatchee River (Lowndes/Monroe Counties, MS), Luxapalila (Lowndes County, MS) and Yellow Creek, Sipsey River (Greene/Pickens/Tuscaloosa Counties, AL), Sucarnoochee River (Sumter County, Alabama), Coalfire Creek (Pickens County, AL), Alabama River tributaries (Sturdivant Creek and McCalls Creek), Cahaba River (above and below the fall line), Uphapee Creek (Tallapoosa River drainage).	Sand and gravel shoals and runs of small rivers and large streams with moderate to high flow; intolerant impoundments or channelization	Yes	Unknown; however gravid females have been observed in June and July	Unknown
Southern clubshell mussel	(MS, AL, GA): East Fork Tombigbee River (Itawamba/Monroe Counties, MS), Bull Mountain Creek (Itawamba County, MS), Buttahatchee River (Monroe/Lowndes Counties, MS), Luxapalila and Yellow Creeks (Lowndes County, MS), Lubbub Creek (Pickens County, AL), and Sipsey River (Greene/Pickens/Tuscaloosa Counties, AL) in the Tombigbee drainage; a short reach of the Alabama River (above Selma between RR trestle and AL 41 bridge), Oakmulgee Creek (Cahaba River Drainage, Dallas County, AL), and Bogue Chitto Creek (Dallas County, AL); Uphapee Creek (Macon County, AL); Chewacla Creek (Macon County, AL) in the Tallapoosa drainage; Coosa River below Weiss Dam (Cherokee County, AL) and below Logan Martin Dam (St. Clair/Talladega Counties, AL) and tributaries Yellowleaf Creek (Shelby County, AL), Big Canoe Creek (St. Clair County, AL), Terrapin Creek (Cherokee County, AL), Conassauga River	Shoals and runs of small rivers and large streams with sand/gravel/cobble substrate and highly oxygenated waters; intolerant of impoundment or channelization	Yes	Unknown; however gravid females with mature glochidia have been collected in June and July	Alabama shiner and tricolor shiner

	(Murray/Whitfield Counties, GA).				
Stirrupshell mussel	(AL): No observations of this species have been recorded in over 20 years; therefore, this species is presumed to be extinct. Only two small areas of viable habitat remain: one in the Sipsey River and the other in a bendway of the Tombigbee River in Sumter County, AL	Moderate to large rivers with moderate current; preferred habitat is riffle-run or shoal areas with stable sandy gravel to gravel-cobble substrates	No	Unknown	Unknown

[a] Critical habitat has been designated for the ovate clubshell mussel and Southern clubshell mussel (USFWS, 2004: 69 FR 40084-40171). Specific locations of the designated critical habitat for these species are provided in Table 2.4.
[b] Tachytictic species have a spring fertilization period, then the glochidia are incubated for a few months and expelled during the summer or early fall. Bradytictic species have a late summer or early fall fertilization period with the glochidia incubating overwinter, and expelled the following spring or summer.

2.6 Designated Critical Habitat

Effective August 2, 2004, the USFWS designated critical habitat for 11 species of freshwater mussels (USFWS, 2004: 69 FR 40084-40171), two of which are assessed as part of this endangered species risk assessment. These species, which both occur in the Tombigbee River Basin, include the ovate clubshell and southern clubshell mussels. 'Critical habitat' is defined in the ESA as the geographic area occupied by the species at the time of the listing where the physical and biological features necessary for the conservation of the species exist, and there is a need for special management to protect the listed species. In addition, critical habitat may also include specific areas outside the geographic area occupied by the species at the time it is listed in accordance with provisions of Section 3(5)(A) of the ESA, upon determination that such areas are essential for conservation of the species. Critical habitat receives protection under Section 7 of the ESA through prohibition against destruction or adverse modification of critical habitat with regard to actions carried out, funded, or authorized by a Federal Agency. Section 7 requires consultation on Federal actions that are likely to result in the destruction or adverse modification of critical habitat.

To be included in a critical habitat designation, the habitat must first be 'essential to the conservation of the species.' Critical habitat designations identify, to the extent known using the best scientific and commercial data available, habitat areas that provide essential life cycle needs of the species (i.e., areas on which the PCEs are found, as defined in 50 CFR 414.12(b)).

Occupied habitat may be included in the critical habitat only if essential features within the habitat may require special management or protection. Therefore, the USFWS does not include areas where existing management is sufficient to conserve the species. Critical habitat is designated outside the geographic area presently occupied by the species only when a designation limited to its present range would be inadequate to ensure the conservation of the species. For the ovate and southern clubshell mussels, critical habitat includes areas of currently occupied habitat as well as areas that are outside of the species current habitat range (i.e., part of the species historical range).

The designated critical habitat areas for the ovate and southern clubshell mussels are considered to have the PCEs that justify critical habitat designation. Activities that may destroy or adversely modify critical habitat are those that alter the PCEs and jeopardize the continued existence of the species. Evaluation of actions related to use of atrazine that may alter the PCEs of the ovate and southern clubshell mussel's critical habitat form the basis of the critical habitat impact analysis. These PCEs, which are specified in the designated critical habitat listing notice (USFWS, 2004), include the following:

- geomorphically stable stream and river channels and banks;
- a flow regime necessary for normal behavior, growth, and survival of all life stages of mussels and their fish hosts in the river environment;

- water quality, including temperature, pH, hardness, turbidity, oxygen content, and other chemical characteristics necessary for normal behavior, growth, and viability of all life stages;
- sand, gravel, and/or cobble substrates with low to moderate amounts of attached filamentous algae and other physical and chemical characteristics necessary for normal behavior, growth, and viability of all life stages;
- fish hosts with adequate living, foraging, and spawning areas for them; and
- few or no competitive or predacious nonnative species present.

A summary of designated critical habitat for the ovate clubshell and southern clubshell mussels, including the river/stream reach name and location, critical habitat unit number, and historical and/or current presence of the species within the critical habitat, is provided in Table 2.4. Critical habitat for these species includes portions of the Tombigbee River drainage in Mississippi and Alabama; portions of the Black Warrior River drainage in Alabama; portions of the Alabama River drainage in Alabama; portions of the Cahaba River drainage in Alabama; portions of the Tallapoosa River drainage in Alabama and Georgia; and portions of Coosa River drainage in Alabama, Georgia, and Tennessee. No critical habitat has been designated for the other six mussel species included in this assessment. Further detail on the designated critical habitat for the ovate and southern clubshell mussels, including critical habitat maps for each species, is provided in Appendix C.

Table 2.4 Designated Critical Habitat for the Southern Clubshell and Ovate Clubshell Mussels[a]				
River/Stream (Critical Habitat Unit #)	**County**	**Species[b]**		**Description (Reach Length)**
		Southern clubshell	Ovate clubshell	
East Fork Tombigbee River (1)	Monroe, Itawaba (MS)	X (H/C)	X (H)	From MS Highway 278, Monroe County, upstream to the confluence of Mill Creek (16 miles)
Bull Mountain Creek (2)	Itawaba (MS)	X (H/C)	X (H)	From MS Highway 25, upstream to U.S. Highway 78, Itawamba County (21 miles)
Buttahatchee River and tributary (3)	Lowndes, Monroe (MS) Lamar (AL)	X (H/C)	X (H/C)	Buttahatchee River: 54 miles extending from its confluence with impounded waters of Columbus Lake, Lowndes/Monroe County, upstream to confluence of Beaver Creek, Lamar County Sipsey Creek: 14 miles from its confluence with the Buttahatchee River, upstream to the MS/AL State Line, Monroe County (68 total miles)
Luxapalila Creek and tributary (4)	Lowndes (MS) Lamar (AL)	X (H/C)	X (H/C)	Luxapalila Creek: 9 miles extending from Waterworks Rd., Columbus, MS, upstream approximately 0.6 miles above Steens Rd., Lowndes County, MS Yellow Creek: 9 miles extending from its confluence with Luxapalila Creek, upstream to the confluence of Cut Bank Creek, Lamar County, AL (18 total miles)
Coalfire Creek (5)	Pickens (AL)	X (H)	X (H/C)	From confluence with impounded waters of Aliceville Lake, upstream to U.S. Highway 82, Pickens County, AL (18 miles)
Lubbub Creek (6)	Pickens (AL)	X (H/C)	X (H)	From confluence with impounded waters of Gainesville Lake, upstream to the confluence of Little Lubbub Creek, Pickens County, AL (19 miles)
Sipsey River (7)	Greene, Pickens, Tuscaloosa (AL)	X (H/C)	X (H/C)	From confluence with impounded waters of Gainesville Lake, Greene/Pickens County, upstream to AL Highway 171 crossing Tuscaloosa County, AL (56 miles)
Trussels Creek (8)	Greene (AL)	X (H)	X (H)	From confluence with impounded waters of Demopolis Lake, upstream to AL Highway 14, Greene County, AL (13 miles
Sucarnoochee River (9)	Sumter (AL)	X (H)	X (H/C)	From confluence with Tombigbee River, upstream to MS/AL State Line, Sumter County, AL (56 miles)

Sipsey Fork and tributaries (10)	Winston, Lawrence (AL)		X (H)	Sipsey Fork: 19 miles from section 11/12 line, Winston County, upstream to the confluence of Hubbard Creek, Lawrence County, AL Thompson Creek: 5 miles from confluence with Hubbard Creek, upstream to section 2 line, Lawrence County, AL Brushy Creek: 22 miles from confluence of Glover Creek, Winston County, upstream to section 9, Lawrence County, AL Capsey Creek: 9 miles from confluence with Bushy Creek, Winston County, upstream to the confluence of Turkey Creek, Lawrence County, AL Rush Creek: 6 miles from confluence with Bushy Creek, upstream to Winston/Lawrence County Line Brown Creek: 2 miles from confluence with Rush Creek, Winston County, upstream to section 24 line, Lawrence County, AL Beech Creek: 2 miles from confluence with Brushy Creek to confluence of East and West Forks, Winston County, AL Caney Creek and North Fork Caney Creek: 8 miles from confluence with Sipsey Fork, upstream to section 14 line, Winston County, AL Borden Creek: 11 miles from confluence with Sipsey Fork, Winston County, upstream to confluence of Montgomery Creek, Lawrence County, AL Flannagin Creek: 6 miles from confluence with Borden Creek, upstream to confluence of Dry Creek, Lawrence County, AL (91 total miles)
North River and tributary (11)	Tuscaloosa, Fayette (AL)	X	(H)	North River: 26 miles from Tuscaloosa County Rd. 38, Tuscaloosa County, upstream to confluence of Ellis Creek, Fayette County, AL Clear Creek: 3 miles from its confluence with the North River to Bays Lake Dam, Fayette County, AL (29 total miles)
Locust Fork and tributary (12)	Jefferson, Blount (AL)	X	(H)	Locust Fork: 58 miles from U.S. Highway 78, Jefferson County, upstream to the confluence of Little Warrior River, Blount County, AL Little Warrior River: 5 miles from confluence with Locust Fork, upstream to the confluence of Calvert Prong and Blackburn Fork, Blount County, AL (63 total miles)
Cahaba River and tributary (13)	Jefferson, Shelby, Bibb (AL)	X (H)	X (H)	Cahaba River: 65 miles from U.S. Highway 82, Centerville, Bibb County, upstream to Jefferson County Rd. 143, Jefferson County, AL Little Cahaba River: 12 miles from confluence with the Cahaba River, upstream to the confluence of Mahan and Shoal Creeks, Bibb County, AL (77 total miles)

Alabama River (14)	Autauga, Lowndes, Dallas (AL)	X (H/C)		From the confluence of the Cahaba River, Dallas County, upstream to the confluence of Big Swamp Creek, Lowndes County, AL (45 miles)
Bogue Chitto Creek (15)	Dallas (AL)	X (H/C)		From its confluence with the Alabama River, Dallas County, upstream to U.S. Highway 80, Dallas County, AL (32 miles)
Uphapee Complex (17)	Macon, Lee (AL)	X (H/C)	X (H/C)	Uphapee Creek: 18 miles from AL Highway 199, upstream of confluence of Opintlocco and Chewacla Creeks, Macon County (AL) Choctafaula Creek: 7 miles from confluence of Uphapee, upstream to Macon County Rd. 54, Macon County (AL) Chewacla Creek: 18 miles from confluence with Opintlocco Creek, Macon County, AL, upstream to Lee County RD 159, Lee County (AL) Opintlocco Creek: 10 miles from confluence with Chewacla Creek, upstream to Macon County Rd. 79, Macon County, AL (46 total miles)
Coosa River (18)	Cherokee, Calhoun, Cleburne (AL)	X (H/C)	X (H)	Coosa River: 11 miles from the powerline crossing SE of Maple Grove, AL, upstream to Weiss Dam, Cherokee County, AL Terrapin Creek: 33 miles from its confluence with the Coosa River, Cherokee County, upstream to Cleburne County Rd. 55, Cleburne County, AL South Fork Terrapin Creek: 4 miles from its confluence with Terrapin Creek, upstream to Cleburne County Rd. 55, Cleburne County, AL (48 total miles)
Hatchet Creek (19)	Coosa, Clay (AL)	X (H)	X (H)	From the confluence of Swamp Creek at Coosa County Rd. 29, Coosa County, upstream to Clay County Rd. 4, Clay County, AL (41 miles)
Kelly Creek and tributary (21)	Shelby, St. Clair (AL)	X (H/C)	X (H)	Kelly Creek: 16 miles from the confluence with the Coosa River, upstream to the confluence of Shoal Creek, St. Clair County, AL Shoal Creek: 5 miles from confluence with Kelly Creek, St. Clair County, upstream to St. Clair/Shelby County Line, St. Clair County, AL (21 total miles)
Big Canoe Creek (24)	St. Clair (AL)	X (H/C)	X (H)	From its confluence with Little Canoe Creek at the St. Clair/Etowah County Line, St. Clair County, upstream to the confluence of Fall Branch, St. Clair County, AL (18 miles)

Oostanaula complex (25)	Floyd, Gordon, Whitfield, Murray (GA) Bradley, Polk (TN)	X (H/C)	X (H)	Oostanaula River: 48 miles from its confluence with the Etowah River, Floyd County, upstream to the confluence of the Conasauga and Coosawattee River, Gordon County, GA Coosawattee River: 9 miles from confluence with the Conasauga River, upstream to the GA State Highway 136, Gordon County, GA Conasauga River: 61 miles from confluence with the Coosawattee River, Gordon County, upstream through Bradley and Polk Counties, TN, to the Murray County Rd. 2, Murray County, GA Holly Creek: 10 miles from confluence with Conasauga River, upstream to its confluence with Rock Creek, Murray County, GA (128 total miles)
Lower Coosa River (26)	Elmore (AL)	X (H)	X (H)	From the AL State Highway 111 bridge, upstream to Jordan Dam, Elmore County, AL (8 miles)

[a] USFWS, 2004: 69 FR 40084-40171.
[b] H = Historical habitat (i.e., the species is not currently present within the designated critical habitat, unless denoted by "H/C"). C = Current habitat.

2.7 Action Area

For listed species assessment purposes, the action area is considered to be the area affected directly or indirectly by the federal action and not merely the immediate area involved in the action (50 CFR 402.02). It is recognized that the overall action area for the national registration of atrazine uses is likely to encompass considerable portions of the United States based on the large array of both agricultural and non-agricultural uses. Based on the available atrazine monitoring data (discussed further in Section 3.2.5) and the toxicity data for the most sensitive non-vascular aquatic plant, the Agency's LOCs are likely to be exceeded in many watersheds that are in proximity to or downstream of atrazine use sites. Therefore, the overall action area for atrazine is likely to include many watersheds of the United States that co-occur and/or are in proximity to agricultural and non-agricultural atrazine use sites. However, in order to focus this assessment, the scope limits consideration of the overall action area to those geographic portions that may be applicable to the protection of the eight listed mussels and designated critical habitat included in this assessment. Based on the available information on potential atrazine use sites, none of the streams and rivers that are within the range of the eight assessed mussels and their designated critical habitat could be excluded from the action area. Therefore, the portion of the atrazine action area that is assessed as part of this ESA includes the area within the boundary of the watersheds that drain to known current locations of the eight assessed mussels and/or their designated critical habitat.

The eight listed mussels included in this assessment (hereafter defined as the listed mussels) are known to currently exist in a wide range of streams and rivers across the Midwest, Mississippi River valley, Appalachian Mountains, Southern Missouri, and Mobile River Basin. Historically, each species is presumed to have ranged over a much broader area; however, this assessment focuses on the current range of the assessed species, as well as designated critical habitat for the ovate and southern clubshell mussels. In many instances, the location information for the listed mussels is non-specific (e.g. Lower Ohio River for the pink mucket pearly mussel), and in these instances, the entire stream or river reach has been included. Location information on the designated critical habitat of the ovate and southern clubshell mussels, which is summarized in Table 2.4, was obtained from USFWS (2004). The "action area" is the overall geographic scope where effects may occur. However, since this assessment is limited to reviewing potential effects of atrazine use to 8 specific species, we are defining the action area as the geographic scope where effects may occur, either directly or indirectly, to any of these 8 species or the two species critical habitat. Therefore, the initial definition of the action area for these species is defined by the watersheds that drain to the known current locations and designated critical habitats of all mussels included in this assessment.

As shown in Figure 2.5, the action area for the eight assessed mussels stretches from a point near the mouth of the Alabama and Tombigbee Rivers (near Mobile, Alabama) up into, and including, the Ohio River watershed. The action area also includes an isolated section in Arkansas, Missouri, and Nebraska. Deriving the geographical extent of this portion of the action area is the product of consideration of the types of effects atrazine may be expected to have on the environment, the exposure levels to atrazine that are associated with those effects, and the best available information concerning the use of atrazine and its fate and transport within the area identified in Figure 2.5.

Specifically, a list of all current locations for the listed mussels was prepared (Table 2.3) and a map was created using ArcMap GIS. Each of the streams and rivers where the listed mussels are located was added to the map using the Enhanced Reach File (ERF) version 1_2 (http://water.usgs.gov/GIS/metadata/usgswrd/XML/erf1.xml). Stream names in the ERF were matched to those listed in Table 2.3 and added to the map. In addition, critical habitat information for the ovate clubshell and southern clubshell mussels was used in defining the action area (Table 2.4). Duplicate stream reach names occurring in areas outside the immediate range of the species were eliminated from the map. For example, the Green River is a listed location for the pink mucket pearly mussel in Kentucky. However, the Green River is a common river name found in locations throughout the United States including Massachusetts, Wyoming/Utah, and Washington states. This analysis eliminated the stream reaches not associated with the locations defined in Table 2.3. The next step in defining the action area was to assume that all waters, within or draining to the identified stream reaches, are part of the action area. Where non-specific information was available on the location of individual species, or GIS analysis was unable to specify the location on a map, it was assumed that the entire identified stream within the given state was part of the species habitat. For example, the current range of the pink mucket pearly mussel in Kentucky was defined as the Lower Ohio River. For purposes of this analysis, the entire length of the Ohio River was included because of the non-specific nature of this description. Areas draining to the specified stream reaches were defined by identifying all watersheds located upstream of the known species' locations using the USGS' hydrologic unit code (HUC) watersheds.

The USGS has defined watersheds within the entire United States into increasingly smaller HUCs, from coarse scales (Regions, or HUC2 watersheds) to subregions (HUC4 watersheds) to accounting units (HUC6 watersheds) to cataloging units (HUC8 watersheds). The action area definition analysis started at the coarsest scale with regional HUCs (or HUC2 watersheds). For this analysis, the full extent of the area draining to the identified streams extended from the Rocky Mountains to the Atlantic coastal plain. Once a drainage area was defined, the next level of refinement within the HUC classification (HUC4, HUC6, and HUC8) watershed was added to the analysis. Those HUCs not draining to the streams where listed mussels occur and/or their designated critical habitat exists were eliminated from the final map. Ultimately, the action area is defined by those HUC8 watersheds draining to the species' habitat ranges including designated critical habitat. The action area consists of two separate areas that are not hydrologically connected. The first is defined by those watersheds that stretch from western Pennsylvania to southern Alabama, and the second is defined by selected HUC8s in northern Arkansas, Missouri, and eastern Nebraska.

More detail on the Agency's ERF stream data and the USGS' HUC classification scheme may be found at the following websites:

http://www.epa.gov/waters/doc/refs.html

http://water.usgs.gov/GIS/huc.html

Modeled concentrations of atrazine for labeled uses expected to occur within the action area exceed the Agency's LOCs for aquatic plants, suggesting that adverse effects on components of the environment are possible. The results of the screening level assessment suggest that effects on components of the environment are possible anywhere within the defined area. In general, available monitoring data for the action area show that peak concentrations are consistent with modeling and are above the Agency's screening levels of concern for indirect effects. Longer term exposures from monitoring data are difficult to assess relative to the Agency's LOCs. For monitoring data that is not specifically targeted to highly vulnerable areas (described further in Sections 3.2.6.2 through 3.2.6.4), the limited sampling frequency precludes a direct comparison of longer-term exposures (e.g. 30-day average concentrations) with modeling. Comparison of annual average concentrations from non-targeted monitoring data (e.g. data in which the study was not specifically designed to capture atrazine concentrations in high use areas) suggests that long-term exposure in monitoring data are generally below modeled concentrations of atrazine and the Agency's LOCs. However, preliminary analysis of the Ecological Monitoring Program data (Section 3.2.6.1), which is targeted for watersheds most vulnerable to atrazine runoff, suggests that longer-term exposures (e.g. 30-day average concentrations) in selected watersheds exceed the Agency's LOCs. The watersheds from the Ecological Monitoring Program that exceed the Agency's LOCs will ultimately represent a subset of the original population of 1,172 watersheds from which they were selected, however, further analysis to determine how many, if any, watersheds within the action area are represented by these sites is not available. The action area for the mussels is defined as shown in Figure 2.5. Further information on the definition of the action area follows.

In addition, an evaluation of use information was conducted to determine whether any or all of the area described above should be included in the action area. As part of this effort, current labels were reviewed and local use information was evaluated to determine which atrazine uses could potentially be present within the defined area. This data suggest that extensive agricultural uses are present within the defined area and that the existence of non-agricultural uses cannot be precluded. Finally, local land cover data were considered to refine the characterization of potential atrazine use in the areas defined above. The overall conclusion of this analysis was that while certain agricultural uses could likely be excluded (i.e. guava, macadamia nuts, and sugarcane) and some non-agricultural uses of atrazine were unlikely, no areas could be excluded from the final action area based on usage and land cover data.

The environmental fate properties of atrazine were also evaluated to determine which routes of transport are likely to have an impact on the listed species included in this assessment. Review of the environmental fate data, as well as physico-chemical properties of atrazine, suggest that transport via runoff and spray drift are likely to be the dominant routes of exposure. In addition, long-range atmospheric transport of pesticides could potentially contribute to atrazine concentrations in the aquatic habitat used by the mussels. Given the physico-chemical profile for atrazine and data showing that atrazine has been detected in both air and rainfall samples, the potential for long range transport from outside the area defined above cannot be precluded. However, the contribution of atrazine via long-range atmospheric transport is not expected to approach the concentrations predicted by modeling (see Section 3.2).

Atrazine transport away from the site of application by both spray drift and volatilization has been documented. Spray drift is addressed as a localized route of transport from the application site in the exposure assessment. However, quantitative models are currently unavailable to address the longer-range transport of pesticides from application sites. The environmental fate profile of atrazine, coupled with the available monitoring data, suggest that long-range transport of volatilized atrazine is a possible route of exposure to non-target organisms; therefore, the full extent of the action area could be influenced by this route of exposure. However, given the amount of direct use of atrazine within the immediate area surrounding the species, the magnitude of documented exposures in rainfall at or below available surface water and groundwater monitoring data (as well as modeled estimates for surface water), and the lack of modeling tools to predict the impact of long range transport of atrazine, the extent of the action area is defined by the transport processes of runoff and spray drift for the purposes of this assessment.

Based on this analysis, the action area for atrazine as it relates to the eight assessed mussels is defined by the entire watersheds depicted in Figure 2.5.

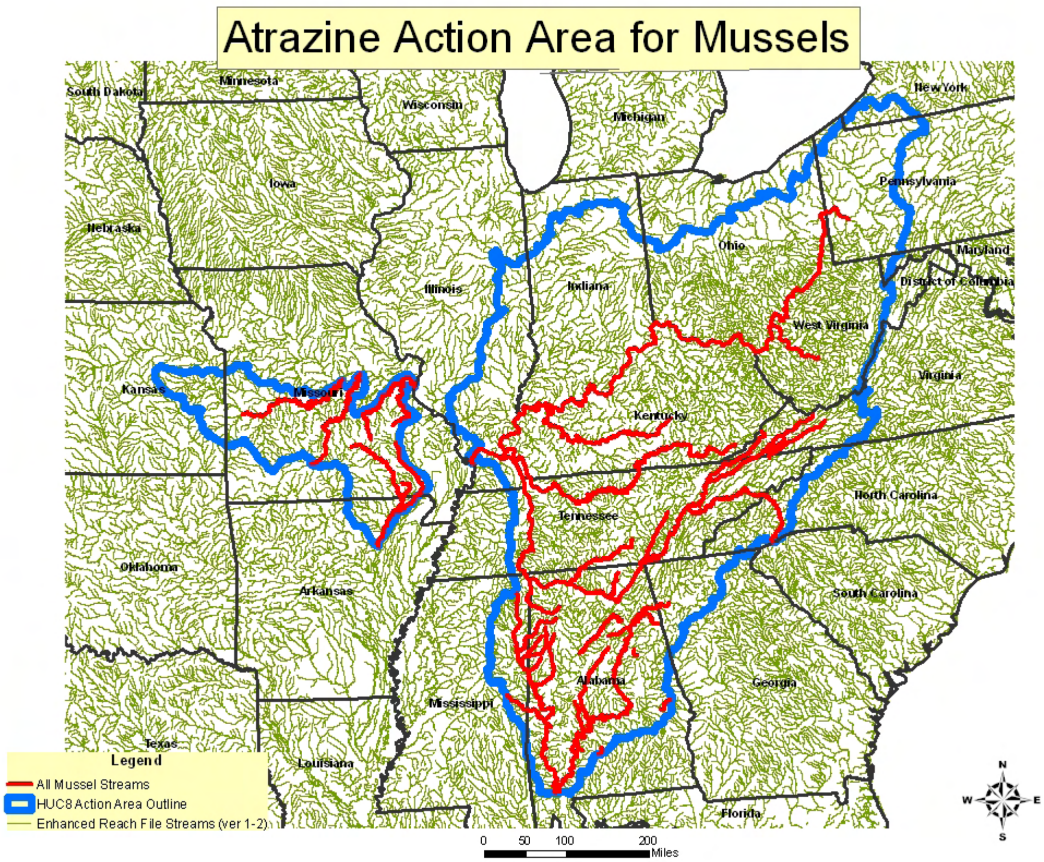

Figure 2.5 Mussel's Action Area Defined by Hydrologic Unit Code (HUC8) Watersheds

2.8 Assessment Endpoints and Measures of Ecological Effect

Assessment endpoints are defined as "explicit expressions of the actual environmental value that is to be protected."[3] Selection of the assessment endpoints is based on valued entities (i.e., eight listed mussels and PCEs of designated critical habitat), the ecosystems potentially at risk (i.e., streams and rivers of the Mississippi Valley, Appalachian Mountains, Southwest Missouri, and Mobile River Basin), the migration pathways of atrazine (i.e., runoff and spray drift), and the routes by which ecological receptors are exposed to atrazine-related contamination (i.e., direct contact).

Assessment endpoints for the eight listed mussels include direct toxic effects on the survival, reproduction, and growth of the mussels, as well as indirect effects, such as reduction of the prey base, perturbation of host fish, and/or modification of its habitat. In addition, potential destruction and/or adverse modification of critical habitat is evaluated via the PCEs, which are components of the habitat areas that provide essential life cycle needs of the ovate clubshell and southern clubshell mussels. Each assessment endpoint

[3] From U.S. EPA (1992). *Framework for Ecological Risk Assessment.* EPA/630/R-92/001.

requires one or more "measures of ecological effect," which are defined as changes in the attributes of an assessment endpoint or changes in a surrogate entity or attribute in response to exposure to a pesticide. Specific measures of ecological effect are evaluated based on a variety of data sources including registrant-submitted studies and information from the open literature. Acute and chronic toxicity information from registrant-submitted guideline tests are required to be conducted on a limited number of organisms. Additional ecological effects data from the open literature, including effects data on aquatic freshwater microcosm and mesocosm data, were also considered. Acute atrazine effects data for freshwater mussels are available; however, chronic data for freshwater mussels are not. Therefore, chronic toxicity data for surrogate species are used to assess potential direct effects to the assessed mussels.

Measures of effect from microcosm and mesocosm data provide an expanded view of potential indirect effects of atrazine on aquatic organisms, their populations and communities in the laboratory, in simulated field situations, and in actual field situations. With respect to the microcosm and mesocosm data, threshold concentrations were determined from realistic and complex time variable atrazine exposure profiles (chemographs) for modeled aquatic community structure changes. Methods were developed to estimate ecological community responses for monitoring data sets of interest based on their relationship to micro- and mescocosm study results, and thus to determine whether a certain exposure profile within a particular use site and/or action area may have exceeded community-level threshold concentrations. Ecological modeling with the Comprehensive Aquatic Systems Model (CASM) (Bartell et al., 2000; Bartell et al., 1999; and DeAngelis et al., 1989) was used to integrate direct and indirect effects of atrazine to indicate changes to aquatic community structure and function.

A complete discussion of all the toxicity data available for this risk assessment, including use of CASM and associated aquatic community-level threshold concentrations, and the resulting measures of ecological effect selected for each taxonomic group of concern, is included in Section 4 of this document. A summary of the assessment endpoints and measures of ecological effect selected to characterize potential assessed mussel risks associated with exposure to atrazine are provided in Table 2.5.

Table 2.5 Summary of Assessment Endpoints and Measures of Ecological Effect for Eight Listed Mussels

Assessment Endpoint	Measures of Ecological Effect
1. Survival, growth, and reproduction of mussel individuals via direct effects	1a. Freshwater mussel LC_{50} 1b. Freshwater invertebrate NOAEC
2. Survival, growth, and reproduction of mussel individuals via indirect effects on food source (i.e., phytoplankton, zooplankton) or host fish (i.e., freshwater fish)	2a. Freshwater fish, invertebrate, and aquatic plant EC_{50} or LC_{50} 2b. Freshwater fish and invertebrate NOAEC 2c. Microcosm/mesocosm threshold concentrations showing aquatic primary productivity community-level effects
3. Survival, growth, and reproduction of mussel individuals via indirect effects on habitat and/or primary productivity (i.e., aquatic plant community)	3a. Vascular plant (duckweed) acute EC_{50} 3b. Non-vascular plant (freshwater algae) acute EC_{50}

	3c. Microcosm/mesocosm threshold concentrations showing aquatic primary productivity community-level effects
4. Survival, growth, and reproduction of mussel individuals via indirect effects on terrestrial vegetation (riparian habitat) required to maintain acceptable water quality and habitat	4a. Monocot and dicot seedling emergence EC_{25} 4b. Monocot and dicot vegetative vigor EC_{25}

Assessment endpoints and measures of ecological effect selected to characterize potential designated critical habitat modification associated with exposure to atrazine are provided in Table 2.6. As previously discussed, the basis of the designated critical habitat analysis is protection of the PCEs identified for the designated critical habitat of the ovate and southern clubshell mussels. PCEs that are identified as assessment endpoints are limited to those that are of a biological nature (i.e., the biological resource requirements for the listed species associated with the critical habitat) and those PCEs for which atrazine effects data are available. Therefore, abiotic PCEs, such as flow regime, pH, and hardness are not evaluated because there is no perceived link between the biotic assessment endpoints and the abiotic PCEs (i.e., atrazine in surface water is unlikely to impact flow, pH, and hardness levels). In addition, the PCE related to the presence of competitive or predacious nonnative species is also not evaluated because there is no ecotoxicity data to differentiate native versus non-native species sensitivity to atrazine.

Table 2.6 Summary of Assessment Endpoints and Measures of Ecological Effect for Primary Constituent Elements of Designated Critical Habitat[a]

Assessment Endpoint	Measures of Ecological Effect
1. Geomorphically stable stream and river channels and banks	1a. Monocot and dicot seedling emergence EC_{25} 1b. Monocot and dicot vegetative vigor EC_{25}
2. Flow regime necessary for normal behavior, growth, and survival of all life stages of mussels and their fish hosts in the river environment	2a. Not evaluated because there is no perceived link between the risk assessment biotic endpoints and abiotic PCE
3. Water quality, including temperature, turbidity, and oxygen content[1]	3a. Monocot and dicot seedling emergence EC_{25} 3b. Monocot and dicot vegetative vigor EC_{25} 3c. Vascular and non-vascular plant (freshwater algae) acute EC_{50} 3d. Microcosm/mesocosm threshold concentrations showing aquatic primary productivity community-level effects
4. Sand, gravel, and/or cobble substrates with low to moderate amounts of attached filamentous algae and sedimentation	4a. Monocot and dicot seedling emergence EC_{25} 4b. Monocot and dicot vegetative vigor EC_{25} 4c. Non-vascular plant (freshwater algae) acute EC_{50} 4d. Microcosm/mesocosm threshold concentrations showing aquatic primary productivity community-level effects
5. Fish hosts with adequate living, foraging, and spawning areas	5a. Freshwater fish, invertebrate, and aquatic plant EC_{50} or LC_{50} 5b. Freshwater fish and invertebrate NOAEC 5c. Monocot and dicot seedling emergence EC_{25} 5d. Monocot and dicot vegetative vigor EC_{25} 5e. Microcosm/mesocosm threshold concentrations showing aquatic primary productivity community-

	level effects
6. Few or no competitive or predacious nonnative species present	6a. Not evaluated because there is no ecotoxicity data to differentiate native versus non-native species sensitivity to atrazine
7. Chemical characteristics necessary for normal behavior, growth, and viability of all life stages of mussels	7a. Freshwater mussel LC_{50} 7b. Freshwater invertebrate NOAEC 7c. Zooplankton and aquatic plant EC_{50} or LC_{50} 7d. Zooplankton NOAEC 7e. Microcosm/mesocosm threshold concentrations showing aquatic primary productivity community-level effects
[a] Water quality parameters including pH and hardness are also included in this PCE; however these components of water quality are not evaluated because there is no perceived link between the risk assessment biotic endpoints and water pH and hardness.	

2.9 Conceptual Model

2.9.1 Risk Hypotheses

Risk hypotheses are specific assumptions about potential adverse effects (i.e., changes in assessment endpoints) and may be based on theory and logic, empirical data, mathematical models, or probability models (U.S. EPA, 1998). For this assessment, the risk is stressor-linked, where the stressor is the release of atrazine to the environment. Based on the results of the 2003 atrazine IRED (U.S. EPA, 2003a), the following risk hypotheses are presumed for this endangered species risk assessment:

- Atrazine in surface water and/or runoff/drift from treated areas within the action area may directly affect one or more of the assessed mussel species by causing mortality or adversely affecting growth or fecundity;
- Atrazine in surface water and/or runoff/drift from treated areas within the action area may indirectly affect one or more of the assessed mussel species by reducing or changing the composition of food supply and/or perturbing fish hosts required for the parasitic glochidial life stage of the assessed mussels;
- Atrazine in surface water and/or runoff/drift from treated areas within the action area may indirectly affect one or more of the assessed mussels by reducing or changing the composition of the aquatic plant community in the rivers and streams comprising the species' current range, thus affecting primary productivity and/or cover;
- Atrazine in surface water and/or runoff/drift from treated areas within the action area may indirectly affect one or more of the assessed mussels by reducing or changing the composition of the terrestrial plant community (i.e., riparian habitat) required to maintain acceptable water quality and habitat in the rivers and streams comprising the species' current range;
- Atrazine in surface water and/or runoff/drift from treated areas within the action area may adversely modify the designated critical habitat of the ovate and southern clubshell mussels by altering the geomorphically stable streams and river channels and banks;
- Atrazine in surface water and/or runoff/drift from treated areas within the action area may adversely modify the designated critical habitat of the ovate and southern

clubshell mussels by impacting water quality, including temperature, turbidity, and oxygen content;

- Atrazine in surface water and/or runoff/drift from treated areas within the action area may adversely modify the designated critical habitat of the ovate and southern clubshell mussels by altering adequate living, foraging, and spawning areas for host fish;
- Atrazine in surface water and/or runoff/drift from treated areas within the action area may adversely modify the designated critical habitat of the ovate and southern clubshell mussels by altering sand, gravel, and/or cobble substrates with low to moderate amounts of attached filamentous algae and sedimentation; and
- Atrazine in surface water and/or runoff/drift from treated areas within the action area may adversely modify the designated critical habitat of the ovate and southern clubshell mussels by altering chemical characteristics necessary for normal behavior, growth, and viability of all life stages.

2.9.2 Diagram

The conceptual model is a graphic representation of the structure of the risk assessment. It specifies the stressor (atrazine), release mechanisms, abiotic receiving media, biological receptor types, and effects endpoints of potential concern. The conceptual models for the atrazine endangered species risk assessment for the eight listed mussel species and designated critical habitat for the ovate and southern clubshell mussels are shown in Figures 2.6 and 2.7, respectively. Exposure routes shown in dashed lines are not quantitatively considered because the resulting exposures are expected to be so low as not to cause adverse effects to the assessed mussel species and/or designated critical habitat.

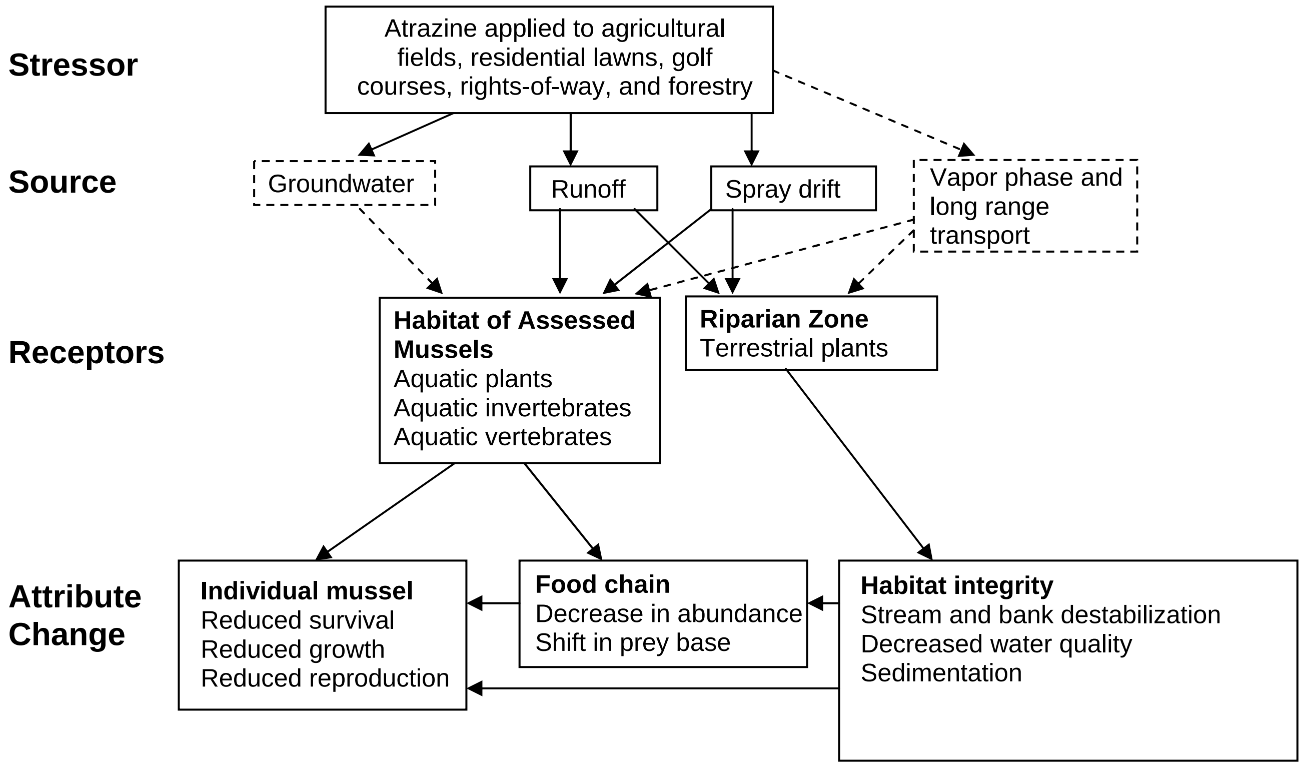

Figure 2.6 Conceptual Model for Eight Assessed Mussel Species

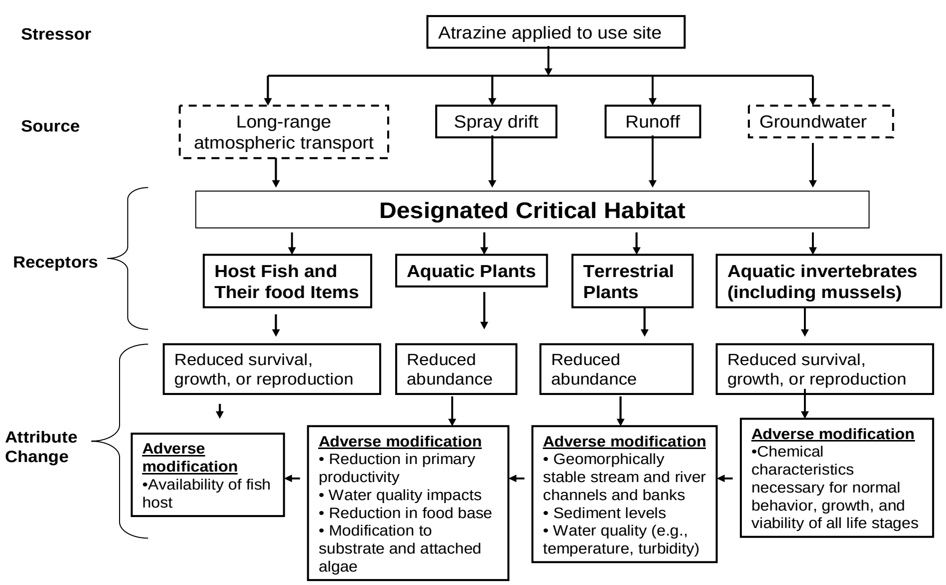

Figure 2.7 Conceptual Model for Designated Critical Habitat Impact Analysis

The conceptual models provide an overview of the expected exposure routes for the assessed mussels and their designated critical habitat within the atrazine action area previously described in Section 2.7. In addition to the mussel species included in this assessment, other aquatic receptors that may be potentially exposed to atrazine include freshwater fish, invertebrates and aquatic plants. Designated critical habitat may also be adversely modified based on alteration of the PCEs, which are those habitat components that support feeding, sheltering, reproduction of the assessed listed mussels.. For freshwater vertebrate and invertebrate species, including the assessed mussels, the major routes of exposure are considered to be via the respiratory surface (gills) or the integument. Direct uptake and adsorption are the major routes of exposure for aquatic plants. Direct effects to freshwater invertebrates and aquatic plants resulting from exposure to atrazine may indirectly affect the assessed mussels and/or adversely modify their designated critical habitat via reduction and/or alteration in food and habitat (i.e., substrate, water quality including oxygen content) availability necessary for normal behavior, growth, and viability of all life stages. The available data indicate that atrazine is not likely to bioconcentrate in aquatic food items, with fish bioconcentration factors (BCFs) ranging from 2 to 8.5 (U.S. EPA, 2003c). Therefore, bioconcentration of atrazine in mussels or in host fish via the diet was not considered as a significant route of exposure.

In addition to aquatic receptors, terrestrial plants may also be exposed to spray drift and runoff from atrazine use in the vicinity of the rivers and streams that comprise the mussel species' current range and designated critical habitat for the ovate and southern clubshell mussels. Detrimental changes in the riparian vegetation adjacent to the mussel's current habitat and designated critical habitat may cause adverse effects to water quality (i.e., temperature and turbidity), stream bank stability, substrate composition, sediment loading, and spawning habitat for host fish. Specifically, changes in the riparian vegetation adjacent to the habitat of the assessed mussels may adversely affect mussel feeding and respiratory efficiency, growth rates, and burrowing activity, and cause increased substrate instability and potential physical smothering via increased sedimentation (Ellis, 1936; Stansbery, 1971; Markings and Bills, 1979; Kat, 1982; Vannote and Minshall, 1982; Aldridge et al., 1987; and Waters, 1995).

The source and mechanism of release of atrazine into surface water are ground application via foliar spray and coated fertilizer granules for agricultural (i.e., corn, sorghum, and fallow/idle land) and non-agricultural uses (i.e., golf courses, residential lawns, rights-of-way, and forestry). Surface water runoff from the areas of atrazine application is assumed to follow topography, resulting in direct runoff to the rivers and streams within the action area. Spray drift and runoff of atrazine may also affect the foliage and seedlings of terrestrial plants that comprise the riparian habitat that may be adjacent to the mussel's habitat including designated critical habitat. Additional release mechanisms include spray drift and atmospheric transport via volatilization, which may potentially transport site-related contaminants to the surrounding air. Atmospheric transport is not considered as a significant route of exposure for this assessment because the magnitude of documented exposures in rainfall are at or below available surface water and monitoring data, as well as modeled estimates of exposure. In addition, modeling tools are not available to predict the potential impact of long-range atmospheric transport of atrazine.

3. Exposure Assessment

3.1 Label Application Rates and Intervals

Atrazine labels may be categorized into two types: labels for manufacturing uses (including technical grade atrazine and its formulated products) and end-use products. Technical products, which contain atrazine of high purity, are not used directly in the environment, but instead are used to make formulated products, which can be applied in specific areas to control weeds. The formulated product labels legally limit atrazine's potential use to only those sites that are specified on the labels.

In the January and October 2003 IREDs (U.S. EPA, 2003a and b), EPA stipulated numerous changes to the use of atrazine including label restrictions and other mitigation measures designed to reduce risk to human health and the environment. Specifically pertinent to this assessment, are provisions of a Memorandum of Agreement (MOA) between the Agency and atrazine registrants. In the MOA, the Agency stipulated that certain label changes must be implemented on all manufacturing-use product labels for atrazine and on all end-use product labels for atrazine prior to the 2005 growing season. These label changes include cancellation of certain uses, reduction in application rates, and requirements for harmonization across labels including setbacks from waterways. Specifically, the label changes prohibit atrazine use within 50 feet of sinkholes, 66 feet of intermittent and perennial streams, and 200 feet of lakes and reservoirs.

While these setbacks were required to reduce atrazine deposition to water bodies as a result of spray drift, it is expected that they will also result in a reduction in loading due to runoff across the setback zone; however, current models do not address this reduction quantitatively. Therefore, these restrictions are not quantitatively evaluated in this assessment. A qualitative discussion of the potential impact of these setbacks on estimated environmental concentrations of atrazine for the assessed mussels is discussed further in Section 3.2.3. Table 3.1 provides a summary of label application rates for atrazine uses evaluated in this assessment.

Currently registered non-agricultural uses of atrazine within the action area include residential areas such as playgrounds and home lawns, turf (golf courses and recreational fields), rights-of-way, and forestry. Agricultural uses within the action area include corn, sorghum, and fallow/idle land[4]. Other agricultural uses (macadamia nut, guava, and sugarcane) are not present in the action area.

Atrazine is formulated as liquid, wettable powder, dry flowable, and granular formulations. Application equipment for the agricultural uses includes ground application (the most common application method), aerial application, band

[4] Fallow or idle land is defined by the Agency as arable land not under rotation that is set at rest for a period of time ranging from one to five years before it is cultivated again, or land usually under permanent crops, meadows or pastures, which is not being used for that purpose for a period of at least one year. Arable land, which is normally used for the cultivation of temporary crops, but which is temporarily used for grazing, is also included.

treatment, incorporated treatment, various sprayers (low-volume, hand held, directed), and spreaders for granular applications. Risks from ground boom and aerial applications are considered in this assessment because they are expected to result in the highest off-target levels of atrazine due to generally higher spray drift levels. Ground boom and aerial modes of application tend to use lower volumes applied in finer sprays than applications coincident with sprayers and spreaders, and thus have a higher potential for off-target movement via spray drift.

Table 3.1 Atrazine Label Application Information for the Endangered Mussels Assessment[a]

Scenario	Maximum Application Rate (lbs/acre)	Maximum Number of Applications	Formulation	Method of Application	Interval Between Applications
Forestry	4.0	1	Liquid	Aerial and Ground	NA
Residential	2.0	2	Granular	Ground	30 days
Residential	1.0	2	Liquid	Ground	30 days
Rights-of-Way	1.0	1	Liquid	Ground	NA
Fallow/ Idle land	2.25	1	Liquid	Ground and Aerial	NA
Corn	2.5[b]	2	Liquid	Ground and Aerial	NA
Sorghum	2.0	1	Liquid	Ground and Aerial	NA
Turf	2.0	2	Granular	Ground	30 days
Turf	1.0	2	Liquid	Ground	30 days

[a] Based on 2003 IRED and Label Change Summary Table memorandum dated June 12, 2006 (U.S. EPA, 2006b).
[b] 2.5 lbs/A is a seasonal maximum limit for corn. The single application maximum is 2.0 lbs/acre. Modeling conducted using a single application at 2.0 lbs/acre but adjusted to account for percent increase expected due to second application.

3.2 Aquatic Exposure Assessment

As discussed in Section 2.5 and Appendix C, the eight listed mussels considered in this assessment reside principally in streams and rivers of the Mississippi River Valley, Missouri, central Appalachian Mountains, and Mobile River Basin. The action area includes the entire watershed of streams and rivers in the areas defined above and are presented graphically in Figure 2.5. In general, the assessed mussels appear to reside in a variety of stream types from second and third order streams to larger fourth and fifth order water bodies in the main stem of major rivers, such as the Lower Ohio, Alabama, and Tombigbee Rivers. As such, the range of hydrologic conditions represents a wide range of moderate to strong flow regimes where the species currently reside. Further discussion of the assumed flow regimes may be found in Section 2.5 and Appendix C, which details the life history information for the mussels included in this assessment.

3.2.1 Introduction

The assessment of exposure within the action area is dependent upon a combination of modeling and monitoring data. In accordance with the Overview Document (U.S. EPA, 2004), screening-level exposures were based on modeling which assumes a static water body. Available monitoring data for atrazine, as well as refined flow-adjusted modeling (adjusted based on flow data from streams where the listed mussels occur), were used to refine the screening-level modeled exposures.

For this assessment, screening-level modeling using a static water body indicates long-term (e.g. 30-day average) exposure concentrations significantly higher than concentrations seen in monitoring data. Refined modeling based on flowing water suggests that concentrations in flowing water are significantly lower than screening-level EECs. Although monitoring targeted to the upper 20th percentile vulnerable watersheds (based on WARP modeling[5]) indicates that, under certain conditions, long-term atrazine concentrations can be higher than those estimated by flow-adjusted modeling, monitored concentrations were similar to, or lower than the flow-adjusted modeling in 60% to 75% of samples. Most of the targeted monitoring sites with concentrations higher than the refined flow-adjusted modeling are within a factor of 2 to 3 times of the refined modeling and represent low flow streams.

Considering that the targeted monitoring data were collected from watersheds representing the upper 20th percentile vulnerable watersheds, it is not unexpected that in some cases the results show atrazine concentrations are higher than predicted by the refined modeling.

Available non-targeted monitoring data (i.e., monitoring data in which the study design was not specifically targeted to detect atrazine in high use areas) suggest a similar pattern of exposure as the targeted data; however, many of these sites are located in the most vulnerable areas represented by the targeted data. Given that these targeted sites

[5] Watershed Regression of Pesticides model (USGS 2005) at http://pubs.usgs.gov/circ/2005/1291/

represent the most vulnerable water bodies out of all atrazine use sites, are generally low order streams (2nd and 3rd order by the Strahler system) with flow rates less then the 5th percentile for all streams occupied by the listed mussels, it is unlikely that exposures outside the vulnerable watersheds are higher than the refined exposures based on flow-adjusted modeling.

The methods used to derive screening-level and refined estimated environmental concentrations (EECs) for use in this endangered species risk assessment are summarized in Table 3.2. As summarized below, screening-level EECs based on the PRZM/EXAMS static water body are used in the risk estimation to derive initial RQs and distinguish between "no effect" and may affect" determinations. Refined EECs are used to characterize exposure in the risk description for listed mussels and designated critical habitat based on a combination of flow-adjusted EECs and available monitoring data. Targeted monitoring data are used to refine exposure within the vulnerable watersheds, whereas flow-adjusted modeled EECs and non-targeted monitoring data are used to refine exposures for watersheds located outside of the boundary of these most vulnerable watersheds. These refined exposure estimates are used to distinguish whether each of the mussels and the critical habitat, is likely or not likely to be adversely affected by the action.

Based on the analysis described in Section 3.2.6.1, the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels inhabit streams that are at least partially located within the boundary of the vulnerable watersheds. The other assessed listed species, including the shiny pigtoe, heavy pigtoe, ovate clubshell and southern clubshell mussels, as well as all designated critical habitat for the ovate and southern clubshell mussels, are located outside of the boundary of the vulnerable watersheds.

Table 3.2 Methodology for EEC Derivation and Use in Risk Assessment		
EEC	**Use**	**Comment**
Screening-Level: PRZM/EXAMS Static Water Body EECs	Risk Estimation: Calculation of initial RQs used to distinguish "no effect" vs. "may effect" determinations	Peak and longer term screening-level EECs are essentially equivalent (highest peak and annual average EECs = 103 µg/L). Therefore, alternative modeling and monitoring data are used to refine longer-term EECs (see below).
Refined: PRZM/EXAMS Flow-Adjusted Index Reservoir EECs Non-Targeted Monitoring Data (Sections 3.2.6.2. to 3.2.6.4)[a]	Risk Description: Refinement of longer-term EECs for shiny pigtoe, heavy pigtoe, ovate clubshell, southern clubshell mussels and designated critical habitat located outside of the boundary of the upper 20th percentile vulnerability watersheds (hereafter called "vulnerable") identified using WARP. Refined longer-term EECs used to distinguish "LAA" vs. "NLAA" determinations for listed mussels and designated critical habitat occurring in watersheds outside of the boundary of "vulnerable" watersheds. Non-targeted monitoring data[a] were used to support PRZM/EXAMS Flow-Adjusted Index Reservoir EECs.	Alternative modeling data suggests that screening-level EECs >14-days are reduced by >70% by incorporating flow. Non-targeted monitoring data[a] discussed in Sections 3.2.6.2 through 3.2.6.4 also suggest that screening-level EECs over-predict long-term exposures. Annual time weighted mean concentrations from NAWQA (Section 3.2.6.2) are reduced by approximately 70% – 99% compared to their respective peak values. Only data from the Heidelberg college data set (Section 3.2.6.4) allow for derivation of longer-term average concentrations. 14-, 30-, 60-, and 90-day EECs from the non-targeted Heidelberg monitoring data approximately 2-fold higher than the PRZM/EXAMS flow-adjusted EECs of equivalent duration; however, use of either data would result in equivalent risk conclusions as discussed in Section 5.2.
Refined: Targeted Monitoring Data (Section 3.2.6.1)	Risk Description: Refinement of peak and longer-term EECs for the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels located in the "vulnerable" watersheds identified using WARP. Refined EECs used to distinguish "LAA" vs. "NLAA" determinations for listed mussels occurring in vulnerable watersheds.	Targeted monitoring data from "vulnerable" watersheds suggest that EECs in these watersheds could be considerably higher than in less vulnerable watersheds. Longer term (14 to 90 days) EECs from targeted monitoring watersheds are approximately 2 to 3 times higher than the flow-adjusted PRZM/EXAMS EECs.
[a] Non-targeted in this case refers to monitoring data in which the study design was not specifically targeted to detect atrazine in high use areas. However, some non-targeted study sites are located in highly vulnerable watersheds and correlated with high atrazine use.		

More detail on the standard modeling, refined modeling, monitoring data evaluation, and characterization of exposure is presented in the following sections.

3.2.2 Modeling Approach

Risk quotients (RQs) were initially based on EECs derived using the Pesticide Root Zone Model/Exposure Analysis Modeling System (PRZM/EXAMS) standard ecological pond scenario according to the methodology specified in the Overview Document (U.S. EPA, 2004). While peak concentrations predicted with the static water body are generally two times lower than monitored values, longer-term EECs likely overestimate exposure based on modeling with the static water body. Further, all of the assessed mussel species reside in flowing waters. Therefore, additional modeling (adjusted for flow) (Section 3.2.5.1) together with available monitoring data (Section 3.2.6) is used to characterize and refine potential exposures of the assessed mussels. Where LOCs for direct/indirect effects and/or adverse habitat modification are exceeded based on the modeled EEC using the static water body (i.e., “may affect”), the refined modeling and available monitoring data were used to differentiate “may affect, but not likely to adversely affect” from “may affect and likely to adversely affect” determinations for the assessed listed mussels and designated critical habitat.

The general conceptual model of exposure for this assessment is that the highest exposures are expected to occur in the headwater streams adjacent to agricultural fields and other non-agricultural use sites (residential, right-of-way, turf, and forestry). Many of the streams and rivers within the action area defined for this assessment are in close proximity to both agricultural and non-agricultural uses sites (Figures 2.2 and 2.3).   The action area was divided into representative regions and modeling scenarios were selected to represent each area. These areas (described in more detail in Section 3.2.3) represent the northern tier of the action area (eastern Illinois, Indiana, Kentucky, western Pennsylvania, and Ohio), the western tier (Arkansas, Missouri, and eastern Nebraska), the southern tier (Mississippi, Alabama, Georgia, and western Tennessee), and the eastern tier (West Virginia, southwestern Virginia, western North Carolina, and eastern Tennessee) (Figure 3.1). A summary of the distribution of the assessed mussels, including known occurrences and designated critical habitat, within the four geographical regions of the action area is provided in Table 3.3.

Table 3.3 Regional Distribution of the Assessed Mussels[a]				
Assessed Species	Region			
	North	East	South	West
Pink pearly mucket	X	X	X	X
Rough pigtoe	X	X	X	--
Shiny pigtoe	X	X	X	--
Fine-rayed pigtoe	X	X	X	--
Heavy pigtoe	--	--	X	--
Ovate clubshell	--	--	X	--
Southern clubshell	--	--	X	--
Stirrupshell[b]	--	--	--	--

[a] The distribution of the assessed mussels includes known occurrences and designated critical habitat.
[b] The stirrupshell mussel is presumed to be extinct (Hartfield, 2006).

Figure 3.1 Regionalization of Mussel Action Area

Available usage data (Kaul, et al., 2005) suggest that the heaviest usage of atrazine relative to the action area is likely to be in Illinois with decreasing intensity south and east of this area. As noted above, the action area was segmented into regions to allow for modeling that covers the expected range of runoff vulnerability. All existing PRZM scenarios were evaluated, and a subset was selected for use in this assessment. The scenarios were selected to provide a spatial context to predicted exposures.

Currently a suite of 63 PRZM standard scenarios and 7 Barton Springs scenarios (recently developed for use in the Barton Springs salamander endangered species risk assessment (U.S. EPA, 2006c), are available for use in ecological risk assessments representing predominantly agricultural uses. Each scenario is intended to represent a high-end exposure setting for a particular crop. Each scenario location is selected based on various factors including crop acreage, runoff and erosion potential, climate, and agronomic practices. Once a location is selected, a scenario is developed using locally specific soil, climatic, and agronomic data. Each PRZM scenario is assigned a specific climatic weather station providing 30 years of daily weather values.

Specific scenarios were selected for use in this assessment using two criteria. First, an evaluation of all available PRZM scenarios was conducted, and those scenarios that represent atrazine uses (e.g. Ohio corn) were selected for modeling. Weather information was assigned to these scenarios at development. Second, an additional suite of scenarios was identified to represent both agricultural and non-agricultural uses for which scenarios within the action area is not available (e.g. Barton Springs residential). These scenarios were used in the assessment as surrogates for atrazine uses without current scenarios (e.g. Oregon Christmas tree as surrogate for forestry) and to provide geographic coverage where no current scenario exists (e.g. Ohio corn scenario modeled using Springfield, Missouri weather data).

Each scenario selected as a surrogate for this assessment is considered to be a conservative representation of exposure in the action area because the surrogate scenarios (Oregon Christmas tree and Kansas sorghum) were developed using a hydrologic group C soil with relatively high curve numbers and moderate slopes. These are the most important parameters within a PRZM scenario for generating runoff coupled with rainfall, which is higher within the action area than the areas where the scenarios were originally developed. In addition, the curve numbers and slopes are expected to be higher than those present in the action area, which generally have lower slopes and less runoff prone soils.

Further description (metadata) and copies of the existing PRZM scenarios may be found at the following websites.

http://www.epa.gov/oppefed1/models/water/index.htm#przmexamsshell

http://www.epa.gov/oppefed1/models/water/przmenvironmentdisclaim.htm

For this assessment, available PRZM weather stations were associated with watersheds highly vulnerable to atrazine runoff. As shown in Figure 3.2**,** weather stations associated with Indianapolis and Evansville, Indiana represent highly vulnerable locations. As such, surrogate scenarios used to model this region were run using weather data from these locations to represent exposures within the entire region.

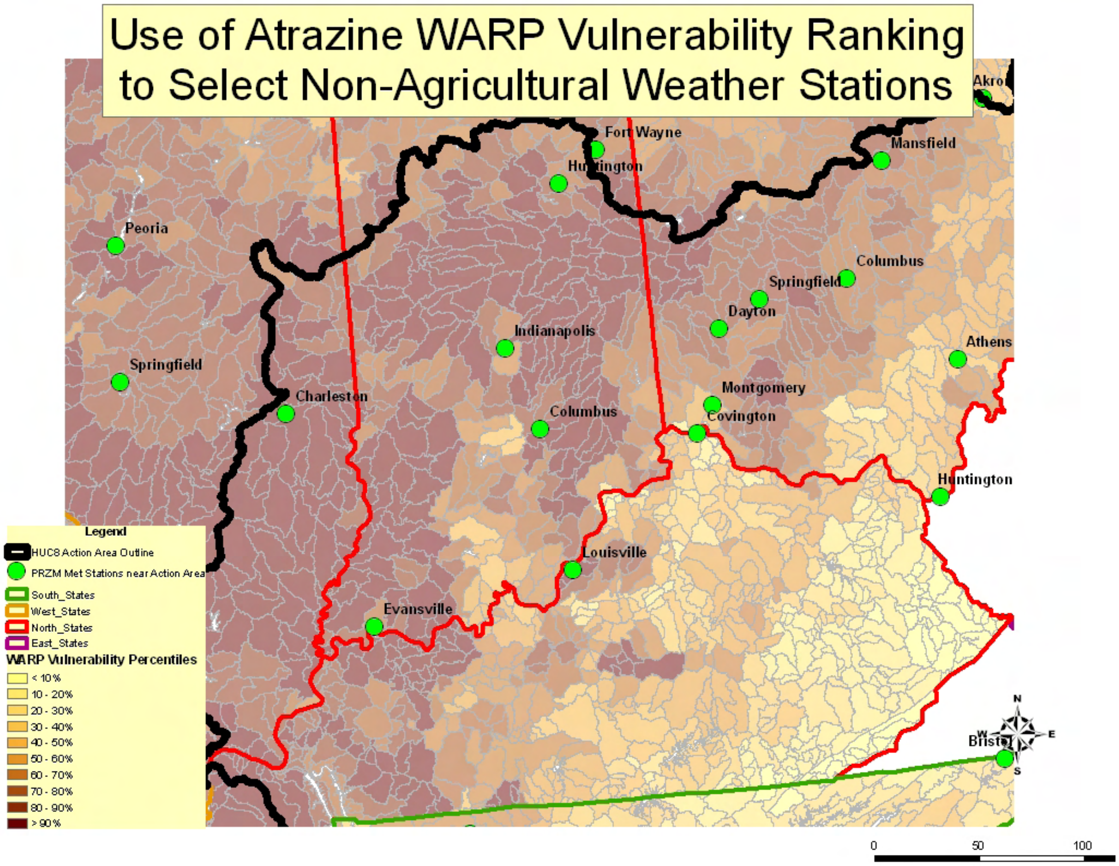

Figure 3.2 WARP Vulnerability Ranking for Atrazine Relative to Weather Stations

For this assessment, the following corn scenarios were modeled to represent all the various regions of the action area: Illinois and Ohio (these are standard scenarios using weather data from Peoria and Dayton, respectively) scenarios are representative of the northern tier states; Mississippi scenario using the weather data from Mobile, Alabama is representative of the southern tier states; Ohio scenario using weather data from Chattanooga, Tennessee is representative of the eastern tier states; and Ohio scenario using the Springfield, Missouri weather data is representative of the western states. The sorghum scenario was modeled with local weather stations including Topeka, Kansas (western states), Evansville, Indiana (northern states), Mobile, Alabama (southern states), and Chattanooga, Tennessee (eastern states).

Currently, the only non-agricultural scenarios available for use in aquatic exposure assessment are those developed specifically for the Barton Springs Salamander Endangered Species Risk Assessment (U.S. EPA, 2006c). For the Barton Springs assessment, a suite of non-agricultural scenarios was developed including a residential, impervious (to be used in tandem with the residential scenario), and rights-of-way scenarios. These scenarios were used in this assessment in a manner similar to the agricultural scenarios described above. Each scenario was modeled using a representative weather station for each region. For example, the residential scenario was modeled using the Mobile, Alabama weather data to represent exposures in the southern states, while the same scenario was modeled with the Chattanooga, Tennessee weather data, the Indianapolis, Indiana weather data, and the Springfield, Missouri weather data to represent the eastern, northern, and western states, respectively. Figure 3.3 shows the locations of these weather stations relative to the action area. A summary of all the modeled scenarios along with associated weather information is included in Table 3.4.

Both the agricultural and non-agricultural scenarios were used within the standard framework of PRZM/EXAMS modeling using the standard graphical user interface (GUI) shell, PE4v01.pl.

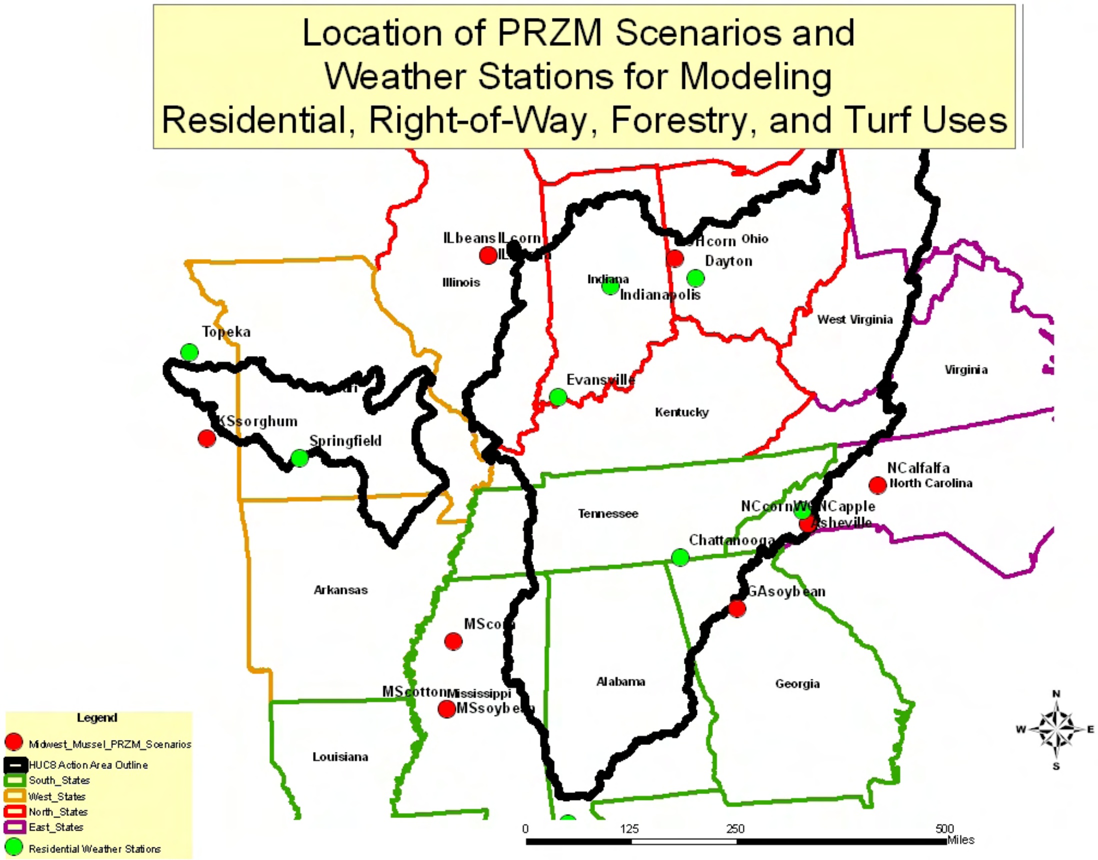

Figure 3.3 Location of Various Weather Stations Used to Model Non-Agricultural Uses (Residential, Right-of-Way, Turf, and Forestry)

Table 3.4 Summary of PRZM Scenarios				
Region	**Use**	**Scenario**	**First Application**	**Weather Station (WBAN #)**
South	Corn	MS corn	April 1	Mobile, AL (13894)
	Sorghum	KS sorghum	May 1	Mobile, AL (13894)
	Fallow	BSS meadow[a]	November 15	Mobile, AL (13894)
	Residential	BSS residential	April 1	Mobile, AL (13894)
	Right-of-way	BSS row	June 1	Mobile, AL (13894)
	Forestry	OR xmastree	June 1	Mobile, AL (13894)
	Turf	BSS turf	April 1	Mobile, AL (13894)
North	Corn	OH corn IL corn	April 15	Dayton, OH (93815) Moline, IL (14923)
	Sorghum	KS sorghum	May 1	Evansville, IN (93817)
	Fallow	BSS meadow	October 15	Evansville, IN (93817)
	Residential	BSS residential	May 1	Indianapolis, IN (93819)
	Right-of-way	BSS row	June 1	Indianapolis, IN (93819)
	Forestry	OR xmastree	June 1	Evansville, IN (93819)
	Turf	BSS turf	May 1	Indianapolis, IN (93819)
West	Corn	IL corn	April 15	Springfield, MO (13995)
	Sorghum	KS sorghum	May 1	Topeka, KS (13996)
	Fallow	BSS meadow	November 1	Springfield, MO (13995)
	Residential	BSS residential	April 15	Springfield, MO (13995)
	Right-of-way	BSS row	June 1	Springfield, MO (13995)
	Forestry	OR xmastree	June 1	Springfield, MO (13995)
	Turf	BSS turf	April 15	Springfield, MO (13995)
East	Corn	OH corn	April 1	Chattanooga, TN (13882)
	Sorghum	KS sorghum	May 1	Chattanooga, TN (13882)
	Fallow	BSS meadow	November 1	Chattanooga, TN (13882)

Table 3.4 Summary of PRZM Scenarios				
Region	**Use**	**Scenario**	**First Application**	**Weather Station (WBAN #)**
	Residential	BSS residential	May 1	Chattanooga, TN (13882)
	Right-of-way	BSS row	June 1	Chattanooga, TN (13882)
	Forestry	OR xmastree	June 1	Chattanooga, TN (13882)
	Turf	BSS turf	May 1	Chattanooga, TN (13882)

[a] BSS scenarios developed for Barton Springs Salamander (BSS) Endangered Species Risk Assessment (U.S. EPA, 2006c).

Peak concentrations, as well as rolling time-weighted averages of 14 days, 21 days, 30 days, 60 days, and 90 days were derived for comparison with the appropriate ecotoxicity endpoints (including the community-level threshold concentrations) for atrazine. The 30-year time series output file was used to recalculate the peak, 14-day, 21-day, 30-day, 60-day, and 90-day rolling averages at the 90th percentile. All model outputs were post-processed manually using Microsoft Excel to provide the equivalent of the standard one in ten year return frequency exposures, as predicted by PRZM/EXAMS. A sample of how this post-processing was conducted may be found in the previous atrazine assessments for the Chesapeake Bay and Alabama Sturgeon (EPA, 2006c, EPA 2006d, and EPA 2006e).

Additional information on the modeling approach for the non-agricultural residential, rights-of-way, and forestry use scenarios may be found in the previous atrazine endangered species risk assessments (U.S. EPA, 2006c, d, and e).

3.2.3 Model Inputs

The estimated water concentrations from surface water sources were calculated using Tier II PRZM (Pesticide Root Zone Model) and EXAMS (Exposure Analysis Modeling System). PRZM is used to simulate pesticide transport as a result of runoff and erosion from a standardized watershed, and EXAMS estimates environmental fate and transport of pesticides in surface waters. The linkage program shell (PE4v01.pl) that incorporates the site-specific scenarios was used to run these models.

Scenarios used in this assessment consist of agricultural scenarios for corn and sorghum developed previously for other geographic areas. Scenarios developed for the Barton Springs Salamander assessment (U.S. EPA, 2006c) not specific to watersheds included in the action area, are used in this assessment for one agricultural use (fallow/idle land) and several non-agricultural uses (residential, turf, forestry, and rights-or-way). All scenarios were modeled using local weather data as described above. Linked use site-specific scenarios and meteorological data were used to estimate exposure as a result of specific use for each modeling scenario. The PRZM/EXAMS model was used to calculate concentrations using the standard ecological water body scenario in EXAMS. Weather and agricultural practices were simulated over 30 years so that the 1 in 10 year exceedance probability at the site was estimated for the standard ecological water body.

of drift reaching a surface water body. The resulting spray drift percentages, which are incorporated into the PRZM/EXAMS modeling, are 0.6% for ground applications and 6.5% for aerial applications.

Models to estimate the effect of setbacks on load reduction for runoff are not currently available. It is well documented that vegetated setbacks can result in a substantial reduction in pesticide load to surface water (USDA, NRCS, 2000). Specifically for atrazine, data reported in the USDA study indicate that well vegetated setbacks have been documented to reduce atrazine loading to surface water by as little as 11% and as much as 100% of total runoff compared to the loading without a setback. It is expected that the presence of a well-vegetated setback between the site of atrazine application and receiving water bodies could result in reduction in loading. Therefore, the aquatic EECs presented in this assessment are likely to over-estimate exposure in areas with well-vegetated setbacks. While the extent of load reduction cannot be accurately predicted through each relevant stream reach in the action area, data from USDA (USDA, 2000) suggest reductions could range from 11 to 100%.

The date of first application was developed based on several sources of information including data provided by BEAD and Crop Profiles maintained by the USDA. More detail on the crop profiles and the previous assessments may be found at:

http://pestdata.ncsu.edu/cropprofiles/cropprofiles.cfm

The appropriate PRZM input parameters were selected from the environmental fate data submitted by the registrant and in accordance with US EPA-OPP EFED water model parameter selection guidelines, Guidance for Selecting Input Parameters in Modeling the Environmental Fate and Transport of Pesticides, Version 2.3, February 28, 2002. These parameters are consistent with those used in both the 2003 IRED (U.S. EPA, 2003a) and the cumulative triazine risk assessment (U.S. EPA, 2006a) and are summarized in Table 3.5. More detail on these assessments may be found at:

http://www.epa.gov/oppsrrd1/REDs/atrazine_ired.pdf

http://www.epa.gov/pesticides/cumulative/common_mech_groups.htm#chloro

Table 3.5 Summary of PRZM/EZAMS Environmental Fate Data Used for Aquatic Exposure Inputs for Atrazine Endangered Mussels Species Assessment		
Fate Property	**Value**	**MRID[a] (or source)**
Molecular Weight	215.7	MRID 41379803
Henry's constant	2.58 x10 -9	MRID 41379803
Vapor Pressure	3 x 10 -7	MRID 41379803
Solubility in Water	33 mg/l	MRID 41379803
Photolysis in Water	335 days	MRID 42089904
Aerobic Soil Metabolism Half-lives	152 days	MRID 40431301 MRID 40629303 MRID 42089906
Hydrolysis	stable	MRID 40431319
Aerobic Aquatic Metabolism (water column)	304 days	2x aerobic soil metabolism rate constant
Anaerobic Aquatic Metabolism (benthic)	608 days	MRID 40431323
Koc	88.78 ml/g	MRID 40431324 MRID 41257901 MRID 41257902 MRID 41257904 MRID 41257905 MRID 41257906
Application Efficiency	95 % for aerial 99 % for ground	Default value[c]
Spray Drift Fraction[b]	6.5 % for aerial 0.6 % for ground	AgDrift adjusted values based on label restrictions

[a] Master Record Identification (MRID) is record tracking system used within OPP to manage data submissions to the Agency. Each data submission if given a unique MRID number for tracking purposes.
[b] Spray drift not included in final EEC due to edge-of-field estimation approach.
[c] Inputs determined in accordance with EFED "*Guidance for Chemistry and Management Practice Input Parameters for Use in Modeling the Environmental Fate and Transport of Pesticides*" dated February 28, 2002.

3.2.4 Results

As noted above, a total of eight scenarios were evaluated in this assessment. Of these, four were developed as part of the Barton Springs salamander endangered species risk assessment (U.S. EPA, 2006c). Two of the Barton Springs scenarios (residential and rights-of-way) were used in tandem with an impervious scenario, while two (fallow/idle land and turf) are standard PRZM/EXAMS scenarios. The remaining three scenarios (corn, sorghum, and Christmas trees as surrogate for forestry) were taken from existing scenarios developed for other regions of the United States and modeled using local weather data. No new scenarios were developed specifically for this assessment. The results of the modeling are summarized in Table 3.6. An example of the modeling approach and the model input files may be found in Appendix D of the previous endangered species risk assessments for atrazine (EPA, 2006c, EPA 2006d, and EPA 2006e).

In general, these EECs show a pattern of exposure for all durations that is influenced by the persistence of the compound and the lack of flow through the static water body. Predicted atrazine concentrations, though high across durations of exposure for a single year, do not increase across the 30-year time series; therefore accumulation is not a concern.

Table 3.6 Summary of PRZM/EXAMS Output Screening-Level EECs for all Modeled Scenarios (Using the Standard Water Body)									
Region	**Use Site**	**Application Rate (lbs/acre)**	**No. of Applications**	**90th Percentile of 30 Years of Output**					
				Peak EEC (µg/L)	**14-day EEC (µg/L)**	**21-day EEC (µg/L)**	**30-day EEC (µg/L)**	**60-day EEC (µg/L)**	**90-day EEC (µg/L)**
South	Corn[a]	2.0	2	109.4	108.1	107.4	107.2	104.8	101.7
South	Sorghum	2.0	1	63.6	62.9	62.4	61.7	59.6	57.4
South	Fallow	2.25	1	58.8	58.2	58.0	57.6	56.6	55.6
South	Residential[b] Granular	2.0	2	19.9	19.6	19.4	19.2	18.6	17.9
South	Residential[b] Liquid	1.0	2	14.6	14.4	14.2	14.1	13.7	13.4
South	Rights-of-way	1.0	1	2.4	2.4	2.4	2.4	2.3	2.2
South	Forestry	4.0	1	46.1	45.2	44.7	44.1	42.2	40.8
South	Turf Granular	2.0	2	17.9	17.7	17.7	17.7	17.6	17.1
South	Turf Liquid	1.0	2	14.8	14.6	14.4	14.3	13.7	13.1
North	Corn[a]	2.0	2	84.5	84.1	83.7	83.3	81.6	79.9
North	Sorghum	2.0	1	58.4	57.7	57.4	56.9	54.9	52.8
North	Fallow	2.25	1	51.6	51.5	51.5	51.5	51.0	50.4
North	Residential[b] Granular	2.0	2	9.9	9.9	9.9	9.9	9.9	9.9
North	Residential[b] Liquid	1.0	2	7.6	7.5	7.5	7.5	7.5	7.4
North	Rights-of-way	1.0	1	2.7	2.7	2.7	2.6	2.6	2.5

Table 3.6 Summary of PRZM/EXAMS Output Screening-Level EECs for all Modeled Scenarios (Using the Standard Water Body)									
Region	Use Site	Application Rate (lbs/acre)	No. of Applications	90th Percentile of 30 Years of Output					
				Peak EEC (µg/L)	14-day EEC (µg/L)	21-day EEC (µg/L)	30-day EEC (µg/L)	60-day EEC (µg/L)	90-day EEC (µg/L)
North	Forestry	4.0	1	48.5	47.7	47.2	46.7	44.9	43.3
North	Turf Granular	2.0	2	7.1	7.1	7.1	7.1	7.1	7.1
North	Turf Liquid	1.0	2	6.6	6.6	6.6	6.6	6.5	6.5
West	Corn[a]	2.0	2	80.9	79.8	79.3	78.5	76.2	73.7
West	Sorghum	2.0	1	60.1	59.4	58.9	58.4	57.3	56.3
West	Fallow	2.25	1	103.4	103.1	103.1	103.1	103.0	103.0
West	Residential[b] Granular	2.0	2	11.9	11.8	11.7	11.6	11.3	11.0
West	Residential[b] Liquid	1.0	2	9.9	9.7	9.7	9.6	9.3	9.1
West	Rights-of-way	1.0	1	3.8	3.8	3.8	3.8	3.6	3.5
West	Forestry	4.0	1	27.4	26.9	26.8	26.5	25.6	24.8
West	Turf Granular	2.0	2	7.2	7.1	7.0	7.0	6.7	6.5
West	Turf Liquid	1.0	2	7.6	7.5	7.5	7.5	7.4	7.2
East	Corn[a]	2.0	2	80.1	78.9	78.7	78.4	76.5	74.1
East	Sorghum	2.0	1	69.2	68.3	68.1	67.6	65.9	63.8
East	Fallow	2.25	1	54.7	54.2	54.0	54.0	53.8	53.7

Table 3.6 Summary of PRZM/EXAMS Output Screening-Level EECs for all Modeled Scenarios (Using the Standard Water Body)									
Region	Use Site	Application Rate (lbs/acre)	No. of Applications	90th Percentile of 30 Years of Output					
				Peak EEC (µg/L)	14-day EEC (µg/L)	21-day EEC (µg/L)	30-day EEC (µg/L)	60-day EEC (µg/L)	90-day EEC (µg/L)
East	Residential[b] Granular	2.0	2	13.3	13.3	13.3	13.3	13.3	13.3
East	Residential[b] Liquid	1.0	2	9.6	9.5	9.4	9.3	9.0	8.8
East	Rights-of-way	1.0	1	2.4	2.4	2.3	2.3	2.3	2.2
East	Forestry	4.0	1	44.2	43.5	43.1	42.7	41.2	40.2
East	Turf Granular	2.0	2	13.2	13.2	13.2	13.2	13.2	13.2
East	Turf Liquid	1.0	2	9.1	9.1	9.1	9.1	9.1	9.1

[a] Actual labeled maximum rates are 2.0 lb/acre for a single application with no more than 2.5 lbs/acre per year. The rate and number of applications reported in this table are an approximation of the label maximum given the current limitation in the Agency's PRZM/EXAMS graphical user interface (GUI) PE4v01.pl. Currently, PE4v01.pl allows multiple applications but the rate cannot be varied from one application to the next. The impact of this assumption was assessed using an interim version of the GUI and yielded an approximately 6% increase in concentration. The corn EECs has been adjusted upwards by 6% for each duration of exposure to reflect this issue.
[b] Assumes 1% overspray of atrazine to the impervious surfaces.

3.2.5 Additional Modeling Exercises Used to Characterize Potential Exposures

Additional characterization of these modeling results has been completed, including a detailed analysis of monitoring data, alternative modeling assumptions, and characterization of the importance of flowing water on modeled EECs. These analyses are described in the sections that follow.

3.2.5.1 Impact of Flowing Water on Modeled EECs

The Agency's standard ecological assessment for aquatic organisms relies on estimates of exposure derived from PRZM/EXAMS using the standard water body. The standard water body is a 1-hectare pond that is 2 meters deep with a total volume of 20,000,000 liters and is modeled without flow. The standard water body was developed in order to provide an approximation of high end exposures expected in ponds, lakes, and perennial/intermittent streams adjacent to treated agricultural fields. Typically, this has been interpreted as a stream with little, or low flow. For pesticides with low to moderate persistence, the standard water body provides a reasonably high end estimate of exposure in headwater streams and other low flow water bodies for both acute and longer-term exposures. For more persistent compounds, the non-flowing nature of the standard water body provides a reasonable high end estimate of peak exposure for many streams found in agricultural areas; however, it appears to over-estimate exposure for longer time periods in all but the most static water bodies.

The hydrologic landscape of the listed mussel's action area is diverse and has been broken into four regions (north, west, south, and east), as shown in Figure 3.1. In general, the stream network within each region can be generalized by categorizing the stream network into broad classifications. A simplified approach of categorization for this assessment places the streams in the watershed into several broad classifications including headwater streams, upper tributary streams, main stem of the tributaries, and the major rivers such as the Ohio, Cumberland and Alabama Rivers. The purpose of this classification scheme is to describe the modeled EECs in the context of where these exposures are most representative and where they may be over- or under-estimated. Modeled concentrations derived with the non-flowing standard water body (presented in Table 3.6), are expected to be representative of exposures in headwater streams in areas of low topography. It is also expected that the chronic EECs over-estimate exposure in water bodies with flowing water, including those where the assessed mussel species and their designated critical habitat are found.

In order to characterize the potential impact of flowing water on the longer-term exposures (14-day, 21-day, 30-day, 60-day, 90-day, and annual average), additional modeling and analysis of site-specific flow data was conducted. Alternate approaches to modeling with the standard water body were conducted to provide a general sense of the relative reduction in long term exposure that might occur in water bodies where flow is higher than small headwater streams in low topographic regions of the mussel's action area.

A single scenario was selected from each region (corn for the north, south and east regions and fallow for the west region) that yielded the highest EEC. These scenarios were re-modeled with assumptions of flow (described below). As previously discussed, the EXAMS static ecological water body is typically used as the receiving body for runoff from a 10-hectare field. That ecological water body is intended to represent a pond or an ecologically sensitive stream adjacent to an agricultural field. Typically, this is conceptualized as a headwater stream; however, it may also be representative of higher order streams with very low flow rates (e.g. small tidal inlets, oxbow lakes occasionally fed by stream flow only, etc.).

In order to further characterize the impact of larger water bodies with flow, each selected scenario was also modeled using the Index Reservoir as the receiving water body. The Index Reservoir represents a 5.3-hectare water body draining a 172-hectare watershed. In the case of the Index Reservoir, the standard approach is to allow EXAMS to estimate total runoff accumulated from the 172-hectare watershed and route that volume of water as flow through the reservoir while assuming no change in reservoir volume. The predicted peak EECs from the model runs using the Index Reservoir are higher than those predicted using the static water body with no flow by 10% to 50% (depending on duration of exposure) and are summarized in Table 3.7. More information on the Index Reservoir may be found at:

http://www.epa.gov/oppfead1/trac/science/reservoir.pdf

The USGS has collected historical daily flow rates from 23,452 streams, creeks, and rivers from across the United States. These data were accessed and cross-walked against the streams identified in Table 2.3 where the listed mussels have been found to be present. Flow data was obtained on October 26, 2006 from the USGS National Water Information System (NWIS) web interface. The database was searched for all states included in the action area and each stream/river listed in Table 2.3 was evaluated to determine if daily data were available. For sites where historical daily data were available, data were downloaded only for those sites with more than 10 years of data (many had 30 to 50 years of data) and for which data from the late 1990s/early 2000s was available. Data from a total of 36 stream sites were selected and downloaded, representing each of the regions where modeling occurred. The data included daily mean values that represent the average concentration for each day across all years of data. The data also included percentiles (5^{th}%, 10^{th}%, 20^{th}%, 25^{th}%, 50^{th}%, 75^{th}%, 80^{th}%, 90^{th}%, and 95^{th} %) for each daily value. A single site from each region was selected for further modeling by selecting the site from each region with the lowest flow rates. It has been demonstrated in previous endangered species risk assessments (U.S. EPA, 2006c, d, and e) and in this assessment that modeling with lower flow rates yields higher EECs. For each regional site, the annual average and a seasonal average of the daily mean flow rates in cubic feet per second (ft^3/s) were calculated. The seasonal average was calculated using data from April through September representing the time of year when atrazine is most likely to be applied. In all cases, the seasonal flow rate was lower than the annual average; therefore, this value was used for additional modeling. More information on the USGS flow data may be found at the following website:

http://waterdata.usgs.gov/nwis/sw

In order to test the influence of these flow data on modeled EECs, a final analysis was conducted with the Index Reservoir by modifying the GUI (PE4v01.pl) that runs PRZM/EXAMS (modeling flow through the static water body was tested but yielded significantly lower EEC and was therefore not used in this assessment). The STFLO (stream flow) parameter responsible for reporting flow through the receiving water body is modified by using the seasonal flow data described above. The results of this analysis indicate that use of all seasonal flow rates yields peak EECs similar with the Index Reservoir analysis above; however, longer-term EECs are significantly reduced below those for the non-flowing standard water body, the standard water body with flow, and the Index Reservoir. The results along with the flow rates used in this evaluation are presented in Table 3.7. As expected, the flow-adjusted EECs were lower than EECs from the standard static ecological water body. Impact of flow on the EECs was greater as flow rate and duration increased.

Table 3.7 Comparison of Alternative PRZM Modeling (assuming flow) with EECs Generated Using the Static Water Body

Scenario	Flow (ft^3/sec)	Peak EEC (µg/L)	14-day EEC (µg/L)	21-day EEC (µg/L)	30-day EEC (µg/L)	60-day EEC (µg/L)	90-day EEC (µg/L)
South Region							
South corn with static water body[a]	0	109.4	108.1	107.4	107.2	104.8	101.7
South corn with Index Reservoir[b]	0.58	172	160	154	147	127	109
South corn (IR) with mean seasonal flow from USGS stream data[c]	105	120	15	10	7	3	2
Percent decrease in EEC using USGS mean seasonal flow data		NA	70	81	85	93	95
North Region							
North corn with static water body[a]	0	84.5	84.1	83.7	83.3	81.6	79.9
North corn with Index Reservoir[c]	0.39	80	76	74	72	65	57

Table 3.7 Comparison of Alternative PRZM Modeling (assuming flow) with EECs Generated Using the Static Water Body							
Scenario	**Flow (ft^3/sec)**	**Peak EEC (µg/L)**	**14-day EEC (µg/L)**	**21-day EEC (µg/L)**	**30-day EEC (µg/L)**	**60-day EEC (µg/L)**	**90-day EEC (µg/L)**
North corn (IR) with mean seasonal flow from USGS stream data[c]	22	69	17	13	8	4	3
Percent decrease in EEC using USGS mean seasonal flow data		18	79	85	90	95	96
West Region							
West fallow with static water body[a]	0	103.4	103.1	103.1	103.1	103.0	103.0
West fallow with Index Reservoir[c]	0.49	96	93	91	89	83	81
West fallow (IR) with mean seasonal flow from USGS stream data[c]	90	74	7	5	4	2	1
Percent decrease in EEC using USGS mean seasonal flow data		29	93	95	97	98	99
East Region							
East corn with static water body[a]	0	80.1	78.9	78.7	78.4	76.5	74.1
East corn with Index Reservoir[c]	0.71	109	103	100	94	78	66
East corn (IR) with mean seasonal flow from USGS stream data[c]	110	68	10	6	4	2	2
Percent decrease in EEC using USGS mean seasonal flow data		16	88	92	94	97	98

[a] EECs generated using PE4v01.pl in this table are slightly different from those presented in Table 3.6 due to different duration of exposure and slight differences in the manual estimation technique used in Table 3.6.
[b] Index Reservoir scenarios EEC are typically reported using percent cropped area (PCA) of 46% for corn and 87% for fallow. In this characterization no PCA is applied to the modeled output.
[c] USGS flow data reported as annual mean or annual seasonal (April to September) mean values.

As noted previously, risk estimation is conducted in accordance with the Overview Document (U.S. EPA, 2004) using EECs derived from PRZM modeling using the static water body. However, the species being assessed herein reside exclusively in flowing water. In order to provide context to the relevance of screening-level static water body EECs to flowing waters, additional characterization was conducted. Ideally, EECs would be estimated using a flowing water body representative of the streams and rivers where the listed species reside. However, no such modeled water body is available for use in Agency ecological risk assessments.

The only water body that is available for use in Agency risk assessment is the Index Reservoir (IR), which is used principally to estimate exposure via drinking water for human health risk assessments. However, the IR does incorporate flow as a principal transport mechanism through the water body; therefore, it is used to provide information on the impact of flow on long term (e.g. 30 day average) aquatic EECs. There are limitations with the use of the IR as a flowing water surrogate for streams and rivers. Principal among these is the geometry of the IR (2.7 meters deep, 640 meters long, 82.2 meters wide), the ratio of drainage area to surface area of the IR, the means of estimating flow in the IR scenario (based on accumulated runoff from the small watershed), and the mechanism of transport through the IR compared to a fast flowing stream (non-mixed). In general, the IR represents a low flowing water body relative to streams and rivers.

Ideally, a dedicated stream-type receiving water body would be used to assess the impact of flow through the type of habitat in which the listed mussel species live in (3^{rd} to 6^{th} order streams). However, as previously mentioned, this type of receiving water body is not available for use in risk assessment. Therefore, several refinements of the modeling were completed to provide context to the static water body EECs. First, the IR was used as in a drinking water assessment. While the geometry and other facets of IR parameterization are not representative of smaller streams, some larger rivers may be approximated by the IR geometry, and the amount of flow typically used in drinking water assessments is similar to some of the smallest streams. Second, the IR approach was modified to account for higher flow rates based on actual flow information from streams and rivers where the listed mussels occur (Table 2.3). The refined modeling used the low-end flow information (see Table D-4 of Appendix D) from each region in order to provide the most conservative estimate of the impact of flowing water on the screening-level static EECs.

As noted above, use of this approach is associated with a number of uncertainties; however the magnitude of decrease in the peak and long-term rolling averages in the refined flow-adjusted EECs do provide context to the screening level static water body EECs and are important in refining exposures to consider the impact of flow in the streams and rivers where the listed mussels may occur. Importantly, the decrease in longer-term exposures (e.g., 30-day average) is consistent with the trends seen in monitoring data where the flashiness of streams and rivers yields peak concentrations within a factor of two of the predicted values and longer-term exposures that are significantly lower than those predicted with the static water body.

3.2.6 Existing Monitoring Data

The second step in the process of characterizing EECs used for risk estimation was to compare the modeling results with available surface water monitoring data. A fairly robust set of surface water monitoring data exists for atrazine from a variety of targeted and non-targeted studies. Targeted studies are those studies whose design is specifically tailored to the use pattern for a specific compound. Sample location, number of samples, frequency of sampling, and when the samples are collected are designed specifically to capture exposures for the target compound. Non-targeted monitoring is typically more general in nature and is not designed for a specific compound. The study design for non-targeted studies are typically broad with the intent of capturing as many compounds as possible but not necessarily focused on the main exposure period for a single compound.

Included in this assessment are atrazine data from the USGS NAWQA program (http://water.usgs.gov.nawqa), Watershed Regression for Pesticides (WARP), Heidelberg College, Community Water System (CWS) data from drinking water sources, published USGS studies, and recently submitted data collected by the registrant of atrazine (Ecological Stream Monitoring Program). These monitoring data were characterized in terms of general statistics including number of samples, frequency of detection, maximum concentration, and mean from all detections. In addition, several sample sites from each data set were selected for further analysis including calculation of annual maximum and annual time weighted mean concentrations by site by year. The sample sites chosen for this additional analysis were based on those locations from the national and local data with the highest detected concentrations of atrazine. An interpolation of a single year's worth of data from one sample site in the Heidelberg College data was completed in order to estimate 14-day, 30-day, 60-day, and 90-day averages. In addition, a preliminary analysis of the data from the Ecological Monitoring Program, which is targeted to watersheds most vulnerable to atrazine is also presented.

3.2.6.1 Ecological Monitoring Program Data

The 2003 IRED required the atrazine registrants to conduct watershed monitoring for atrazine as a condition of re-registration. One component of the monitoring program is focused on flowing water bodies, and provides two to three years of monitoring data, accrued over a three-year period (2004-2006), in the most vulnerable watersheds associated with corn and sorghum production. These data are targeted specifically to atrazine use and are designed to represent exposure in the watersheds most prone to atrazine runoff. In this case, vulnerability has been defined using the USGS WARP model. The principal factors influencing WARP predictions of exposure and hence the vulnerability ranking are:

- Atrazine use,
- Rainfall intensity,
- Soil erodibility,
- Watershed area, and
- Dunne overland flow

Surface water data included in this study were collected using a targeted methodology that relied on WARP to identify the upper 20th percentile of vulnerable watersheds and a statistical design to select a subset of 40 watersheds that may be representative of 1,172 vulnerable watersheds. The atrazine use input was derived by calculating the mean annual atrazine concentration (at the 95th percent confidence limit) across all watersheds in the United States where atrazine is used. Given the statistical nature of the sampling design of this study, it is not possible to extrapolate the monitoring data from the 40 watersheds beyond the upper 20th percentile of watersheds (i.e., the 1,172 vulnerable watersheds).

Samples were collected from 20 locations within the designated watersheds every four days during the peak use period for atrazine (April to August) during the 2004-2005 growing season, and a second set of 20 watersheds were sampled during the 2005-2006 growing season (several watersheds from the 2004-2005 sample period were carried over for a third year of monitoring). A complete listing of site names and the corresponding watersheds where samples were collected is presented in Table D-1 of Appendix D. The strength of this data set is the targeted nature of site selection to areas of high atrazine use, the frequency of the sampling (every four days during peak use season), and the collection of multiple samples on selected days from a number of sites that allows for a statistical description of the variability surrounding the time series data. More detail on the approach, methodology and objectives of the surface water Ecological Monitoring Program for atrazine may be found at:

http://www.epa.gov/oppsrrd1/reregistration/atrazine/

A preliminary analysis of this Ecological Monitoring Program data from 2004 to 2006 has been completed. The data have been statistically evaluated for each site/year combination, including number of non-detections, frequency of detection, maximum concentration, mean concentration, median concentration, and number of scheduled samples that ultimately did not occur or samples that were not subsequently analyzed. These statistics provide a general picture of the level of exposures seen in these data relative to the other data sets described in this assessment.

Overall, the data suggest a similar pattern of atrazine exposure in surface water as in the other data sets evaluated as part of this assessment. Atrazine was detected in a total of 2,979 out of 3,601 samples for an overall frequency of detection of 79%. The frequency of detection ranged across all watersheds and years from a maximum of 100% to a minimum of 11%. The maximum concentration detected from all watersheds was 208.8 µg/L from the Indiana 11 site in 2005. The mean annual concentrations ranged from a maximum of 9.5 µg/L from the Missouri 01 site in 2004 to a low of 0.1 µg/L for the Nebraska 06 site in 2006, while the median values ranged from 4.2 µg/L for the Missouri 02 site in 2004 to 0.1 µg/L for the Ohio 03 site in 2004. It should be noted that a number of watersheds, particularly in Nebraska, experienced dry periods where scheduled sampling did not take place; therefore, the statistics for those watersheds may not represent actual conditions expected in normal or wetter years.

This data set is currently releasable only upon completion and submission of an Affirmation of Non-multinational Status form under section 10(g) of FIFRA. Information on how to submit a request to obtain a copy of the data may be obtained from the following website:

http://www.epa.gov/espp/atrazine_ewm_data.htm

A summary of the watershed analysis is presented in Table D-2 of Appendix D.

Although the ecological monitoring data set was targeted specifically to high atrazine use areas, only half the watersheds are within the action area and none appear to be co-located with streams or rivers occupied by the listed mussels or designated as critical habitat for the ovate and southern clubshell mussels. In addition, the site selection process was focused on watersheds deemed to be highly vulnerable to atrazine runoff based on use, soil, and climatic conditions and were selected to be statistically representative of the 1,172 watersheds from the highly vulnerable area (Figure 3.4). As seen in Figure 3.4, only a sub-set of the 1,172 watersheds from which the 40 watersheds were selected are within the action area, and of the watersheds within the action area, only a few of the 1,172 watersheds are co-located and/or adjacent to streams that are occupied with the listed mussels (Figure 3.5).

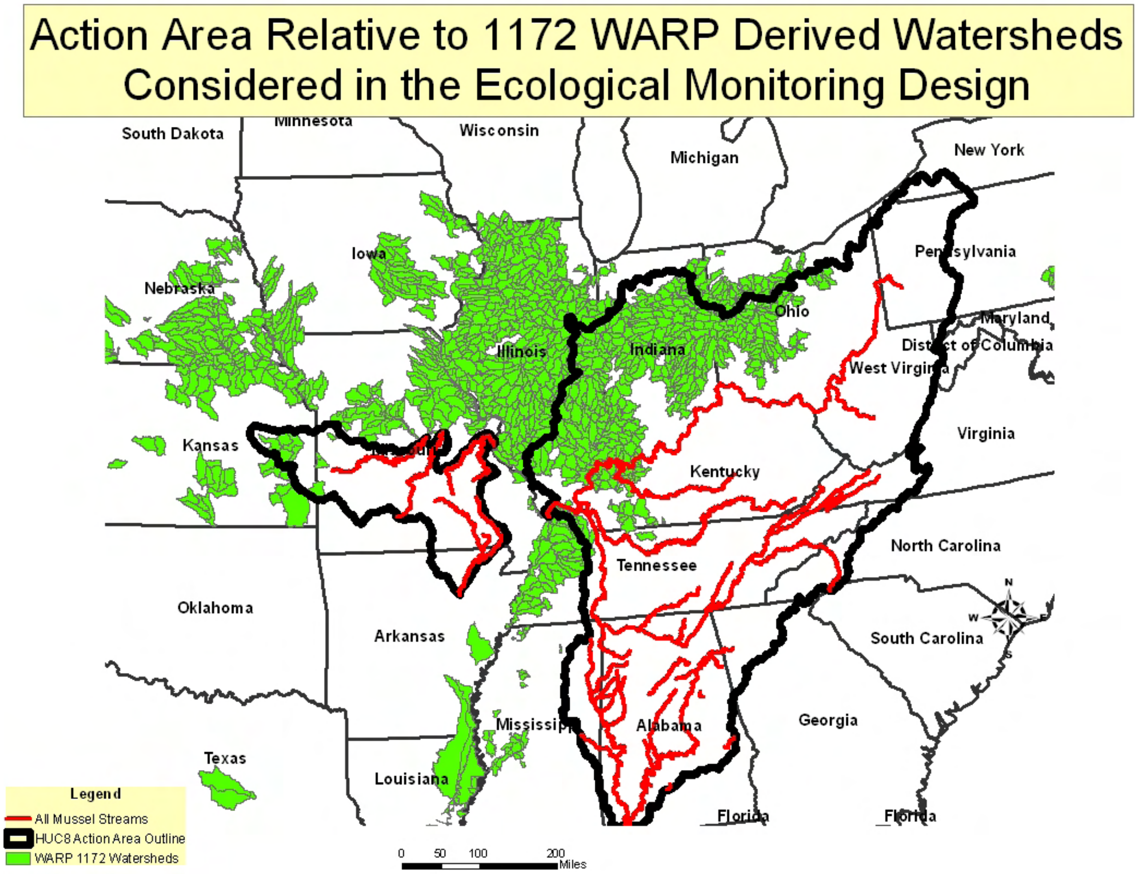

Figure 3.4 Relationship of WARP Vulnerable Watersheds Relative to Action Area

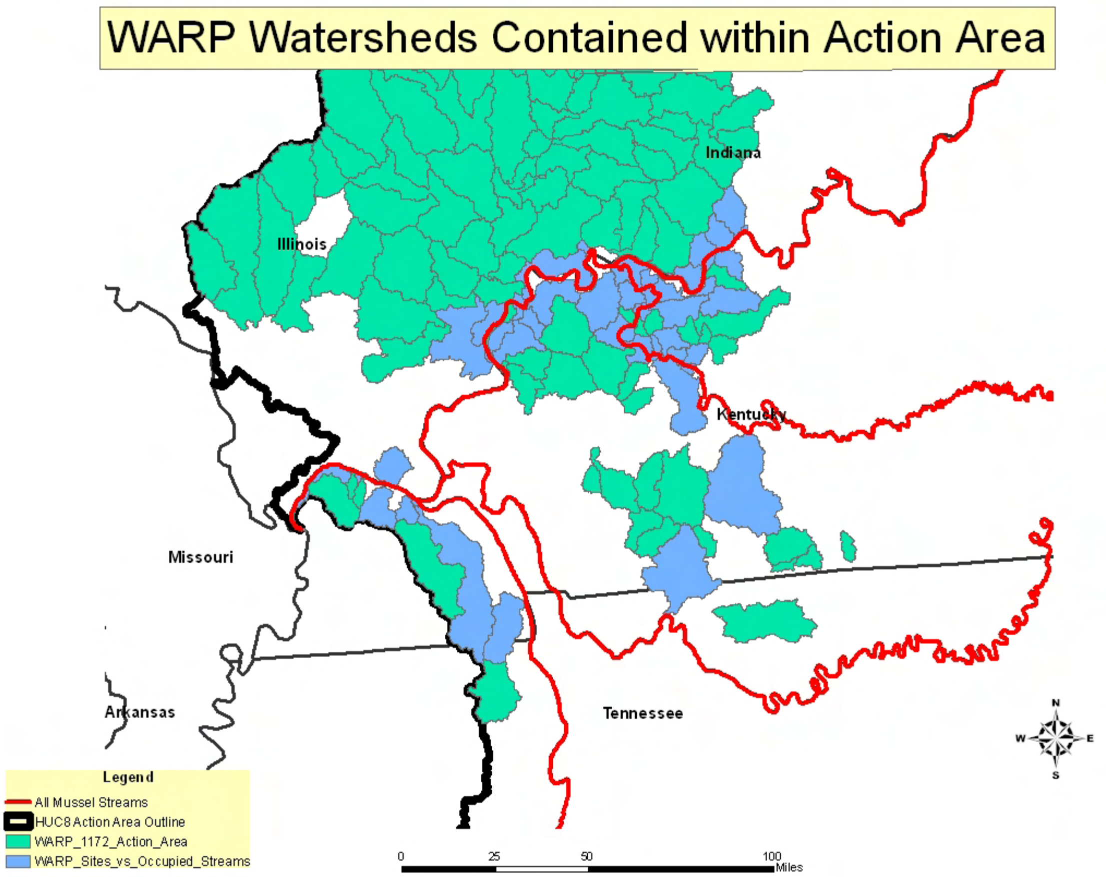

Figure 3.5 WARP Vulnerable Watersheds Containing or Immediately Adjacent to Occupied Streams

The statistical nature of the study design is critical in the selection of the 40 watersheds to sample. Watersheds were selected using a generalized random tessellation stratified (GRTS) method to identify spatially representative locations that can be linked back to the entire population of 1,172 watersheds. In general, most of the sites within watersheds selected for monitoring are second and third order streams in high atrazine use areas deemed vulnerable to runoff (a few of these sites are first and fourth order streams). The sampled watersheds were selected from a set of 1,172 watersheds using a statistical design, and thus are representative of some proportion of the total 1,172 watersheds. Comparison of the site locations from the ecological monitoring data with the action area for the listed mussels indicates that 19 of the sites are within the action area (Figure 3.6), although none of these sites are occupied by the listed species (Figure 3.7).

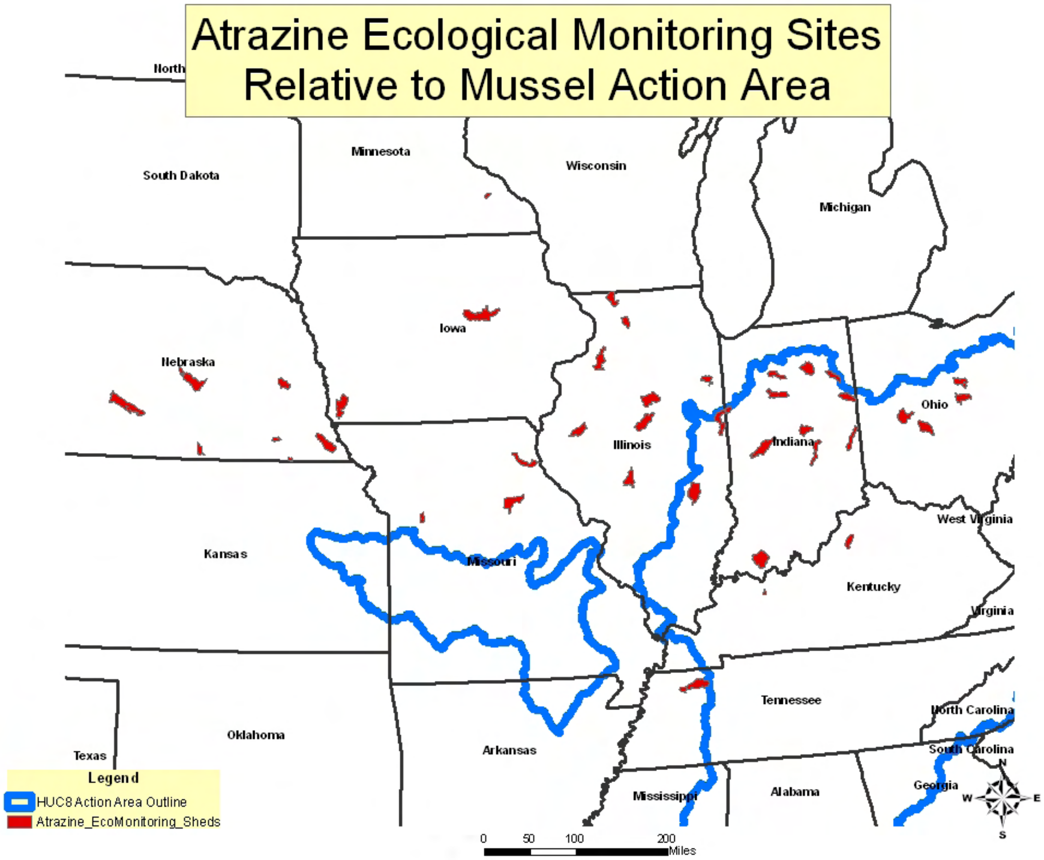

Figure 3.6 Atrazine Ecological Monitoring Sites Relative to Action Area

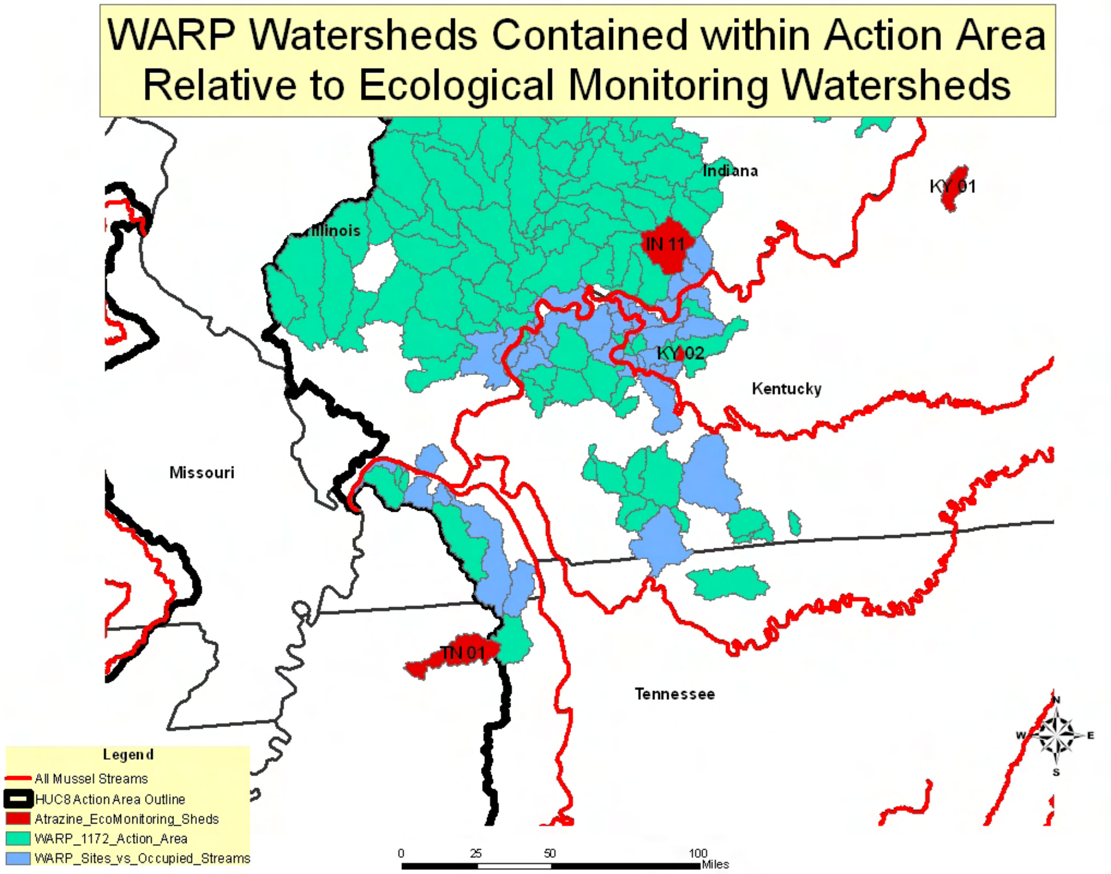

Figure 3.7 Close-up View of WARP Watersheds Containing or Immediately Adjacent to Occupied Streams Relative to Ecological Monitoring Sites

The following analysis represents a preliminary evaluation of the raw data and does not include a statistical analysis required to describe how the conditions in individual watersheds represent the larger population of 1,172 watersheds. That analysis is not currently available. In order to complete this preliminary analysis, each site/year of data was analyzed separately. Each data set was expanded to a 365-day time series and data interpolation was conducted. Preliminary data interpolation used a linear step method where the three un-sampled days after each sampled day were considered to have the same analytical result as the sampled day. Samples prior to the first sample date were considered to have the same result as the first sample date from that year, and a similar approach was taken for the un-sampled dates after the last sampling event. In addition, sample results from each date that were reported as non-detects were conservatively assigned an assumed value of the detection limit. Finally, dates where no sample was collected or analyzed were assumed to be equal to the nearest previous sample with a result. This final assumption results in significant uncertainty for a selected number of sites, particularly in Nebraska, where dry conditions resulted in fewer samples being collected.

Once the time series profile was created, a distribution of 14-day, 30-day, 60-day, and 90-day rolling average concentrations were calculated across the 365-day time series. In addition, an annual average concentration was calculated for comparison with screening-level EECs derived by PRZM modeling. The maximum values for each year with each watershed for each 14-, 30-, 60-, and 90-day duration, and the maximum peak and annual average concentrations are summarized in Table D-3 of Appendix D. Overall, a total of 84 individual site years of data have been collected from the 40 watersheds. Two of the watersheds (NE 04 in 2005 and NE 07 in 2005) represent years when multiple samples were not collected reportedly due to low flow conditions (NE 04 in 2005 with 15 missed samples and NE 07 in 2005 with 8 missed samples). Therefore, the rolling averages for these sites are questionable given the large amount of interpolation needed to infill data gaps. For all 40 watersheds, the exposures cover a range of concentrations for each duration with peak concentrations of 0.13 µg/L to 208.76 µg/L, 14-day concentrations ranging from 0.11 µg/L to 79.98 µg/L, 30-day concentrations from 0.10 µg/L to 45.17 µg/L, 60-day concentrations ranging from 0.1 µg/L to 25.74 µg/L, and 90-day concentrations ranging from 0.10 µg/L to 17.85 µg/L.

Comparison of the calculated duration-magnitude concentrations from the monitoring data with flow-adjusted modeled EECs (Table D-4 of Appendix D) indicates that 5 of the 40 watersheds (13%) exceed the highest peak flow-adjusted EECs, 11 (28%) watersheds exceed the highest 14-day flow-adjusted EECs, 12 (30%) watersheds exceed the highest 30-day flow-adjusted EECs, 17 (43%) watersheds exceed the highest 60-day flow-adjusted EECs, and 17 (43%) watersheds exceed the highest 90-day flow-adjusted EECs. However, the magnitude of under-prediction by the flow-adjusted EEC is brought into context when considering that of these, only 2 watersheds are higher than two times the peak flow adjusted concentration, only 5 watersheds are greater than two times above the 14-day and 30-day average concentrations, and only 7 watersheds are greater than two times above the 60-day and 90-day average concentrations. Table D-5 and Table D-6 in Appendix D present more detail of this comparison of watersheds relative to flow-adjusted EEC and flow rates. In general, flow rates for the monitored sites yielding exposures higher than the flow adjusted modeling are low flow streams suggesting that flow is an important consideration, particularly when considering longer-term durations of exposure.

As shown in Figure 3.6**,** 39 of the 1,172 watersheds are contained within, or drain directly into occupied stream miles. Although none of the 40 ecological monitoring watersheds co-occur with these 39 watersheds, several watersheds (IN 11, KY 01, KY 02, and TN 01) are in the vicinity of occupied streams (Figure 3.8). Based on the analysis of known locations of the listed mussels and their designated critical habitat (Tables 2.3 and 2.4), the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels inhabit streams that are at least partially located within the boundary of the 1,172 WARP vulnerable watersheds. The 40 watersheds sampled in this study were selected using a statistical design intended to allow for extrapolation of monitoring results to the entire 1,172 watersheds including those present in the action area. However, the analysis to allow for such extrapolation is not currently available and it is therefore not possible to determine

the representative nature of these locations to the original 1,172 vulnerable watersheds, including those specific locations where listed mussels may occur.
Therefore, these targeted monitoring data are used quantitatively to assess exposure and potential risk to these listed mussels that may be found within the total 1,172 vulnerable watersheds.

The shiny pigtoe, heavy pigtoe, ovate clubshell and southern clubshell mussels, as well as all designated critical habitat for the ovate and southern clubshell mussels, are located completely outside of the boundary of the 1,172 vulnerable watersheds, in less vulnerable watersheds. In these less vulnerable areas, exposures are best represented by the refined flow-adjusted PRZM EECs presented in Table 3.7. In addition ancillary non-targeted monitoring data from watersheds with sufficient sampling frequency to derive 14 through 90 day rolling average exposure concentrations (i.e., Heidelberg data discussed in Section 3.2.6.3) are also considered to provide context to the refined flow-adjusted EECs for less vulnerable watersheds.

3.2.6.2 USGS NAWQA Data

An analysis was completed of the entire USGS NAWQA data set for atrazine. A data download was conducted from the USGS data warehouse (http://water.usgs.gov/nawqa). Overall, a total of 20,812 samples were analyzed for atrazine. Of these, 16,742 samples had positive detections (including estimated values) yielding a frequency of detection of roughly 80%. The maximum detection from all samples was 201 µg/L from the Bogue Chitto Creek in Alabama near Memphis in 1999. Overall, the average concentration detected was 0.26 µg/L when considering only detections and 0.21 µg/L when considering all detections and non-detections (using the detection limit as the value for estimation). The location of all NAWQA surface water sites relative to the action area and the targeted monitoring data is shown in Figure 3.8.

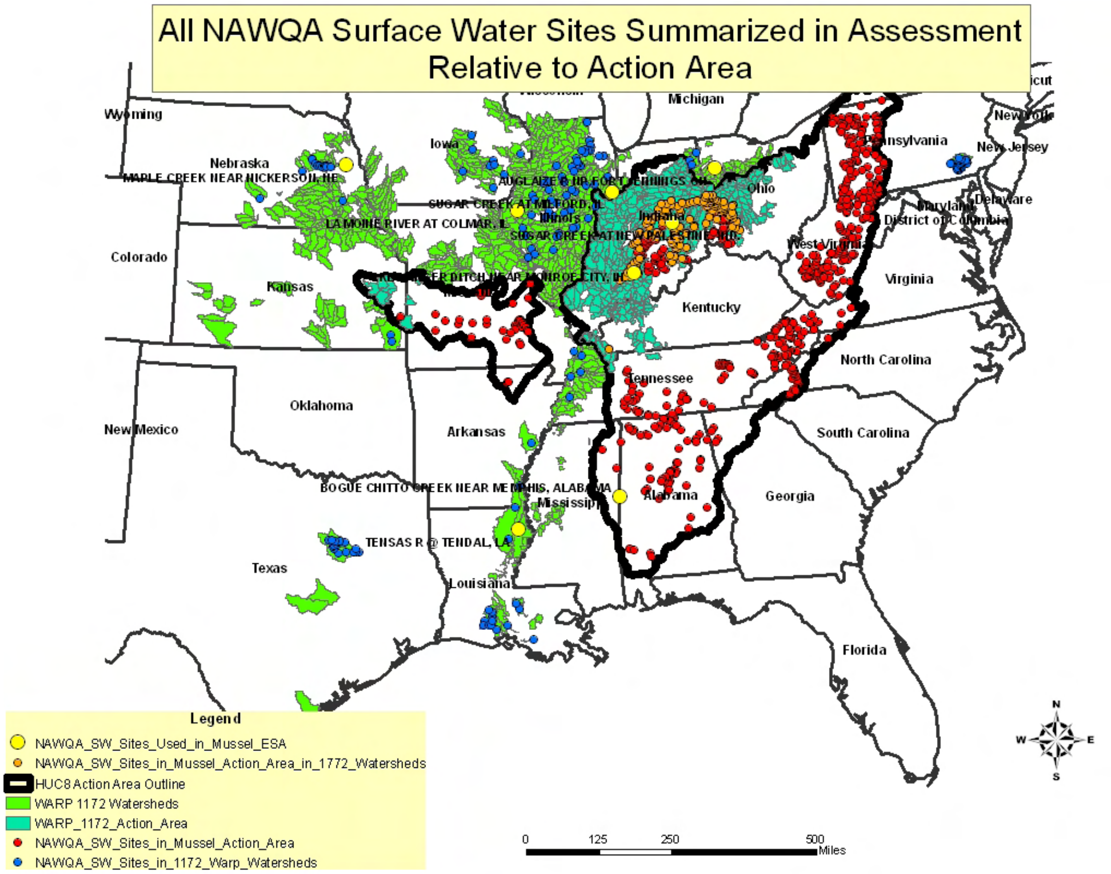

Figure 3.8 All USGS NAWQA Sites Relative to Action Area

The top ten sites with the highest atrazine concentrations from the national NAWQA data were selected for refined analysis of the detections. All values from the national data set were ranked and the top ten sites were selected based on maximum concentration. Each location was analyzed separately by year, and the annual maximum and annual time weighted mean concentrations were calculated. The minimum criterion for calculating time-weighted means for each sampling station was at least 4 samples in a single year. The equation used for calculating the time weighted annual mean is as follows:

$$[((T_{0+1}-T_0) + ((T_{0+2}-T_{0+1})/2))*C\, t_{0+1})] + (((T_{i+1}-T_{i-1})/2)*C_i) + [((T_{end}-T_{end-1}) + ((T_{end-1}-T_{end-2})/2)*C_{Tend-1})]/365$$

where: Ci = Concentration of pesticide at sampling time (Ti)
Ti = Julian time of sample with concentration Ci
T_0 = Julian time at start of year = 0
T_{end} = Julian time at end of year = 365

Generally, the maximum (peak) concentrations from the USGS NAWQA data are consistent with peak concentrations observed from the targeted monitoring data, and roughly two times the values predicted using both the static water body and the flow adjusted approach. The time weighted mean (TWM) values from this analysis are roughly an order of magnitude below the static water body model predictions, two times above those estimated in the refined flow-adjusted EECs, and consistent with the targeted monitoring data. This analysis is somewhat biased because the selected USGS NAWQA data represent those sites with the highest concentrations and the majority of the sampling locations are within the same geographic extent as the targeted data – the 1,172 vulnerable watersheds. In reality, there are many more NAWQA sites within and outside the action area (Figure 3.9) with atrazine detections and these sites would be expected to have lower concentrations (peak and annual average) than those reported for the top ten sites. Also of note is that there appears to be a general downward trend in atrazine exposures over time in these data (e.g. Bogue Chitto Creek), although some exceptions are noted (e.g. Sugar Creek, IL). Downward trends in exposure over time are expected given the label changes that have reduced application rates and implemented setbacks in the 1990's. Comparison of these data with modeled predictions for the intermediate duration exposures (14-day, 30-day, etc.) was not conducted because the NAWQA data generally do not have the frequency needed to conduct a meaningful interpolation between data points. Table 3.8 presents a summary of the annual time weighted mean concentrations, and Table 3.9 presents a summary of the annual maximum concentrations.

Table 3.8 Annualized Time Weighted Mean (TWM) Concentration (μg/L) for the Top Ten NAWQA Surface Water Sites (Ranked by Maximum Concentration Detected)										
	Station Name (ID)									
Year	**Bogue Chitto Creek, near Memphis, TN (02444490)**	**Tributary to S Fork Dry Creek, near Schuyler, NE (06799750)**	**Sugar Creek, New Palestine, IN (394340085524601)**	**Kessinger Ditch, near Monroe City, IN (03360895)**	**LaMoine River @ Colmar, IL (05584500)**	**Sugar Creek @ Milford, IL (05525500)**	**Tensas River @ Tendal, LA (07369500)**	**Maple Creek near Nickerson, NE (06800000)**	**Auglaize River near Ft Jennings, OH (04186500)**	
1992			0.98					1.32		
1993			0.77	3.80				1.43		
1994			0.87	2.56						
1995			2.28	0.74						
1996			1.30				4.32		2.18	
1997			5.36		3.45		5.55	1.03	2.82	
1998			0.82		1.79		2.94	1.21	1.88	
1999	9.62		0.28				2.50	0.68		
2000	6.49		0.56			1.26		0.15		
2001	1.20		0.83			0.78		0.22	1.28	
2002	2.88		0.51			2.22		1.26	0.80	
2003	2.14	4.46	0.70			7.83		2.23	1.42	
2004	1.77	68.78[a]	0.67			1.24		3.31	1.93	

[a] TWM concentration likely biased because the first sample on May 8 is the peak sample from this year.

Table 3.9 Maximum Concentration (µg/L) for the Top Ten NAWQA Surface Water Sites (Ranked by Maximum Concentration Detected)										
	Station Name (ID)									
Year	**Bogue Chitto Creek, near Memphis, TN (02444490)**	**Tributary to S Fork Dry Creek, near Schuyler, NE (06799750)**	**Sugar Creek, New Palestine, IN (394340085524601)**	**Kessinger Ditch, near Monroe City, IN (03360895)**	**LaMoine River @ Colmar, IL (05584500)**	**Sugar Creek @ Milford, IL (05525500)**	**Tensas River @ Tendal, LA (07369500)**	**Maple Creek near Nickerson, NE (06800000)**	**Auglaize River near Ft Jennings, OH (04186500)**	
1992			14					25		
1993			8.5	120				11.2		
1994			11	24						
1995			27	2.6						
1996			14.2						18	
1997			129		108		92.3	10.3	85.2	
1998			7.88		27.7		19.3	30	9.96	
1999	201		2.39				13.9	10.7		
2000	136		3.84			230		0.87		
2001	4.5		14.4			6.96		1.21	10.4	
2002	24.8		4.01			21.3		16.4	2.58	
2003	18.8	21.3	10.5			108		34.8	13.4	
2004	14.6	191	28.3			10.9		91.9	18.7	

3.2.6.3 USGS Watershed Regression of Pesticides (WARP) Data

The NAWQA data were then compared against the percentiles used to develop the USGS WARP model. Comparison against WARP percentiles was conducted because the WARP model has been reported to be a valuable tool for site selection and assessing overall vulnerability. More information on the WARP model may be found at:

http://pubs.usgs.gov/wri/wri034047/wrir034047.pdf

The WARP data were developed using a subset of the national data described above (all WARP data are included in the national data analysis described above). Data collected between 1992 and 1999 from a total of 113 sample sites were used to create the model. Sample sites were selected based on the robustness of the data available at a given site. The model yields predicted daily exposures at various percentiles of occurrence. The Agency compared the national NAWQA data and the model predictions against the mean and 95^{th} percentile values from the data used. The maximum 95^{th} percentile value from the WARP data was 20.2 μg/L as compared to a maximum of 201 μg/L from all data. The maximum mean value used in the WARP model development data was 3.82 μg/L, which is consistent with the annual TWM values discussed above.

3.2.6.4 Heidelberg College Data

Data from Heidelberg College, which consists of two intensively sampled watersheds (Maumee and Sandusky) in Ohio, were also analyzed. These sample sites are on the extreme northern edge of the action area and are also included in this analysis to provide context to the modeled exposures. It appears that the Sandusky watershed is within the boundary of the vulnerable watersheds included in the targeted monitoring study, while the Maumee watershed is outside this boundary. More information on the water quality monitoring program at Heidelberg College may be found at the following website:

http://wql-data.heidelberg.edu/

The Heidelberg data were collected more frequently than other data included in this assessment. The study design was specifically established to capture peak and longer-term trends in pesticide exposures. Data were collected between 1983 and 1999 and consist of an average of roughly 100 samples per year with several days of multiple sampling.

For the Sandusky watershed, a total of 1,597 samples were collected with 1,444 detections of atrazine (90.4% frequency of detection). The maximum concentration detected in the Sandusky watershed was 52.2 μg/L, and the overall average concentration was 4.5 μg/L. For the Maumee watershed, a total of 1,437 samples were collected with 1,305 detections of atrazine (90.8% frequency of detection). The maximum concentration detected in the Maumee watershed was 38.7 μg/L with an overall average concentration of 3.7 μg/L.

This analysis was further refined by deriving the annual TWM and maximum concentrations by sampled watershed by year. The results of this analysis are presented in Table 3.10. The results show a consistent pattern with that seen in other data collected from high atrazine use areas with general TWM concentrations between 1 and 3 μg/L. In addition, these data are generally two times lower than the peak refined flow-adjusted EECs and are generally consistent with the longer-term flow-adjusted average concentrations.

Table 3.10 Annual Time Weighted Mean and Maximum Concentrations (μg/L) for Atrazine in Two Ohio Watersheds from the Heidelberg College Data

Year	Sandusky Watershed		Maumee Watershed	
	TWM	Max	TWM	Max
1983	1.34	7.97	0.98	5.42
1984	1.08	8.73	1.27	11.71
1985	1.83	19.46	1.00	6.21
1986	3.32	24.61	1.64	10.01
1987	1.76	16.45	1.80	9.92
1988	0.41	1.53	0.43	2.15
1989	1.30	15.71	1.07	8.49
1990	1.96	19.31	1.69	14.78
1991	1.49	20.59	2.044	21.45
1992	0.39	40.53	0.51	7.35
1993	1.27	26.34	1.21	22.66
1994	0.86	10.10	0.82	4.02
1995	1.39	15.46	1.30	14.06
1996	1.56	23.40	1.19	16.19

Table 3.10 Annual Time Weighted Mean and Maximum Concentrations (µg/L) for Atrazine in Two Ohio Watersheds from the Heidelberg College Data				
Year	**Sandusky Watershed**		**Maumee Watershed**	
	TWM	**Max**	**TWM**	**Max**
1997[a]	**2.16**	**53.21**	2.09	38.74
1998	1.49	40.03	1.41	27.62
1999	1.57	17.11	1.88	19.37

[a] Sample year 1997 from Sandusky selected for data infilling by interpolation in order to calculate CASM duration exposure values.

Unlike the NAWQA data set, this data set had a sampling frequency adequate to interpolate between data points to estimate 14-day, 30-day, 60-day, and 90-day average concentrations. A final analysis of the data was completed by selecting one year's worth of data from the Heidelberg data. The 1997 sampling year was selected because it was one of the more recent data sets and because the maximum and TWM concentrations were higher than most other year's data. To process these data, it was necessary to "fill in the gaps". A total of 126 samples were collected during 1997 with 50 days with multiple samples yielding a time series of roughly 75 days. A step-wise approach was used to estimate daily concentrations between sampling dates that consisted of simply extending an analytical result from the date of analysis to the next date. For example, on January 6, 1997, atrazine was detected at a concentration of 0.475 µg/L. On the next sample date of January 20, 1997, no atrazine was detected (0 µg/L). In the step-wise interpolation, all dates between January 6 and January 20 were assigned the concentration of 0.475 µg/L. Also, because January 6 was the first sample date of the year, all previous days were also assigned a value of 0.475 µg/L. This process was repeated throughout the year to fill in the time series and yield 365 days worth of data. In addition, where multiple samples were analyzed on any given day, the highest of the values on that day was assigned. There is significant uncertainty with this type of interpolation because there is no information to suggest whether the interpolated value represents actual exposure. For example, where a significant gap in time exists between two samples, it is unlikely that a continuous concentration exists. It is more likely that there are upward and downward fluctuations in exposure, with a greater likelihood that higher exposures are missed between sample times with larger gaps in data points.

Table 3.11 presents the results of this analysis. The analysis suggests that, for the Sandusky watershed, in 1997, the estimated longer-term exposures are similar to those seen in the targeted data at roughly the 90th percentile of the distribution of 14-day, 30-day, 60-day, and 90-day rolling averages. Although the Sandusky watershed is located within the vulnerable watershed boundary defined by WARP, the rolling averages provided in Table 3.11 are used to characterize the potential upper bound of the refined flow-adjusted EECs for listed mussels and designated critical habitat that occur in less vulnerable watersheds. These data are used to provide context to the flow-adjusted EECs because they were derived from non-targeted data with sufficient sampling frequency to derive 14 though 90 day rolling average exposure concentrations, and are considered as conservative estimates of exposure.

Table 3.11 Magnitude and Duration Estimates (µg/L) from the 1997 Data from Sandusky Watershed Using Stepwise Interpolation Between Samples

	14 day	21 day	30 day	60 day	90 day
Maximum	28.26	21.11	18.30	12.38	8.89

3.2.6.5 Summary of Open Literature Sources of Monitoring Data for Atrazine

Atrazine is likely to be persistent in ground water and in surface waters with relatively long hydrologic residence times (such as in some reservoirs) where advective transport (flow) is limited. The reasons for atrazine's persistence are its resistance to abiotic hydrolysis and direct aqueous photolysis, its only moderate susceptibility to biodegradation, and its limited volatilization potential as indicated by a relatively low Henry's Law constant. Atrazine has been observed to remain at elevated concentrations longer in some reservoirs than in flowing surface water or in other reservoirs with presumably much shorter hydrologic residence times in which advective transport (flow) greatly limits its persistence.

A number of open literature studies cited in the 2003 IRED (U.S. EPA, 2003a), document the occurrence of atrazine and its degradates in both surface water and groundwater. These data support the general conclusion that higher exposures tend to occur in the most vulnerable areas in the Midwest and South and that the most vulnerable water bodies tend to be headwater streams and water bodies with little or no flow.

The analysis in the IRED also documents the occurrence of atrazine in the atmosphere. The data indicate that atrazine can enter the atmosphere via volatilization and spray drift. The data also suggest that atrazine is frequently found in rain samples and tends to be seasonal, related to application timing. Finally, the data suggest that although frequently detected, atrazine concentrations detected in rain samples are less than those seen in the monitoring data and modeling conducted as part of this assessment and support the contention that runoff and spray drift are the principal routes of exposure. More details on these data can be found in the 2003 IRED (U.S. EPA, 2003a).

3.2.6.6 Miscellaneous Drinking Water Monitoring Data Derived from Surface Water

A number of surface water data sets were evaluated as part of the 2003 IRED. Included in that analysis were data from Acetochlor Registration Partnership (ARP) Monitoring

Study, the Novartis Population Linked Exposure (PLEX) Database, the USGS 1992-1993 Study of 76 Mid-Western Reservoirs (USGS Open File Report 96-393), the USGS 1989-1990 Reconnaissance Study of Mid-Western Streams (USGS Open File Report 93-457), the USGS 1994-1995 Reconnaissance Study of Mid-Western Streams (USGS Open File Report 98-181), the USGS 1990-1992 Study of 9 Mid-Western Streams (USGS Open File Report 94-396), USGS NAWQA data available in 2002, as well as numerous open literature studies. In general, these data show a pattern of atrazine exposure in various water body types (streams vs. reservoirs), collected with a variety of study objectives (human health vs. ecological health) consistent with those summarized previously in this assessment. The maximum reported concentration from the studies (excluding open literature) was 108 µg/L from the USGS study (Open File Report 93-457) for Mid-Western Streams sampled between 1989 and 1990. Atrazine exposure in rivers, streams, lakes, and reservoirs documented in the open literature cited in the 2003 IRED were consistent with these results with no concentrations above 100 µg/L (except edge of field runoff concentrations in mg/l range which were reported as diluted to µg/L ranges when reaching surface water bodies). In addition, the 2003 IRED summarized reports from the Agency's 6(a)(2) incident database and found the highest concentration at 62 µg/L.

More detail on the individual studies and analysis of the data may be found in the 2003 IRED at the following website:

http://www.epa.gov/oppsrrd1/reregistration/atrazine/efed_redchap_22apr02.pdf

Subsequent to the completion of the 2003 IRED, additional monitoring data from surface water sources used for drinking water were submitted to the Agency for review. Atrazine monitoring results from 2003 to 2005 were collected as part of the Atrazine Monitoring Program (AMP) for purposes of assessing dietary risk for human health. In this study, data were collected from over 100 community water systems (CWS) in 10 states including many in the action area of this assessment. Monitoring was weekly through the growing season (generally April through July) with biweekly monitoring for the rest of the year. Both raw and finished water were monitored. In general, the results were consistent with those discussed above, with maximum detected concentrations of 33.1 µg/L in 2002, 39.7 µg/L in 2004, and 84.8 µg/L in 2005.

3.2.7 Comparison of Modeling and Monitoring Data

Modeling with the static water body provides screening-level EECs for use in risk estimation (Section 5.1). These screening-level EECs are also refined and used in the risk description to characterize the relevance of predicted screening-level modeled exposures to the streams and rivers that are occupied by the listed mussels and designated as critical habitat. In this case, the listed species reside in streams and rivers (Table 2.3) with relatively fast flows (Table D-7 of Appendix D). Therefore, additional characterization of the modeled static water body screening-level EECs used for risk estimation is necessary to determine its relevance (and hence the RQs) to the species habitat. In order to complete this characterization, additional refinement of the screening-level EECs is completed based on evaluation of modeled flow-adjusted EECs and available atrazine monitoring data.

Available monitoring data consists of both targeted and non-targeted data, as described above. Targeted monitoring data (i.e. Ecological Monitoring Program; discussed in Section 3.2.6.1) is designed specifically to capture atrazine concentrations in watersheds with high atrazine use and exposure patterns in the most runoff prone settings and is used for direct comparison with effects data where the species resides in streams and rivers located within the boundary of vulnerable watersheds. Non-targeted data (e.g. USGS NAWQA) is typically designed to capture the general pattern of pollutants in the environment and is not designed specifically for any one chemical.

In this assessment, data from the Ecological Monitoring Program provide a robust data set targeted to the most vulnerable watersheds in areas of atrazine use. In this case, vulnerability is defined by the USGS WARP model and is determined by ranking model output (95th percent confidence interval of annual mean concentration). Sampled watersheds are deemed highly vulnerable (based on the upper 20th percentile in the WARP predictions) and were selected to be statistically representative of the total watershed population from which they were selected (1,172 upper 20th percentile WARP watersheds). Based on the statistical nature of the site selection, the monitoring results can be used to indicate where within these highly vulnerable watersheds similar concentrations may be expected. It should be noted that because of the statistical nature of the study design, the results cannot be quantitatively comparable to less vulnerable watersheds outside the study design area.

In general, the targeted monitoring and refined flow-adjusted modeling provide a reasonable consistent picture of overall exposure. Of the 40 watersheds sampled, between 60% and 75% (depending on the duration of exposure) of the sampled sites are similar to, or less than the flow-adjusted model EEC. Of the targeted watersheds that exceed the refined flow-adjusted EECs, all but 10% to 15% of these exposures are within 2 times the refined modeling. Given that the targeted monitoring data represent the most vulnerable watersheds for the entire country and that the conditions modeled (low flow rates) are generally at or above those seen in the targeted monitoring data, it is not unexpected that there are a few excursions above the modeling. Another way of considering this is to understand that 40% of sites higher than refined modeling from the upper 20th percentile of vulnerable sites represents approximately 8% of all atrazine watersheds nationally. In other words, 8% of all atrazine watersheds nationally are expected to be higher than the flow-adjusted EEC (assuming lower exposures in the lower vulnerability areas). If it assumed that only 10% of sites are higher than 2 times the refined modeling (and this is considered to be within the normal uncertainty of a model run), only 2% of all atrazine watersheds nationally would be expected to be higher than the flow adjusted modeling. This suggests that relative to the targeted monitoring the refined flow-adjusted EECs, though exceeded occasionally, represent reasonably high end exposures for all watersheds nationally where atrazine is used.

A similar comparison of non-targeted monitoring data with refined flow-adjusted modeling yields similar conclusions. Non-targeted monitoring also provides a sense of how well the screening and refined modeling predict exposures in portions of the action area not directly represented by the targeted data. Comparison with modeling suggests that under certain conditions (low flow rates) concentrations can be higher in the non-targeted monitoring data than those predicted by the refined flow-adjusted modeling, however, it appears that most of these sites are located within the same watersheds as the targeted monitoring (i.e. WARP 1,172 highly vulnerable watersheds). However, much of the non-targeted monitoring data considered in this assessment are from the same general geographic area as the targeted data described above (Figure 3.9), although these non-targeted data have differing study objectives and are generally less robust.

In general, the trends in the non-targeted data are similar to those seen in the targeted data. Peak concentrations (though generally more than 10 years old) are twice as high as those predicted in screening and refined modeling. Given the less robust nature of these data, a direct comparison of various rolling averages with refined flow-adjusted rolling averages is not possible for the NAWQA data. However, rolling averages were considered for the Heidelberg data and like some of the targeted data is approximately 2 times higher than the refined modeling. For the NAWQA data, the annual mean concentrations can be compared and generally show the same pattern as the targeted data. The ranked percentiles (99^{th}, 95^{th}, 90^{th}, 75^{th}, 50^{th}, 25^{th}, 10^{th}, and 5^{th}) from the non-targeted data are comparable to those seen in the targeted data. This information is further summarized in Table D-8 of Appendix D.

An important consideration when comparing the monitoring results to modeling is stream type and flow rate relative to each other. Several lines of evidence were evaluated to determine whether trends in the targeted and non-targeted data could be determined which would provide context to the overall exposure assessment. The range of flow rates in the targeted data was compared to the flow rates used in the refined modeling (i.e., those flows specific to streams and rivers where the listed mussels occur). In general, the watersheds where the species reside are third and higher order streams, while the targeted monitoring data are generally from 2^{nd} and 3^{rd} order streams. Flow rates used in refined modeling were between 20 ft^3/sec and 110 ft^3/sec, while flow rates for the targeted monitoring ranged from roughly < 10 ft^3/sec to 180 ft^3/sec. This suggests that the flow rates used in modeling were a reasonable approximation of flow in the targeted monitoring study.

Additionally, because the flow rates were drawn from the pool of streams, creeks and rivers where the species reside, the data were compared to the full range of flow in all occupied streams with flow data. Because the majority of the occupied streams are from areas not represented by the targeted data, a comparison of flow between the two types of data provides context to whether exposure-related trends seen in the targeted data (some rolling averages higher than the refined modeling) could be expected in areas outside the most vulnerable sites and whether the exposures in the targeted data are relevant to the occupied streams. Table D-7 of Appendix D summarizes the range of flow rates found in the occupied streams.

Comparison of flow rates from the targeted monitoring data with occupied streams shows that there is some overlap around the 25th percentile and below of occupied stream flow, indicating that, in general, the occupied streams identified in Table 2.3 have generally higher flow (sometimes two to three orders of magnitude) than the watersheds sampled as part of the targeted data. For example, comparison of the 50th percentile of flow from all targeted monitoring sites (30 ft^3/sec) and occupied streams (487 ft^3/sec) indicates that flow is approximately 16-fold higher in streams that are occupied by the listed mussels. Although there is some overlap between the two data sets, overall this analysis suggests that most of the occupied streams are higher flow rate habitats than those represented by the targeted monitoring data. This analysis suggests that these modeled concentrations are unlikely to be in all but the lowest flow streams and that most of these low flow streams are not occupied by the listed species. This analysis is summarized in Table 3.12**.**

Table 3.12 Comparison of Ranked Percentile of Flow Rates (ft^3/sec) from Occupied Streams versus Ecological (Targeted) Steams Sites

Occupied Sites		**Ecological Steam Monitoring Sites**	
Max Value	12011	Max Value	177
99th Percentile	11570	99th Percentile	177
95th Percentile	9243	95th Percentile	141
90th Percentile	5603	90th Percentile	105
75th Percentile	982	75th Percentile	67
50th Percentile	487	50th Percentile	30
25th Percentile	148	25th Percentile	18
10th Percentile	99	10th Percentile	7
5th Percentile	72	5th Percentile	4

In order to provide additional context to the monitoring data, a comparison of the targeted and non-targeted data was completed. This analysis was conducted to determine if distinctions could be made between the portions of the action area represented by the vulnerable watersheds (i.e., targeted data) and the remaining portions of the action area outside the boundary of vulnerable watersheds (where most of the non-targeted sites are located) (Figure 3.9). A ranking of percentiles for the non-targeted data was conducted for both peak, rolling averages (Heidelberg only), and annual average concentrations. The lack of robustness of the non-targeted NAWQA data precludes estimation of other long-term average concentrations (e.g. 30-day rolling average). These peak and annual values (and rolling averages for the Heidelberg data) were compared directly with the targeted data. In general, peak concentrations were similar between targeted and non-targeted data while the annual averages in the NAWQA data were higher, and the annual averages from the Heidelberg data were comparable. Finally, the rolling averages from the Heidelberg data are between the 90th and 95th percentile from the targeted monitoring data. Overall, this suggests that for the highest monitoring data from the areas not represented by the targeted data, that the trends in the data are similar to the targeted data. This suggests that for sites similar in character to the targeted data (2nd and 3rd order streams with relatively low flow rates) that similar exposures could occur. These analysis are summarized in Table 3.13 and Table 3.14.

Table 3.13 Summary Comparing Ranked Percentiles of Peak Exposures (µg/L) for Targeted and Non-targeted Monitoring Data

	Eco Sites	NAWQA[a]	Sandusky	Maumee
Percentile	**PEAK**	**PEAK**	**PEAK**	**PEAK**
Max Value	208.8	201.0	53.2	38.7
99th Percentile	187.4	195.9	51.2	37.0
95th Percentile	81.6	132.2	43.1	29.8
90th Percentile	48.5	108.0	40.2	24.6
75th Percentile	20.7	28.7	24.6	19.4
50th Percentile	10.5	17.2	19.3	11.7
25th Percentile	5.6	10.4	15.5	7.4
10th Percentile	2.2	3.9	8.4	4.9
5th Percentile	1.3	2.5	6.7	3.6

[a] Based on top ten NAWQA sites with highest atrazine concentrations

Table 3.14 Summary Comparing Ranked Percentiles of Annual versus Time Weighted Mean Exposures (µg/L) for Targeted and Non-targeted Monitoring Data

	Eco Sites	NAWQA[a]	Sandusky	Maumee
Percentile	**Annual Average**	**TWM**	**TWM**	**TWM**
Max Value	4.6	9.6	3.3	2.1
99th Percentile	4.6	8.7	3.1	2.1
95th Percentile	3.6	6.0	2.4	2.1
90th Percentile	2.7	4.5	2.0	1.9
75th Percentile	1.1	2.7	1.8	1.7
50th Percentile	0.6	1.4	1.5	1.3
25th Percentile	0.2	0.8	1.3	1.0
10th Percentile	0.2	0.7	0.7	0.7

Table 3.14 Summary Comparing Ranked Percentiles of Annual versus Time Weighted Mean Exposures (µg/L) for Targeted and Non-targeted Monitoring Data				
	Eco Sites	**NAWQA[a]**	**Sandusky**	**Maumee**
5th Percentile	0.1	0.4	0.4	0.5

[a] Based on top ten NAWQA sites with highest atrazine concentrations

The previous analysis was conducted using site-specific information from the top ten NAWQA surface water sites as determined by peak concentration. Additional characterization comparing atrazine detections from all NAWQA surface water sites with all detections from the Ecological Stream Monitoring data was completed. In this analysis, all samples, regardless of site location or year, were ranked for both data sets**.** Table 3.15 presents the results of this analysis. Direct comparison indicates that peak values are roughly equivalent for both data sets; however, the distribution across the entire spectrum of atrazine detections is dramatically different. As the percentile decreases, the Ecological Stream Monitoring data becomes increasingly higher in concentration relative to the NAWQA data, with a two-fold difference at the 99.9th%, an order of magnitude difference at the 50th%, and nearly two orders of magnitude difference at the 10th%. A simple comparison of the two distributions of Ecological Stream Monitoring and NAWQA data was conducted using the t-test (two samples assuming unequal variances) in Microsoft Excel for both raw data and log-normalized data. In both cases the p values were less than 0.05 indicating that the distributions are significantly different. This analysis confirms that there are significant differences between the Ecological Stream Monitoring data and the entire NAWQA data, which are likely due to differences in the sampling design (i.e., the Ecological Monitoring data are focused on the upper 20th% of vulnerable watersheds while the NAWQA data cover the entire range of atrazine use areas).

Table 3.15 Comparison of all NAWQA Atrazine Surface Water Data with the Ecological Stream Monitoring Data

Percentile	All NAWQA Surface Water Data (µg/L)	Ecological Stream Monitoring Data (µg/L)	Difference (µg/L)	Percent Difference
Max Value	201.00	237.50	36.50	18%
99.9th Percentile	61.25	137.21	75.96	124%
99.5th Percentile	20.09	59.51	39.41	196%
99th Percentile	11.70	33.37	21.67	185%
95th Percentile	1.96	10.70	8.74	446%
90th Percentile	0.63	4.97	4.34	685%
75th Percentile	0.13	1.12	0.99	762%
50th Percentile	0.02	0.32	0.30	1233%

Table 3.15 Comparison of all NAWQA Atrazine Surface Water Data with the Ecological Stream Monitoring Data				
Percentile	**All NAWQA Surface Water Data (µg/L)**	**Ecological Stream Monitoring Data (µg/L)**	**Difference (µg/L)**	**Percent Difference**
25th Percentile	0.01	0.11	0.10	1471%
10th Percentile	0.00	0.10	0.10	9900%
5th Percentile	0.00	0.10	0.10	9900%

3.2.8 Impact of Typical Usage Information on Exposure Estimates

A final piece of the exposure characterization includes an evaluation of usage information. Label application information was provided by EPA's Biological and Economic Analysis Division and summarized in Table 2.2. This information suggests that atrazine use on corn and sorghum (non-agricultural usage data is not available as part of this analysis) is typically 1.2 lbs/acre and 1.3 lbs/acre in the states considered within the action area of this assessment. This suggests that if typical application rates were used, atrazine exposures would be reduced below those modeled with the label maximum application rate by 40% for corn and 35% for sorghum. Typically usage information is not incorporated into these assessments, but does provide context to the exposures predicted. Caution is used when evaluating "typical" application rate information because this represents the average of all reported applications and thus roughly 50% of the time higher application rates are being applied.

3.2.9 Uncertainties in the Aquatic Exposure Assessment

A number of factors add uncertainty to the direct comparison of flow-adjusted modeling EECs with the monitoring data (including other sources discussed previously). For example, the selection process for the ecological monitoring sites was focused on the most vulnerable sites relative to atrazine runoff, and as seen in Figure 3.5, do not directly correlate with the majority of steams that are occupied by the listed mussels or are designated as critical habitat. The ecological monitoring sites represent highly vulnerable 2nd and 3rd order streams (by the Strahler system), while the occupied streams and designated critical habitat are dominated by higher order streams (mostly 3rd through 5th order with an occasional 2nd order stream). In addition, a number of the listed mussels (i.e., shiny pigtoe, heavy pigtoe, ovate clubshell and southern clubshell mussels) and designated critical habitat for the ovate and southern clubshell mussels are located in watersheds that are not located in highly vulnerable areas. This is significant because the flow values used in the flow-adjusted modeling are generally from higher order streams with flow rates that are higher than those found in most of the ecological monitoring sites. There are also uncertainties associated with modeling using the Index Reservoir water body (used principally for human health exposure assessments) because the water body volume of the Index Reservoir may not be representative of the more vulnerable monitoring sites. Finally, the modeled EECs represent a 1 in 10 year return frequency from 30 years of data, while the monitoring values represent a single year maximum with most of the sites having two years of data (a selected number of sites have three years of data). Given these factors, there are many uncertainties that should be considered when directly comparing the flow-adjusted modeling EECs to the available monitoring data; however, the analysis suggests that flow-adjusted EECs may under-predict exposures in some portions of the action area, particularly areas of low flow and high atrazine use.

Additional uncertainties should be considered when comparing the modeled static water body EECs with various habitat types and monitoring data. Specifically, the modeled water body represents static water; however, in reality, many water bodies have some amount of flow. For the action area, it is expected that no-flow and low-flow water bodies are representative of the headwater streams adjacent to agricultural field. In general, it is expected that modeled atrazine concentrations in the static water body will over-estimate exposure in settings where flow is greater than those modeled and where the volume of the water body is greater than that modeled (20,000,000 liters). As demonstrated in the various comparisons between modeling and monitoring data described above, it is apparent that peak concentrations are well represented by modeling with both the static water body and flow-adjusted modeling using the Index Reservoir although some of the more vulnerable sites may be under-represented. However, longer-term concentrations (e.g. 14-, 30-, 60-, and 90-day averages) appear to be over-represented by modeling with the static water body, while these same duration-exposure concentrations may be under-represented by flow-adjusted modeling in the most vulnerable watersheds with low flow rates.

3.3 Terrestrial Plant Exposure Assessment

Terrestrial plants in riparian areas may be exposed to atrazine residues carried from application sites via surface water runoff or spray drift. Exposures can occur directly to seedlings breaking through the soil surface and through root uptake or direct deposition onto foliage to more mature plants. Riparian vegetation is important to the water and stream quality of the listed mussels because it serves as a buffer and filters out sediment, nutrients, and contaminants before they enter the watersheds associated with mussels' current and designated critical habitat. Riparian vegetation has been shown to be essential in the maintenance of a stable stream (Rosgen, 1996). Destabilization of the stream can have an adverse effect on mussel habitat quality by increasing sedimentation within the watershed.

Concentrations of atrazine on the riparian vegetation were estimated using OPP's TerrPlant model (U.S. EPA, 2005; Version 1.2.1), considering use conditions likely to occur in the watersheds associated with the listed mussel's action area. The TerrPlant model evaluates exposure to plants via runoff and spray drift and is EFED's standard tool for estimating exposure to non-target plants. The runoff loading of TerrPlant is estimated based on the solubility of the chemical and assumptions about the drainage and receiving areas. The spray drift component of TerrPlant assumes that 1% and 5% of the application rate deposits in the receiving area for ground boom and aerial applications, respectively.

Although TerrPlant calculates exposure values for terrestrial plants inhabiting two environments (i.e., dry adjacent areas and semi-aquatic areas), only the exposure values from the dry adjacent areas are used in this assessment. The 'dry, adjacent area' is considered to be representative of a slightly sloped area that receives relatively high runoff and spray drift levels from upgradient treated fields. In this assessment, the 'dry, adjacent area' scenario is used to estimate screening-level exposure values for terrestrial plants in riparian areas. The 'semi-aquatic area' is considered to be representative of depressed areas that are ephemerally flooded, such as marshes, and, therefore, is not used to estimate exposure values for terrestrial riparian vegetation.

The following input values were used to estimate terrestrial plant exposure to atrazine from all uses: solubility = 33 ppm; minimum incorporation depth = 0 (from product labels); application methods: ground boom, aerial, and granular (from product labels). The following agricultural and non-agricultural scenarios were modeled: ground/aerial application to fallow/idle land at 2.25 lbs ai/A, corn/sorghum at 2.0 lb ai/A, and forestry at 4.0 lbs ai/A, and granular application to residential lawns at 2 lbs ai/A.

Terrestrial plant EECs for non-granular and granular formulations is summarized in Table 3.16. EECs resulting from spray drift are derived for non-granular applications only.

Table 3.16 Screening-Level Exposure Estimates for Terrestrial Plants to Atrazine

Use/ App. Rate (lbs/acre)	Application Method	Total Loading to Dry Adjacent Areas (lbs/acre)	Drift EEC (lbs/acre)
Fallow/idle land / 2.25	Aerial	0.16	0.11
	Ground	0.07	0.02
Corn and Sorghum / 2.0	Aerial	0.14	0.10
	Ground	0.06	0.02
Forestry / 4.0	Aerial	0.28	0.20
	Ground	0.12	0.04
Residential / 2.0	Granular	0.04	NA

For non-granular applications of atrazine, the highest off-target loadings of atrazine predicted by TerrPlant are approximately 7% of the application rate for dry adjacent areas. As expected, resulting exposure estimates for terrestrial plants are higher for aerial than ground boom applications. Granular applications associated with residential use of atrazine result in estimated exposures, as a percentage of the associated application rate, of 2% for adjacent areas.

4. Effects Assessment

This assessment evaluates the potential for atrazine to directly or indirectly affect the listed assessed mussels and/or adversely modify designated critical habitat for the ovate and southern clubshell mussels. As previously discussed in Section 2.8, assessment endpoints for the listed mussels include direct toxic effects on the survival, reproduction, and growth of the assessed mussels, as well as indirect effects, such as reduction of the prey base and/or modification of its habitat. In addition, potential destruction and/or adverse modification of critical habitat are assessed by evaluating effects to the PCEs, which are components of the critical habitat areas that provide essential life cycle needs of the ovate clubshell and southern clubshell mussels. Critical habitat for the other assessed mussels has not been designated. Toxicity data used to evaluate direct effects, indirect effects, and adverse modification to critical habitat are summarized in Table 4.1.

Table 4.1 Summary of Toxicity Data Used to Assess Direct and Indirect Effects and Adverse Modification to Critical Habitat

Toxicity Data	Assessment Endpoint	Comment
Acute and chronic studies in freshwater aquatic invertebrates	- Direct effects to listed mussels - Indirect effects to listed mussels via reduction in food supply - Adverse Modification: Effects to food of host fish; chemical characteristics suitable to support normal behavior, growth, and viability of listed mussels	Preference given to tested species closest in taxonomy to assessed species and appropriate dietary items of assessed mussels and potential host fish
Acute and chronic studies in freshwater fish	- Indirect effects to listed mussel species via effects to host fish - Adverse Modification: Effects to fish host	Most sensitive studies used for assessment; refinements not made because identity of host fish for all assessed mussels is unknown.
Acute studies in vascular and non-vascular aquatic plants	- Indirect effects to eight listed mussels via reduction in food supply, habitat, and primary productivity - Adverse Modification: Alteration to water quality; filamentous algae on substrates; chemical characteristics suitable to support normal behavior, growth, and viability of listed mussels	Most sensitive vascular and non-vascular aquatic plant studies initially used for screening-level RQ calculations; refinements include use of threshold concentrations to predict community-level effects.
Terrestrial plant toxicity data	- Indirect effects to eight listed mussels via potential effects to habitat and water quality Adverse Modification: Alteration to stream bank stability; water quality; silt-free substrates; host fish spawning area;	Distribution of seedling emergence and vegetative vigor terrestrial plant data used in combination with toxicity data for woody vegetation, and riparian habitat characteristics.

Acute (short-term) and chronic (long-term) effects toxicity information is characterized based on registrant-submitted studies and a comprehensive review of the open literature on atrazine, consistent with the Overview Document (U.S. EPA, 2004). In addition to registrant-submitted and open literature toxicity information, indirect effects to the listed mussels, via impacts to aquatic plant community structure and function are also evaluated based on community-level threshold concentrations. Other sources of information, including use of the acute probit dose response relationship to establish the probability of an individual effect and reviews of the Ecological Incident Information System (EIIS), are conducted to further refine the characterization of potential ecological effects associated with exposure to atrazine. A summary of the available freshwater and terrestrial plant ecotoxicity information, the community-level endpoints, use of the probit dose response relationship, and the incident information for atrazine are provided in Sections 4.1 through 4.4, respectively.

With respect to atrazine degradates, including hydroxyatrazine (HA), deethylatrazine (DEA), deisopropylatrazine (DIA), and diaminochloroatrazine (DACT), it is assumed that each of the degradates are less toxic than the parent compound. As shown in Table 4.2, comparison of available toxicity information for HA, DIA, and DACT indicates lesser aquatic toxicity than the parent for freshwater fish, invertebrates, and aquatic plants.

Table 4.2 Comparison of Acute Freshwater Toxicity Values for Atrazine and Degradates

Substance Tested	Fish LC_{50} (µg/L)	Daphnid EC_{50} (µg/L)	Aquatic Plant EC_{50} (µg/L)
Atrazine	5,300	3,500	1
HA	>3,000 (no effects at saturation)	>4,100 (no effects at saturation)	>10,000
DACT	>100,000	>100,000	No data
DIA	17,000	126,000 (NOAEC: 10,000)	2,500
DEA	No data	No data	1,000

Although degradate toxicity data are not available for terrestrial plants, lesser or equivalent toxicity is assumed, given the available ecotoxicological information for other taxonomic groups including aquatic plants and the likelihood that the atrazine degradates are expected to lose efficacy as an herbicide.

Therefore, given the lesser toxicity of the degradates, as compared to the parent, concentrations of the atrazine degradates are not assessed, and the focus of this assessment is limited to parent atrazine. The available information also indicates that aquatic organisms are more sensitive to the technical grade (TGAI) than the formulated products of atrazine; therefore, the focus of this assessment is on the TGAI. A detailed summary of the available ecotoxicity information for all atrazine degradates and formulated products is presented in Appendix A.

As previously discussed in the problem formulation, the available toxicity data show that other pesticides may combine with atrazine to produce synergistic, additive, and/or antagonistic toxic interactions. The results of available toxicity data for mixtures of atrazine with other pesticides are presented in Section A.6 of Appendix A. Synergistic effects with atrazine have been demonstrated for a number of organophosphate insecticides including diazanon, chlorpyrifos, and methyl parathion, as well as herbicides including alachlor. If chemicals that show synergistic effects with atrazine are present in the environment in combination with atrazine, the toxicity of the atrazine mixture may be increased relative to the toxicity of each individual chemical, offset by other environmental factors, or even reduced by the presence of antagonistic contaminants if they are also present in the mixture. The variety of chemical interactions presented in the available data set suggest that the toxic effect of atrazine, in combination with other pesticides used in the environment, can be a function of many factors including but not necessarily limited to (1) the exposed species, (2) the co-contaminants in the mixture, (3) the ratio of atrazine and co-contaminant concentrations, (4) differences in the pattern and duration of exposure among contaminants, and (5) the differential effects of other physical/chemical characteristics of the receiving waters (e.g. organic matter present in sediment and suspended water). Quantitatively predicting the combined effects of all these variables on mixture toxicity to any given taxa with confidence is beyond the capabilities of the available data. However, a qualitative discussion of implications of the available pesticide mixture effects data involving atrazine on the confidence of risk assessment conclusions for the freshwater mussels is addressed as part of the uncertainty analysis for this effects determination.

4.1 Evaluation of Aquatic Ecotoxicity Studies

Toxicity endpoints are established based on data generated from guideline studies submitted by the registrant, and from open literature studies that meet the criteria for inclusion into the ECOTOX database maintained by EPA/Office of Research and Development (ORD) (U.S. EPA, 2004). Open literature data presented in this assessment were obtained from the 2003 atrazine IRED as well as ECOTOX information obtained on October 30, 2006. The October 2006 ECOTOX search included all open literature data for atrazine (i.e., pre- and post-IRED). In order to be included in the ECOTOX database, papers must meet the following minimum criteria:

(1) the toxic effects are related to single chemical exposure;
(2) the toxic effects are on an aquatic or terrestrial plant or animal species;
(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.

Meeting the minimum criteria for inclusion in ECOTOX does not necessarily mean that the data are suitable for use in risk estimation. Data that pass the ECOTOX screen are evaluated along with the registrant-submitted data, and may be incorporated qualitatively or quantitatively into this endangered species risk assessment. In general, effects data in the open literature that are more conservative than the registrant-submitted data are considered. Based on the results of the 2003 IRED for atrazine, potential adverse effects on sensitive aquatic plants and non-target aquatic organisms including their populations and communities, are likely to be greatest when atrazine concentrations in water equal or exceed approximately 10 to 20 μg/L on a recurrent basis or over a prolonged period of time (U.S. EPA, 2003a). Given the large amount of microcosm/mesocosm and field study data for atrazine, only effects data that are less than or more conservative than the 10 μg/L aquatic-community effect level identified in the 2003 atrazine IRED were considered. The degree to which open literature data are quantitatively or qualitatively characterized is dependent on whether the information is relevant to the assessment endpoints (i.e., maintenance of listed mussel survival, reproduction, and growth; alteration of PCEs in the critical habitat impact analysis) identified in the problem formulation. For example, endpoints such as biochemical modifications are likely to be qualitatively evaluated, because it is not possible to quantitatively link these endpoints with reduction in mussel species survival, reproduction, and/or growth (e.g., the magnitude of effect on the biochemical endpoint needed to result in effects on survival, growth, or reproduction is not known).

Citations of all open literature not considered as part of this assessment because it was either rejected by the ECOTOX screen or accepted by ECOTOX but not used (e.g., the endpoint is less sensitive and/or not appropriate for use in this assessment) are included in Appendix G. Appendix G also includes a rationale for rejection of those studies that did not pass the ECOTOX screen and those that were not evaluated as part of this ESA.

As described in Agency's Overview Document (U.S. EPA, 2004), the most sensitive endpoint for each taxa is evaluated. For this assessment, evaluated taxa include freshwater fish, freshwater aquatic invertebrates, freshwater aquatic plants, and terrestrial plants. Table 4.3 summarizes the most sensitive ecological toxicity endpoints for the eight listed mussels and their designated critical habitat, based on an evaluation of both the submitted studies and the open literature, as previously discussed. A brief summary of submitted and open literature data considered relevant to this ecological risk assessment for the eight listed mussels is presented below. Additional information is provided in Appendix A. It should be noted that Appendix A also includes ecotoxicity data for taxonomic groups that are not relevant to this assessment (i.e., birds, estuarine/marine fish and invertebrates) because the Agency is completing endangered species risk assessments for other species concurrently with this assessment.

Table 4.3 Freshwater Aquatic and Terrestrial Plant Toxicity Profile for Atrazine

Assessment Endpoint	Species	Toxicity Value Used in Risk Assessment	Citation MRID # (Author & Date)	Comment
Acute Direct Toxicity to Listed Mussels and Adverse Modification to Critical Habitat PCE (chemical characteristics essential for mussel viability)	Freshwater mussel (*Anodonta imbecillis*)	24- and 48-hour LC_{50} = >36 mg/L Probit slope unavailable	ECOTOX #50679 (Johnson et al., 1993)	Open literature study
Chronic Direct Toxicity to Listed Mussels, Indirect Toxicity to Listed Mussels via Chronic Toxicity to Zooplankton (i.e., food items), and Adverse Modification to Critical Habitat PCE (chronic effects to fish host food items)	Scud	NOAEC = 60 μg/L LOAEC = 120 μg/L	000243-77 (Macek et al., 1976)	Acceptable: 25 % reduction in development of F_1 to seventh instar at the LOAEC
Indirect Toxicity to Mussel	Rainbow	96-hour LC_{50} = 5,300	000247-16	Acceptable

Table 4.3 Freshwater Aquatic and Terrestrial Plant Toxicity Profile for Atrazine				
Assessment Endpoint	**Species**	**Toxicity Value Used in Risk Assessment**	**Citation MRID # (Author & Date)**	**Comment**
Glochidia via Direct Acute Effects to Host Fish and/or Adverse Modification to Critical Habitat PCE (acute effects to host fish)	trout	µg/L Probit slope = 2.72	(Beliles and Scott, 1965)	
Indirect Toxicity to Mussel Glochidia via Direct Chronic Effects to Host Fish and/or Adverse Modification to Critical Habitat PCE (chronic effects to host fish)	Brook trout	NOAEC = 65 µg/L LOAEC = 120 µg/L	000243-77 (Macek et al., 1976)	Acceptable full life-cycle study: 7.2% reduction in length; 16% reduction in weight occurred at the LOAEC
Indirect Toxicity to Listed Mussels via Acute Toxicity to Zooplankton (i.e., food items) and Adverse Modification to Critical Habitat PCE (acute effects to fish host food items)	Midge	48-hour LC_{50} = 720 µg/L Probit slope unavailable	000243-77 (Macek et al., 1976)	Supplemental: raw data unavailable
Indirect Toxicity to Listed Mussels and/or Adverse Modification to Critical Habitat PCE (chemical characteristics essential for mussel viability, habitat, primary productivity, water quality, filamentous algae on substrate)	4 species of freshwater algae	1-week EC_{50} = 1 µg/L	000235-44 (Torres & O'Flaherty, 1976)	Supplemental study
Indirect Toxicity to Listed Mussels and/or Adverse Modification to Critical Habitat PCE (primary productivity and water quality)	Duckweed	14-day EC_{50} = 37 µg/L	430748-04 (Hoberg, 1993)	Supplemental study: NOAEC not determined
Indirect Toxicity to Listed Mussels and/or Adverse Modification to Critical Habitat PCE (streambank stability, water quality, silt-free substrate, and host fish spawning areas)	Oat (monocot)	Tier II Seedling Emergence EC_{25} = 0.004 lb ai/A	420414-03 (Chetram, 1989)	Acceptable: EC_{50} based on reduction in dry weight
Indirect Toxicity to Listed Mussels and/or Adverse Modification to Critical Habitat PCE (streambank stability, water quality, silt-free substrate, and host fish spawning areas)	Carrot (dicot)	Tier II Seedling Emergence EC_{25} = 0.003 lb ai/A	420414-03 (Chetram, 1989)	Acceptable: EC_{50} based on reduction in dry weight

Toxicity to aquatic fish and invertebrates is categorized using the system shown in Table 4.4 (U.S. EPA, 2004). Toxicity categories for aquatic plants have not been defined.

Table 4.4 Categories of Acute Toxicity for Aquatic Organisms	
LC/EC_{50} (mg/L)	**Toxicity Category**
< 0.1	Very highly toxic
> 0.1 - 1	Highly toxic
> 1 - 10	Moderately toxic
> 10 - 100	Slightly toxic
> 100	Practically nontoxic

4.1.1 Toxicity to Freshwater Mussels

Available freshwater mussel toxicity data were used to assess potential direct acute effects of atrazine to the assessed mussel species. A summary of acute and chronic freshwater mollusk and bivalve toxicity data is provided below in Sections 4.1.1.1 and 4.1.1.2. No freshwater mussel studies were submitted; therefore, all freshwater mussel studies were located in the open literature.

4.1.1.1 Freshwater Mussels: Acute Exposure Studies

The results of two acute toxicity tests using juvenile (*i.e.,* glochidial) and mature freshwater mussels suggest that two species of unionid mussels, *Anodonta imbecillis* and *Utterbackia imbecillis*, are less sensitive to atrazine on an acute exposure basis than other freshwater invertebrates commonly used in aquatic toxicity tests (e.g., cladocerans and amphipods) (Johnson et al., 1993; Conners and Black, 2004). The results of the freshwater mussel studies obtained from the open literature are summarized in Table A-21 of Appendix A. Johnson *et al*. (1993) exposed juvenile mussels (20/concentration) to atrazine under static conditions at nominal concentrations up to 36 mg/L and evaluated survival of exposed individuals for 48 hours. Glochidia (1 to 2 days old and 7 to 10 days old) were exposed in a separate experiment for 24 hours under similar environmental conditions and exposure concentrations and evaluated for survival. The study reported LC_{50} values that were >60 mg/L for all life stages; therefore, it appears that the relative sensitivity of both the glochidial and mature mussel life stages to atrazine is similar. No acute toxicity was observed at any concentration tested. However, the methods did not report that 60 mg/L was tested either in a definitive or range-finding study. Therefore, the LC_{50} for this study is assumed to be >36 mg/L (corresponding NOAEC = 36 mg/L, the highest concentration reportedly tested). Using methods similar to the Johnson et al. (1993) study, Conners and Black (2004) report a 24-hr LC_{50} value of 214 mg/L for *U. imbecillis* glochidia for a formulated product (Atrazine 4L, 40.8% a.i.).

Guideline acute toxicity data for atrazine are also available for the Eastern oyster (*Crassostrea virginica*); however, this species inhabits estuarine/marine habitats. The results of Eastern oyster acute shell deposition studies report EC_{50} values ranging from >1,000 to >1,700 µg/L, with no effects reported at the highest atrazine test concentrations (MRIDs 466482-01 and 466482-01).

Given that the unionid mussel toxicity data from the open literature is more representative of the freshwater adult and juvenile mussel species being assessed as part of this effects determination than other tested species, and the available guideline data on estuarine/marine Eastern oysters shows no effects at the highest test concentrations of atrazine, the LC_{50} endpoint for *A. imbecillis* of >36 mg/L is used to calculate risk quotients for direct acute effects to the assessed mussels and potential critical habitat modification related to direct effects.

4.1.1.2 Freshwater Mussels: Chronic Exposure Studies

Chronic atrazine toxicity data for bivalves that are suitable for quantitative use in this risk assessment are not available from submitted studies or the open literature. However, several mollusk chronic exposure studies were located, with study durations ranging from approximately 6 to 12 weeks and endpoints including survival, fecundity, growth, and behavior. Baturo et al. (1995) did not observe any effects to *Lymnaea palustris* in a 12-week mesocosm study at concentrations up to 125 µg/L (the highest concentration tested). Streit and Peter (1978) evaluated effects to the river limpet and to leeches from a 40-day exposure duration at atrazine concentrations of 1,000 to 16,000 µg/L. Effects at the LOAEC of 1000 µg/L included increased mortality (although statistical significance was not indicated), increased ingestion, and reduced egg development. Although these studies were not considered appropriate for use in RQ calculations due to limitations in the study design and the lack of definitive NOAEC values (see Table A-21b of Appendix A), collectively, they suggest that effects to freshwater mollusks may occur at chronic exposure concentrations between 125 µg/L (NOAEC from Baturo et al., 1995) and 1,000 µg/L (LOAEC from Streit and Peter, 1978).

In the absence of appropriate chronic toxicity data for freshwater animals of similar taxa as mussels, the most sensitive endpoint across all freshwater aquatic invertebrate data was used to derive risk quotients. Uncertainties in using the most sensitive value across all species tested are discussed in Section 5.2. The most sensitive chronic endpoint for freshwater invertebrates is based on a 30-day flow-through study on the scud (*Gammarus fasciatus*), which showed a 25% reduction in the development of F_1 to the seventh instar at atrazine concentrations of 140 µg/L; the corresponding NOAEC is 60 µg/L (MRID # 000243-77).

4.1.2 Toxicity to Freshwater Fish

Freshwater fish toxicity data were used to assess potential indirect effects to the assessed mussels because the presence of suitable host fish is considered an essential elemental in the glochidial stage of the mussel's life cycle. These data are also used in the critical habitat impact analysis to assess whether atrazine may adversely modify critical habitat for the ovate and southern clubshell mussels by altering the PCE associated with the presence of fish hosts for these species. Specific host fish species for the southern clubshell mussel include the Alabama shiner and tricolor shiner (Table 2.3); however, specific host fish species for the ovate southern clubshell are unknown. Given that atrazine toxicity data are not available for the shiner, and fish hosts for the ovate southern clubshell are unknown, the most sensitive acute and chronic freshwater fish data are used in the critical habitat impact analysis. A summary of acute and chronic freshwater fish atrazine toxicity data, in addition to data from the open literature on sublethal effects, is provided below in Sections 4.1.2.1 through 4.1.2.3.

4.1.2.1 Freshwater Fish: Acute Exposure (Mortality) Studies

Freshwater fish acute toxicity studies were used to assess potential indirect effects to the glochidial stage of the assessed mussels because all assessed mussels occur within freshwater rivers and/or streams and all identified fish hosts for the assessed mussels are presumably freshwater species (see Table 2.3). Atrazine toxicity has been evaluated in numerous freshwater fish species, including rainbow trout, brook trout, bluegill sunfish, fathead minnow, tilapia, zebrafish, goldfish, and carp, and the results of these studies demonstrate a wide range of sensitivity. The range of acute freshwater fish LC_{50} values for atrazine spans one order of magnitude, from 5,300 to 60,000 µg/L; therefore, atrazine is categorized as moderately (>1,000 to 10,000 µg/L) to slightly (>10,000 to 100,000 µg/L) toxic to freshwater fish on an acute basis. The freshwater fish acute LC_{50} value of 5,300 µg/L is based on a static 96-hour toxicity test using rainbow trout (*Oncorhynchus mykiss*) (MRID # 000247-16). No sublethal effects were reported as part of this study. A complete list of all the acute freshwater fish toxicity data for atrazine is provided in Table A-8 of Appendix A.

4.1.2.2 Freshwater Fish: Chronic Exposure (Growth/Reproduction) Studies

Chronic freshwater fish toxicity studies were used to assess potential indirect effects to mussel glochidia via growth and reproduction to mussel's host fish. Freshwater fish full life-cycle studies for atrazine are available and summarized in Table A-12 of Appendix A. Following 44 weeks of exposure to atrazine in a flow-through system, statistically significant reductions in brook trout mean length (7.2%) and body weight (16%) were observed at a concentration of 120 µg/L, as compared to the control (MRID # 000243-77). The corresponding NOAEC for this study is 65 µg/L. Although the acute toxicity data for atrazine show that rainbow trout are the most sensitive freshwater fish, available chronic rainbow trout toxicity data indicate that it is less sensitive to atrazine, on a chronic exposure basis than the brook trout with respective LOAEC and NOAEC values of 1,100 µg/L and 410 µg/L. Further information on chronic freshwater fish toxicity data for atrazine is provided in Section A.2.2 of Appendix A.

4.1.2.3 Freshwater Fish: Sublethal Effects and Additional Open Literature Information

In addition to submitted studies, data were located in the open literature that report sublethal effect levels to freshwater fish that are less than the selected measures of effect summarized in Table 4.1. Although these studies report potentially sensitive endpoints, effects on survival, growth, or reproduction were not observed in the four available full life-cycyle studies at concentrations that induced the reported sublethal effects described below and in Appendix A.

Reported sublethal effects in adult largemouth bass show increased plasma vitellogenin levels in both female and male fish at 50 μg/L and decreased plasma testosterone levels in male fish at atrazine concentrations greater than 35 μg/L (Wieser and Gross, 2002 [MRID 456223-04]). Vitellogenin (Vtg) is an egg yolk precursor protein expressed normally in female fish and dormant in male fish. The presence of Vtg in male fish is used as a molecular marker of exposure to estrogenic chemicals. It should be noted, however, 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.

Effects of atrazine on freshwater fish behavior, including a preference for the dark part of the aquarium following one week of exposure (Steinberg et al., 1995 [MRID 452049-10]) and a reduction in grouping behavior following 24-hours of exposure (Saglio and Trijase, 1998 [MRID 452029-14]), have been observed at atrazine concentrations of 5 μg/L. In addition, alterations in rainbow trout kidney histology have also been observed at atrazine concentrations of 5 μg/L and higher (Fischer-Scherl et al., 1991 [MRID 452029-07]).

In salmon, atrazine effects on gill physiology and endocrine-mediated olfactory functions have been studied. Data from Waring and Moore (2004; ECOTOX #72625) suggest that salmon smolt gill physiology, represented by changes in Na-K-ATPase activity and increased sodium and potassium levels, was altered at 1 μg/L atrazine and higher. It should be noted, however, that a non-recommended solvent (methylated industrial spirits) was used in this study. Also, since the assessed freshwater mussels are located in the southeastern and midwestern United States, seawater survival is not a relevant endpoint for potential host fish. Moore and Lower (2001; ECOTOX #67727) reported that endocrine-mediated functions of male salmon parr were affected at 1 μg/L atrazine. The reproductive priming effect of the female pheromone prostaglandin $F_{2\alpha}$ on the levels of expressible milt in males was reduced after exposure to atrazine at 1 μg/L. Although the hypothesis was not tested, the study authors suggest that exposure of smolts to atrazine during the freshwater stage may potentially affect olfactory imprinting to the natal river and subsequent homing of adults. However, no quantitative relationship is established between reduced olfactory response of male epithelial tissue to the female priming hormone in the laboratory and reduction in salmon reproduction (i.e., the ability of male salmon to detect, respond to, and mate with ovulating females). A negative control was not included as part of the study design; therefore, potential solvent effect cannot be evaluated. Furthermore, the study did not determine whether the decreased response of olfactory epithelium to specific chemical stimuli would likely impair similar responses in intact fish.

Although these studies raise questions about the effects of atrazine on plasma steroid levels, behavior modifications, gill physiology, and endocrine-mediated functions in freshwater and anadromous fish, it is not possible to quantitatively link these sublethal effects to the selected assessment endpoints for the listed mussels (i.e., survival, growth, and reproduction of individuals and maintenance of critical habitat PCEs). Also, effects on survival, growth, or reproduction were not observed in the four available full life-cycle studies at concentrations that induced these reported sublethal effects. Therefore, potential sublethal effects on fish are evaluated qualitatively in Section 5.2 and not used as part of the quantitative risk characterization. Further detail on sublethal effects to fish is provided in Sections A.2.4a and A.2.4b of Appendix A.

4.1.3 Toxicity to Freshwater Invertebrates

Although the primary component of the listed mussel's diet is phytoplankton, they have also been observed to filter zooplankton. Direct effects to zooplankton resulting from exposure to atrazine could indirectly affect the listed mussels via reduction in available food. As previously discussed, freshwater mussels are capable of filter-feeding only smaller sized zooplankton (i.e., $\leq$ 250 µm); however, toxicity data on the relative sensitivity of various sizes of freshwater invertebrates to atrazine are not available. Therefore, toxicity data for the most sensitive freshwater invertebrate are used to assess potential indirect effects of atrazine to the listed mussels via reduction in available zooplankton as food.

Acute and chronic freshwater invertebrate data are also used in the critical habitat impact analysis to assess whether atrazine may adversely modify critical habitat foraging areas for the ovate and southern clubshell mussels and their host fish. This analysis is completed by considering indirect effects to the listed mussels and their host fish, based on direct effects to dietary food items. As previously discussed, specific host fish species for the southern clubshell mussel include the Alabama shiner and tricolor shiner; however, specific host fish species for the ovate southern clubshell are unknown. Shiners and other warmwater fish likely to be hosts for the glochidial stage of the ovate and southern clubshell mussels are assumed to primarily consume aquatic invertebrates including aquatic insect larvae; however the relative percentage of various aquatic invertebrates in the diet is unknown.

A summary of acute and chronic freshwater invertebrate data is provided below in Sections 4.1.3.1 and 4.1.3.2, respectively. All available open literature data for freshwater aquatic invertebrates that may be consumed by the listed mussels and/or fish hosts of the ovate and southern clubshell mussels are less sensitive than the submitted atrazine toxicity data.

4.1.3.1 Freshwater Invertebrates: Acute Exposure Studies

Atrazine is classified as highly toxic to slightly toxic to aquatic invertebrates. There is a wide range of EC_{50}/LC_{50} values for freshwater invertebrates with values ranging from 720 to >33,000 µg/L. The lowest freshwater LC_{50} value of 720 µg/L is based on an acute 48-hour static toxicity test for the midge, *Chironomus tentans* (MRID # 000243-77). Further evaluation of the available acute toxicity data for the midge shows high variability with the LC_{50} values, ranging from 720 to >33,000 µg/L. With the exception of the midge, reported acute toxicity values for the other five freshwater invertebrates tested (including the water flea, scud, stonefly, leech, and snail) are 3,500 µg/L and higher. Because the listed mussels are likely to consume smaller, pelagic invertebrates, such as the water flea, the lowest water flea LC_{50} value of 3,500 µg/L (MRID # 450874-13) is used to characterize and refine the potential acute toxicity of atrazine to zooplankton. Further evaluation of the available acute toxicity data for the water flea also shows high variability similar to other freshwater invertebrates with LC_{50} values ranging from 3,500 to >30,000 µg/L. All of the available acute toxicity data for freshwater invertebrates are provided in Section A.2.5 and Table A-18 of Appendix A. The LC_{50}/EC_{50} distribution for freshwater invertebrates is graphically represented in Figure 4.1. The columns represent the lowest reported value for each species, and the positive y error bar represents the maximum reported value. Values in parentheses represent the number of studies included in the analyses.

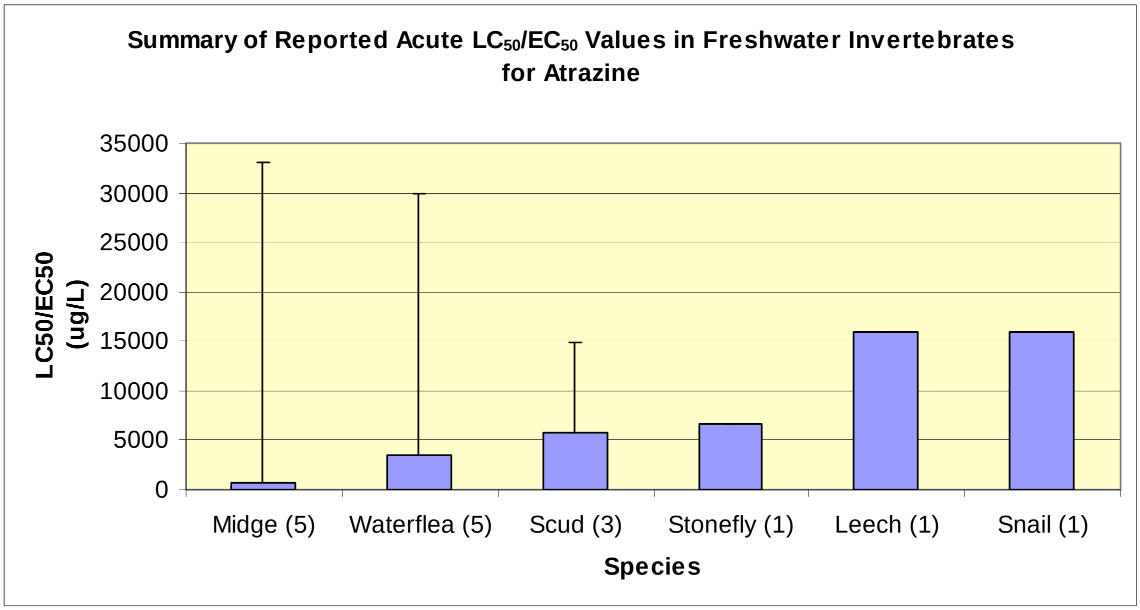

Figure 4.1 Summary of Reported Acute LC_{50}/EC_{50} Values in Freshwater Invertebrates for Atrazine

4.1.3.2 Freshwater Invertebrates: Chronic Exposure Studies

The most sensitive chronic endpoint for freshwater invertebrates is based on a 30-day flow-through study on the scud (*Gammarus fasciatus*), with respective NOAEC and LOAEC values of 60 and 140 µg/L, based on a 25% reduction in the development of F_1 to the seventh instar (MRID # 000243-77) (see Section 4.1.1.2). Although the acute toxicity data for atrazine show that the midge (*Chironomus tentans*) is the most sensitive freshwater invertebrate, available chronic midge toxicity data indicate that it is less sensitive to atrazine, on a chronic exposure basis, than the scud, with respective LOAEC and NOAEC values of 230 µg/L and 110 µg/L. The most sensitive chronic endpoint for zooplankton is based on a 21-day flow-through study on the water flea (*Daphnia magna*), which showed a 54% reduction in survival of F_0 young/female at atrazine concentrations of 250 µg/L; the corresponding NOAEC is 140 µg/L (MRID # 000243-77). Additional information on the chronic toxicity of atrazine to freshwater invertebrates is provided in Section A.2.6 and Table A-20 of Appendix A.

4.1.4 Toxicity to Aquatic Plants

Aquatic plant toxicity studies were used as one of the measures of effect to evaluate whether atrazine may affect primary production. In addition, aquatic plants including phytoplankton are a primary food source of both the juvenile and adult life stages of the listed freshwater mussels. In the watersheds within the action area for the mussels, primary productivity is essential for supporting the growth and abundance of the listed mussels. In addition, freshwater vascular and non-vascular plant data are used to evaluate a number of the PCEs associated with the critical habitat impact analysis. Specifically, non-vascular plant data are used to determine whether adverse modification to critical habitat may occur via changes in the amount of attached filamentous algae on substrates. In addition, both vascular and non-vascular aquatic plant data are used in the critical habitat impact analysis to determine whether water quality parameters including oxygen content, and suitable habitat for host fish of the ovate and southern clubshell mussels may be adversely modified.

Two types of studies were used to evaluate the potential of atrazine to affect primary productivity. Laboratory studies were used to determine whether atrazine may cause direct effects to aquatic plants. In addition, the community-level effect threshold concentrations, described in Section 4.2, were used to further characterize potential community-level effects to the listed mussel species resulting from potential effects to aquatic plants. A summary of the laboratory data for aquatic plants is provided in Section 4.1.4.1. A description of the threshold concentrations used to evaluate community-level effects is included in Section 4.2.

4.1.4.1 Aquatic Plants: Laboratory Data

Numerous aquatic plant toxicity studies have been submitted to the Agency. A summary of the data for freshwater vascular and non-vascular plants is provided below. Section A.4.2 and Tables A-40 and A-41 of Appendix A include a more comprehensive description of these data.

The Tier II results for freshwater aquatic plants produced EC_{50} values for four different species of freshwater algae at concentrations as low as 1 µg/L, based on data from a 7-day acute study (MRID # 000235-44). Vascular plants are less sensitive to atrazine than freshwater non-vascular plants with an EC_{50} value of 37 µg/L, based on reduction in duckweed growth (MRID # 430748-04).

Comparison of atrazine toxicity levels for three different endpoints in algae suggests that the endpoints in decreasing order of sensitivity are cell count, growth rate and oxygen production (Stratton, 1984). Walsh (1983) exposed *Skeletonema costatum* to atrazine and concluded that atrazine is only slightly algicidal at relatively high concentrations (i.e., 500 and 1,000 µg/L). Caux et al. (1996) compared the cell count IC_{50} and fluorescence LC_{50} and concluded that atrazine is algicidal at concentrations affecting cell counts. Abou-Waly et al. (1991) measured growth rates on days 3, 5, and 7 for two algal species. The pattern of atrazine effects on growth rates differs sharply between the two species.

Atrazine had a strong early effect on *Anabaena flos-aquae* followed by rapid recovery in clean water (i.e., EC_{50} values for days 3, 5, and 7 are 58, 469, and 766 µg/L, respectively). The EC_{50} values for *Selenastrum capricornutum* continued to decline from day 3 through 7 (i.e., 283, 218, and 214 µg/L, respectively). Based on these results, it appears that the timing of peak effects for atrazine may differ depending on the test species.

It should be noted that recovery from the effects of atrazine and the development of resistance to the effects of atrazine in some vascular and non-vascular aquatic plants have been reported and may add uncertainty to these findings. However, reports of recovery are often based on differing interpretations of recovery. Thus, before recovery can be considered as an uncertainty, an agreed upon interpretation is needed. For the purposes of this assessment, recovery is defined as a return to pre-exposure levels for the *affected population*, not for a replacement population of more tolerant species. Further research would be necessary in order to quantify the impact that recovery and resistance would have on aquatic plants.

4.1.5 Freshwater Field Studies

Microcosm and mesocosm studies with atrazine provide measurements of primary productivity that incorporate the aggregate responses of multiple species in aquatic plant communities. Because plant species vary widely in their sensitivity to atrazine, the overall response of the plant community may be different from the responses of the individual species measured in laboratory toxicity tests. Mesocosm and microcosm studies allow observation of population and community recovery from atrazine effects and of indirect effects on higher trophic levels. In addition, mesocosm and microcosm studies, especially those conducted in outdoor systems, incorporate partitioning, degradation, and dissipation, factors that are not usually accounted for in laboratory toxicity studies, but that may influence the magnitude of ecological effects.

Atrazine has been the subject of many mesocosm and microcosm studies in ponds, streams, lakes, and wetlands. The durations of these studies have ranged from a few weeks to several years at exposure concentrations ranging from 0.1 µg/L to 10,000 µg/L. Most of the studies have focused on atrazine effects on phytoplankton, periphyton, and macrophytes; however, some have also included measurements on animals.

As described in the 2003 IRED for atrazine (U.S. EPA, 2003a), potential adverse effects on sensitive aquatic plants and non-target aquatic organisms including their populations and communities are likely to be greatest when atrazine concentrations in water equal or exceed approximately 10 to 20 µg/L on a recurrent basis or over a prolonged period of time. A summary of all the freshwater aquatic microcosm, mesocosm, and field studies that were reviewed as part of the 2003 IRED is included in Section A.2.8a and Tables A-22 through A-24 of Appendix A. Given the large amount of microcosm and mesocosm and field study data for atrazine, only effects data less than or more conservative than the 10 µg/L aquatic community effect level identified in the 2003 IRED were considered from the open literature search that was completed in October 2006. Based on the selection criteria for review of new open literature, all of the available studies show effects levels to freshwater fish, invertebrates, and aquatic plants at concentrations greater than 10 µg/L.

It should be noted that the 10 to 20 µg/L community effect level has been further refined, since completion of the 2003 IRED. The community-level effects thresholds for various durations of exposure from 14 to 90 days are described in further detail in Section 4.2. In summary, the potential for atrazine to induce community-level effects depends on both atrazine concentration and duration. As the exposure duration increases, atrazine concentrations that may produce community level effects decrease. For example, 14-day atrazine concentrations of 38 µg/L or lower are not considered likely to result in aquatic community level effects, whereas 90-day atrazine concentrations of 12 µg/L or lower are not expected to produce community level effects.

Community-level effects to aquatic plants that are likely to result in indirect effects to the rest of the aquatic community, including the listed mussel species, are evaluated based on threshold concentrations. These threshold concentrations, which are discussed in greater detail in Section 4.2 and Appendix B, incorporate the available micro- and mesocosm data included in the 2003 IRED (U.S. EPA, 2003a) as well as additional information gathered following completion of the 2003 atrazine IRED (U.S. EPA, 2003e).

4.1.6 Toxicity to Terrestrial Plants

Terrestrial plant toxicity data are used to evaluate the potential for atrazine to affect riparian zone vegetation within the action area for the listed mussels. Riparian zone effects may result in increased sedimentation, which may impact the assessed mussel species by reducing feeding and respiratory efficiency from clogged gills, disrupting metabolic processes, reducing growth rates, increasing substrata instability, limiting burrowing activity, and physical smothering (Ellis, 1936; Stansbery, 1971; Markings and Bills, 1979; Kat, 1982; Vannote and Minshall, 1982; Aldridge et al., 1987; and Waters, 1995). As previously discussed in Section 2.5 and Appendix C, the listed mussels require stable substrates for maintenance of viable mussel beds. In addition, many of the PCEs associated with designated critical habitat for the ovate and southern clubshell mussels (i.e., geomorphically stable banks, water quality, and substrate composition, spawning habitat for host fish) rely on the presence of riparian vegetation.

Plant toxicity data from both registrant-submitted studies and studies in the scientific literature were reviewed for this assessment. Registrant-submitted studies are conducted under conditions and with species defined in EPA toxicity test guidelines. Sub-lethal endpoints such as plant growth, dry weight, and biomass are evaluated for both monocots and dicots, and effects are evaluated at both seedling emergence and vegetative life stages. Guideline studies generally evaluate toxicity to ten crop species. A drawback to these tests is that they are conducted on herbaceous crop species only, and extrapolation of effects to other species, such as the woody shrubs and trees and wild herbaceous species, contributes uncertainty to risk conclusions. Atrazine is labeled for use on conifers and softwoods; therefore, effects to evergreens would not be anticipated at exposure concentrations less than the application rate. In addition, preliminary data submitted to the Agency (discussed below) suggests that sensitive woody plant species exist; however, damage to most woody species at labeled application rates of atrazine is not expected.

Commercial crop species have been selectively bred, and may be more or less resistant to particular stressors than wild herbs and forbs. The direction of this uncertainty for specific plants and stressors, including atrazine, is largely unknown. Homogenous test plant seed lots also lack the genetic variation that occurs in natural populations; therefore, the range of effects seen from these tests is likely to be smaller than would be expected from wild populations.

Based on the results of the submitted terrestrial plant toxicity tests, it appears that seedlings are more sensitive to atrazine via soil/root uptake exposure than emerged plants via foliar routes of exposure. However, all tested plants, with the exception of corn in the seedling emergence and vegetative vigor tests and ryegrass in the vegetative vigor test, exhibited adverse effects following exposure to atrazine. Tables 4.5 and 4.6 summarize the respective seedling emergence and vegetative vigor terrestrial plant toxicity data used to derive risk quotients in this assessment.

In Tier II seedling emergence toxicity tests, the most sensitive monocot and dicot species are oats and carrots, respectively. EC_{25} values for carrots and oats, which are based on a reduction in dry weight, are 0.003 and 0.004 lb ai/A, respectively; NOAEC values for both species are 0.0025 lb ai/A. Dry weight was the most sensitive parameter evaluated; emergence was not significantly affected at any level tested.

For Tier II vegetative vigor studies, the most sensitive dicot and monocot species are the cucumber and onion, respectively. In general, dicots appear to be more sensitive than monocots via foliar routes of exposure with all tested dicot species showing a significant reduction in dry weight at EC_{25} values ranging from 0.008 to 0.72 lb ai/A. In contrast, two of the four tested monocots showed no effect to atrazine (corn and ryegrass), while EC_{25} values for onion and oats were 0.61 and 2.4 lb ai/A, respectively.

Table 4.5 Non-target Terrestrial Plant Seedling Emergence Toxicity (Tier II) Data

Surrogate Species	% ai	EC_{25} / NOAEC (lbs ai/A) Probit Slope	Endpoint Affected	MRID No. Author/Year	Study Classification
Monocot - Corn (*Zea mays*)	97.7	> 4.0 / > 4.0	No effect	420414-03 Chetram 1989	Acceptable
Monocot - Oat (*Avena sativa*)	97.7	**0.004 / 0.0025**	red. in dry weight	420414-03 Chetram 1989	Acceptable
Monocot - Onion (*Allium cepa*)	97.7	0.009 / 0.005	red. in dry weight	420414-03 Chetram 1989	Acceptable
Monocot - Ryegrass (*Lolium perenne*)	97.7	0.004 / 0.005	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Root Crop - Carrot (*Daucus carota*)	97.7	**0.003 / 0.0025**	red. in dry weight	420414-03 Chetram 1989	Acceptable

Table 4.5 Non-target Terrestrial Plant Seedling Emergence Toxicity (Tier II) Data					
Surrogate Species	**% ai**	**EC_{25} / NOAEC (lbs ai/A) Probit Slope**	**Endpoint Affected**	**MRID No. Author/Year**	**Study Classification**
Dicot - Soybean (*Glycine max*)	97.7	0.19 / 0.025	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Lettuce (*Lactuca sativa*)	97.7	0.005 / 0.005	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Cabbage (*Brassica oleracea alba*)	97.7	0.014 / 0.01	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Tomato (*Lycopersicon esculentum*)	97.7	0.034 / 0.01	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Cucumber (*Cucumis sativus*)	97.7	0.013 / 0.005	red. in dry weight	420414-03 Chetram 1989	Acceptable

Table 4.6 Non-target Terrestrial Plant Vegetative Vigor Toxicity (Tier II) Data					
Surrogate Species	**% ai**	**EC_{25} / NOAEC (lbs ai/A)**	**Endpoint Affected**	**MRID No. Author/Year**	**Study Classification**
Monocot - Corn	97.7	> 4.0 / > 4.0	No effect	420414-03 Chetram 1989	Acceptable
Monocot - Oat	97.7	2.4 / 2.0	red. in dry weight	420414-03 Chetram 1989	Acceptable
Monocot - Onion	97.7	**0.61 / 0.5**	red. in dry weight	420414-03 Chetram 1989	Acceptable
Monocot - Ryegrass	97.7	> 4.0 / > 4.0	No effect	420414-03 Chetram 1989	Acceptable
Dicot - Carrot	97.7	1.7 / 2.0	red. in plant height	420414-03 Chetram 1989	Acceptable
Dicot - Soybean	97.7	0.026 / 0.02	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Lettuce	97.7	0.33 / 0.25	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Cabbage	97.7	0.014 / 0.005	red. in dry weight	420414-03 Chetram 1989	Acceptable
Dicot - Tomato	97.7	0.72 / 0.5	red. in plant height	420414-03 Chetram 1989	Acceptable
Dicot - Cucumber	97.7	**0.008 / 0.005**	red. in dry weight	420414-03 Chetram 1989	Acceptable

In addition, a report on the toxicity of atrazine to woody plants (Wall et al., 2006; MRID 46870400-01) was reviewed by the Agency. A total of 35 species were tested at application rates ranging from 1.5 to 4.0 lbs ai/A. Twenty-eight species exhibited either no or negligible phytotoxicity. Seven of 35 species exhibited >10% phytotoxicity. However, further examination of the data indicate that atrazine application was clearly associated with severe phytotoxicity in only one species (Shrubby Althea). These data suggest that, although sensitive woody plants exist, atrazine exposure to most woody plant species at application rates of 1.5 to 4.0 lbs ai/A is not expected to cause adverse effects. A summary of the available woody plant data is provided in Table A-39b of Appendix A.

4.2 Community-Level Endpoints: Threshold Concentrations

In this ESA, direct and indirect effects to the listed mussels are evaluated in accordance with the screening-level methodology described in the Agency's Overview Document (U.S. EPA, 2004). If aquatic plant RQs exceed the Agency's non-listed species LOC (because the assessed mussels do not have an obligate relationship with any one particular plant species, but rather rely on multiple plant species), based on available EC_{50} data for vascular and non-vascular plants, risks to individual aquatic plants are assumed.

It should be noted, however, that the indirect effects and components of the critical habitat impact analyses in this assessment are unique, in that the best available information for atrazine-related effects on aquatic communities is significantly more extensive than for other pesticides. Hence, atrazine effects determinations can utilize more refined data than is generally available to the Agency. Specifically, a robust set of microcosm and mesocosm data and aquatic ecosystem models are available for atrazine that allowed EPA to refine the indirect effects and critical habitat impact analysis associated with potential aquatic community-level effects (via aquatic plant community structural change and subsequent habitat modification) to the listed mussels. Use of such information is consistent with the guidance provided in the Overview Document (U.S. EPA, 2004), which specifies that "the assessment process may, on a case-by-case basis, incorporate additional methods, models, and lines of evidence that EPA finds technically appropriate for risk management objectives" (Section V, page 31 of EPA, 2004). This information, which represents the best scientific data available, is described in further detail below and in Appendix B. This information is also considered a refinement of the 10-20 µg/L range reported in the 2003 IRED (U.S. EPA, 2003a).

The Agency has selected an atrazine level of concern (LOC) in the 2003 IRED (U.S. EPA, 2003a and b) that is consistent with the approach described in the Office of Water's (OW) draft atrazine aquatic life criteria (U.S. EPA, 2003c). Through these previous analyses (U.S. EPA, 2003a, b, and c), which reflect the current best available information, predicted or monitored aqueous atrazine concentrations can be interpreted to determine if a water body is likely to be significantly affected via indirect effects to the aquatic community. Potential impacts of atrazine to plant community structure and function that are likely to result in indirect effects to the rest of the aquatic community, including the listed mussels, are evaluated as described below.

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

To avoid having to routinely run the CASM model, simulations were conducted for a variety of actual and synthetic atrazine chemographs to determine 14-, 30-, 60-, and 90-day average concentrations that discriminated among exposures that were unlikely to exceed the CASM-based index (i.e., 5% change in the index). It should be noted that the average 14-, 30-, 60-, and 90-day concentrations were originally intended to be used as screening values to trigger a CASM run (which is used as a tool to identify the 5% index change LOC), rather than actual thresholds to be used as an LOC (U.S. EPA, 2003e). The following threshold concentrations for atrazine were identified (U.S. EPA, 2003e):

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

Effects of atrazine on aquatic plant communities that have the potential to subsequently pose indirect effects to the listed mussels and their designated critical habitat are best addressed using the robust set of micro- and mesocosm studies available for atrazine and the associated risk estimation techniques (U.S. EPA, 2003a, b, c, and e). The 14-, 30-, 60-, and 90-day threshold concentrations developed by EPA (2003e) are used to evaluate potential indirect effects to aquatic communities for the purposes of this ESA. Use of these threshold concentrations is considered appropriate because: (1) the CASM-based index meets the goals of the defined assessment endpoints for this assessment; (2) the threshold concentrations provide a reasonable surrogate for the CASM index; and (3) the additional conservatism built into the threshold concentration, relative to the CASM-based index, is appropriate for an endangered species risk assessment (i.e., the threshold concentrations were set to be conservative, producing a low level (1%) of false negatives relative to false positives). Therefore, these threshold concentrations are used to identify potential indirect effects (via aquatic plant community structural change) to the listed mussels and their designated critical habitat. If modeled atrazine EECs exceed the 14-, 30-, 60- and 90-day threshold concentrations following refinements of potential atrazine concentrations with available monitoring data, the CASM model could be employed to further characterize the potential for indirect effects. A step-wise data evaluation scheme incorporating the use of the threshold concentrations is provided in Figure 4.2. Further information on threshold concentrations is provided in Appendix B.

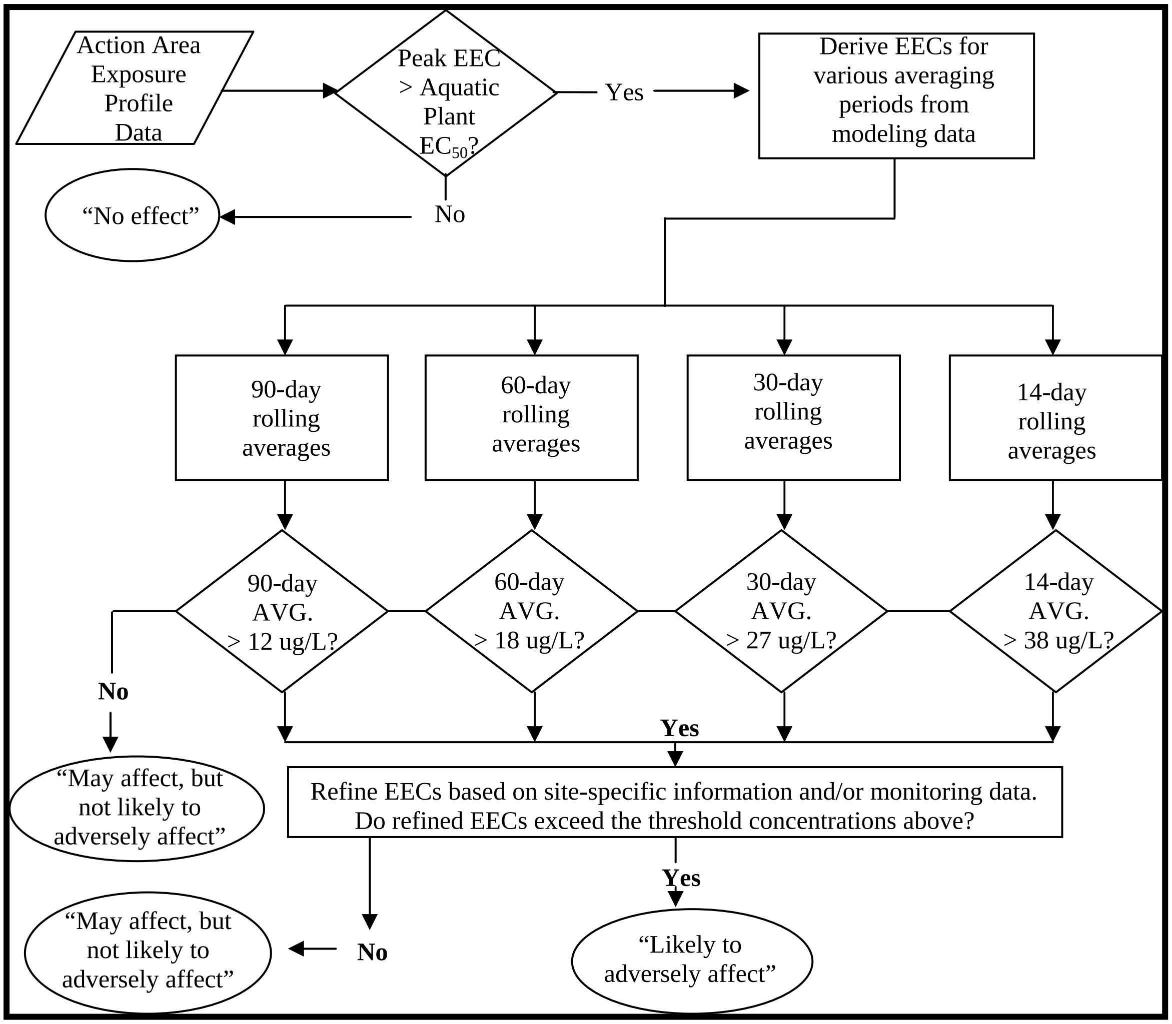

Figure 4.2 Use of Threshold Concentrations in Endangered Species Assessment

4.3 Use of Probit Slope Response Relationship to Provide Information on the Endangered Species Levels of Concern

The Agency uses the probit dose response relationship as a tool for providing additional information on the potential for acute direct effects to individual listed species and aquatic animals that may indirectly affect the listed species of concern (U.S. EPA, 2004). As part of the risk characterization, an interpretation of acute RQ for listed species is discussed. This interpretation is presented in terms of the chance of an individual event (i.e., mortality or immobilization) should exposure at the EEC actually occur for a species with sensitivity to atrazine on par with the acute toxicity endpoint selected for RQ calculation. To accomplish this interpretation, the Agency uses the slope of the dose response relationship available from the toxicity study used to establish the acute toxicity measures of effect for each taxonomic group that is relevant to this assessment. The individual effects probability associated with the acute RQ is based on the mean estimate of the slope and an assumption of a probit dose response relationship. In addition to a single effects probability estimate based on the mean, upper and lower estimates of the effects probability are also provided to account for variance in the slope, if available. The upper and lower bounds of the effects probability are based on available information on the 95% confidence interval of the slope. A statement regarding the confidence in the estimated event probabilities is also included. Studies with good probit fit characteristics (i.e., statistically appropriate for the data set) are associated with a high degree of confidence. Conversely, a low degree of confidence is associated with data from studies that do not statistically support a probit dose response relationship. In addition, confidence in the data set may be reduced by high variance in the slope (i.e., large 95% confidence intervals), despite good probit fit characteristics. In the event that dose response information is not available to estimate a slope, a default slope assumption of 4.5 (lower and upper bounds of 2 to 9) (Urban and Cook, 1986) is used.

Individual effect probabilities are calculated using an Excel spreadsheet tool IECV1.1 (Individual Effect Chance Model Version 1.1) developed by the U.S. EPA, OPP, Environmental Fate and Effects Division (June 22, 2004). The model allows for such calculations by entering the mean slope estimate (and the 95% confidence bounds of that estimate) as the slope parameter for the spreadsheet. In addition, the acute RQ is entered as the desired threshold.

4.4 Incident Database Review

A number of incidents have been reported in which atrazine has been associated with some type of environmental effect. Incidents are maintained and catalogued by EFED in the Ecological Incident Information System (EIIS). Each incident is assigned a level of certainty from 0 (unrelated) to 4 (highly probable) that atrazine was a causal factor in the incident. As of the writing of this assessment, 358 incidents are in EIIS for atrazine spanning the years 1970 to 2005. Most (309/358, 86%) of the incidents involved damage to terrestrial plants, and most of the terrestrial plant incidences involved damage to crops treated directly with atrazine. Of the remaining 49 incidents, 47 involved aquatic animals and 2 involved birds. Because the species included in this effects determination are aquatic species, incidents involving aquatic animals assigned a certainty index of 2 (possible) or higher (N=33) were re-evaluated. Results are summarized below, and additional details are provided in Appendix E. The 33 aquatic incidents were divided into three categories:

1. Aquatic incidents in which atrazine concentrations were confirmed to be sufficient to either cause or contribute to the incident, including directly via toxic effects to aquatic organisms or indirectly via effects to aquatic plants, resulting in depleted oxygen levels;
2. Aquatic incidents in which insufficient information is available to conclude whether atrazine may have been a contributing factor – these may include incidents where there was a correlation between atrazine use and a fish kill, but the presence of atrazine in the affected water body was not confirmed; and
3. Aquatic incidents in which causes other than atrazine exposure are more plausible (e.g., presence of substance other than atrazine confirmed at toxic levels).

The presence of atrazine at levels thought to be sufficient to cause either direct or indirect effects was confirmed in 3 (9%) of the 33 aquatic incidents evaluated. Atrazine use was also correlated with 11 (33%) additional aquatic incidents where its presence in the affected water was not confirmed, but the timing of atrazine application was correlated with the incident. Therefore, a definitive causal relationship between atrazine use and the incident could not be established. The remaining 19 incidents (58%) were likely caused by some factor other than atrazine. Other causes primarily included the presence of other pesticides at levels known to be toxic to affected animals. Although atrazine use was likely associated with some of the reported incidents for aquatic animals, they are of limited utility to this assessment for the following reasons:

- No incidents in which atrazine is likely to have been a contributing factor have been reported after 1998. A number of label changes, including cancellation of certain uses, reduction in application rates, and harmonization across labels to require setbacks for applications near waterbodies, have occurred since that time. For example, several incidents occurred in ponds that are adjacent to treated fields. The current labels require a 66-foot buffer between application sites and water bodies.

- The habitat of the assessed species is not consistent with environments in which incidents have been reported. For example, no incidents in streams or rivers were reported.

Although the reported incidents suggest that high levels of atrazine may result in impacts to aquatic life in small ponds that are in close proximity to treated fields, the incidents are of limited utility to the current assessment. However, the lack of recently reported incidents in flowing waters does not indicate that effects have not occurred. Further information on the atrazine incidents and a summary of uncertainties associated with all reported incidents are provided in Appendix E.

5. Risk Characterization

Risk characterization is the integration of the exposure and effects characterizations to determine the potential ecological risk from varying atrazine use scenarios within the action area and likelihood of direct and indirect effects on the listed mussels and their designated critical habitat. The risk characterization provides an estimation (Section 5.1) and a description (Section 5.2) of the likelihood of adverse effects; articulates risk assessment assumptions, limitations, and uncertainties; and synthesizes an overall conclusion regarding the likelihood of adverse effects to the listed mussels and/or their designated critical habitat (i.e., “no effect,” “likely to adversely affect,” or “may affect, but not likely to adversely affect”). In accordance with the Agency’s Overview Document (U.S. EPA, 2004), RQs derived in the risk estimation are based on screening-level EECs using the PRZM-EXAMS static water body modeling. In the risk description, atrazine exposures are refined by considering the available targeted and non-targeted monitoring data, flow-adjusted EECs, and variability in flow rates between streams and rivers where the listed mussels occur and those vulnerable watersheds where targeted monitoring data was collected.

As previously discussed in the problem formulation, the atrazine effects determination for the stirrupshell mussel is ‘no effect’ because the species is presumed to be extirpated (Hartfield, 2006). Therefore, risks associated with exposure to atrazine in the defined action area are characterized for the remaining seven assessed listed mussel species (i.e., pink pearly mucket, rough pigtoe, shiny pigtoe, fine-rayed pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell).

As previously discussed in the effects assessment (Section 4), the toxicity of the atrazine degradates has been shown to be less than the parent compound based on the available toxicity data for freshwater fish, invertebrates, and aquatic plants; therefore, the focus of the risk characterization is parent atrazine (i.e., RQ values were not derived for the degradates).

5.1 Risk Estimation

Risk was estimated by calculating the ratio of the screening-level estimated environmental concentration (EEC) (Table 3.6) and the appropriate toxicity endpoint (Table 4.3). This ratio is the risk quotient (RQ), which is then compared to pre-established acute and chronic levels of concern (LOCs) for each category evaluated (Appendix F). Screening-level RQs are based on the most sensitive endpoints and the following surface water concentration scenarios for atrazine:

- corn use @ 2.5 lbs ai/A; 2 applications
- sorghum use @ 2 lbs ai/A; 1 application
- fallow/idle land use @ 2.25 lb ai/A; 1 application
- forestry use @ 4.0 lb ai/A; 1 application
- residential granular use @ 2 lb ai/A; 2 applications with 30 days between applications
- residential liquid use @ 1 lb ai/A; 2 applications with 30 days between applications
- turf granular use @ 2 lb ai/A; 2 applications with 30 days between applications
- turf liquid use @ 1 lb ai/A; 2 applications with 30 days between applications
- rights-of-way liquid use @ 1 lb ai/A; 1 application

EECs are also derived for terrestrial plants, as discussed in Section 3.3, based on the highest application rates of atrazine relevant within the action area.

As previously discussed in Section 3.2, the action area for the listed mussels was divided into four regions representing the northern, eastern, southern, and western portions of the listed mussel’s range and designated critical habitat. As shown in Table 3.2, all seven of the assessed mussel species, including designated critical habitat for the ovate and southern clubshell mussels, are known to occur in the southern region; four of the seven species also occur in the northern and eastern regions (i.e., pink pearly mucket, rough pigtoe, shiny pigtoe, and fine-rayed pigtoe), and the western region is inhabited by only the pink pearly mucket. The highest screening-level EEC (corn in the southern region) was initially used to derive risk quotients. In cases where LOCs were not exceeded based on the highest EEC, additional RQs were not derived because it was assumed that RQs for lower EECs would also not exceed LOCs. However, if LOCs were exceeded based on the highest EEC, use/region-specific RQs were also derived. The highest EEC used in the risk estimation is based on atrazine use in the southern region because it was the location of the highest EEC, and all species are known to occur and/or have designated critical habitat in this region of the action area.

In cases where the screening-level RQ exceeds one or more LOCs (i.e., "may affect"), additional factors, including the listed mussels life history characteristics, refinement of the screening-level EECs using site-specific information, available monitoring data, and consideration of community-level threshold concentrations, are considered and used to characterize the potential for atrazine to adversely affect the listed mussels and their designated critical habitat. Risk estimations of direct and indirect effects of atrazine to the seven listed mussels are provided in Sections 5.1.1 and 5.1.2, respectively. Risk estimates of potential adverse modification to designated critical habitat for the ovate and southern clubshell mussels are presented in Section 5.1.3.

5.1.1 Direct Effects

Direct effects to the listed mussels associated with acute and chronic exposure to atrazine are based on the most sensitive toxicity data available for freshwater mussels and other surrogate aquatic invertebrates. Acute toxicity data specific for freshwater mussels are available; however, no chronic data for freshwater mussels exist. RQs used to estimate acute direct effects to the listed mussels are provided in Table 5.1 below. The peak screening-level EEC (109 μg/L) used to derive acute RQs for all of the assessed listed mussels is representative of the highest modeled EEC from the southern corn use scenario. Based on the highest screening-level EEC modeled for atrazine use patterns within the four regions, acute RQs do not exceed the endangered species LOC of 0.05. Therefore, atrazine is not expected to result in acute direct effects to listed mussels within the action area. These RQs are further characterized in Section 5.2.1.1.

Table 5.1 Summary of Direct Effect Acute RQs for the Listed Mussels

Effect to Listed Mussels	Surrogate Species	Toxicity Value (μg/L)	EEC (μg/L)	RQ	Probability of Individual Effect	LOC Exceedance and Risk Interpretation
Acute Direct Toxicity	Freshwater Mussel	LC_{50} = >36,000[a]	Peak = 109[b]	0.003	1 in 2.7E+29 (1 in 4.4E+06 to 1 in 5.1E+113)[c]	No[d]

[a] Based on 48-hour LC50 value of >36,000 μg/L for freshwater mussels and glochidia (ECOTOX #50679).
[b] Based on peak southern corn screening-level EEC (Table 3.6).
[c] A probit slope value for the acute mussel toxicity test is not available; therefore the effect probability was calculated based on a default slope assumption of 4.5 with upper and lower 95% confidence intervals of 2 and 9 (Urban and Cook, 1986).
[d] RQ < acute endangered species LOC of 0.05.

In the absence of chronic toxicity data to freshwater mussels, the most sensitive NOAEC value from the available freshwater invertebrate data was used as a surrogate to derive chronic risk quotients for freshwater mussels. RQs used to estimate chronic direct effects to the listed mussels are provided in Table 5.2. Chronic RQs exceed LOCs based on atrazine use on corn and sorghum in all four regions of the action area, with RQs ranging from 1 to 1.8. In addition, chronic RQs based on atrazine use on fallow/idle land in the western and southern regions of the action area also exceed LOCs. Because all seven of the assessed mussels are known to occur in the southern region of the action area, direct chronic effects may occur based on atrazine use on fallow/idle land, as well as corn and sorghum. Chronic RQs based on atrazine use on forestry, residential, turf, and rights-of-way are less than LOCs; therefore, direct chronic effects to the listed mussels are not expected based on these use patterns. In summary, the chronic RQs derived using screening-level EECs and the most sensitive aquatic invertebrate NOAEC resulted in LOC exceedance for atrazine use on corn, sorghum, and fallow/idle land within the action area; however, atrazine use patterns related to forestry, residential, turf, and rights-of-way did not exceed the chronic LOC. These RQs are further characterized in Section 5.2.1.1.

Table 5.2 Summary of Direct Effect Chronic RQs for the Listed Mussels

Effect to Listed Mussels	Use (appl. Method; rate; # appl.; interval between appl.)	Range of 21-day EECs (µg/L)[a]	Freshwater Invertebrate Chronic RQ (NOAEC= 60 µg/L[b])	LOC Exceedance
Chronic Direct Toxicity	Corn (aerial liquid; 2.5 lb ai/A; 2 appl.)	78.7 - 107	**1.3 – 1.8**	Yes[c]
	Sorghum (aerial liquid; 2 lb ai/A; 1 appl.)	57.4 - 68.1	**1.0 – 1.1**	Yes[c]
	Fallow/Idle land (aerial liquid; 2.25 lb ai/A; 1 appl.)	West: 103 South: 58.0 East: 54.0 North: 51.5	**1.7** **1.0** 0.9 0.9	West: Yes[c] South: Yes[c] East: No[d] North: No[d]
	Forestry (aerial liquid; 4 lb ai/A; 1 appl.)	26.8 - 47.2	0.4 – 0.8	No[d]
	Residential (granular; 2 lb ai/A; 2 appl.; 30 d interval) and (liquid; 1 lb ai/A; 1 appl.)	7.5 – 19.4	0.1 – 0.3	No[d]
	Turf (granular; 2 lb ai/A; 2 appl.; 30 d interval) and (liquid; 1 lb ai/A; 1 appl.)	6.6 – 17.7	0.1 – 0.3	No[d]
	Rights-of-Way (liquid; 1 lb ai/A; 1 appl.)	2.3 – 3.8	<0.1	No[d]

[a] 21-day screening-level EECs include the range of modeled concentrations from all four regions of the action area (Table 3.6). 21-day screening-level EECs from each of the four regions are provided for the fallow/idle land scenario in order to differentiate the specific region where chronic freshwater invertebrate RQs exceed LOCs.
[b] Based on 30-day NOAEC value of 60 µg/L for the scud (MRID # 000243-77).
[c] RQ > chronic LOC of 1.0. Further evaluation of the RQs is necessary to determine if atrazine is likely to adversely affect the assessed species.
[d] RQ < chronic LOC of 1.0.

5.1.2 Indirect Effects

Pesticides have the potential to exert indirect effects upon listed species by inducing changes in structural or functional characteristics of affected communities. Perturbation of forage or prey availability, adverse impacts to host fish, and alteration of the extent and nature of habitat are examples of indirect effects. A number of these indirect effects, including impacts to host fish, reduction of available food for listed mussels, and alteration of habitat, are also considered as part of the critical habitat adverse modification evaluation in Section 5.1.3.

In conducting a screen for indirect effects, direct effects LOCs for each taxonomic group (i.e., freshwater fish, invertebrates, aquatic plants, and terrestrial plants) are employed to make inferences concerning the potential for indirect effects upon listed species that rely upon non-listed organisms in these taxonomic groups as resources critical to their life cycle (U.S. EPA, 2004). This approach used to evaluate indirect effects to listed species is endorsed by the Services (USFWS/NMFS, 2004b). If no direct effect listed species LOCs are exceeded for non-endangered organisms that are critical to the listed mussel's life cycle, indirect effects to the listed mussels are not expected to occur.

If LOCs are exceeded for freshwater invertebrates (i.e., zooplankton) or aquatic non-vascular plants (i.e., phytoplantkon) that are food items of the listed mussels, there is a potential for atrazine to indirectly affect the listed mussels by reducing available food supply. In addition, if LOCs are exceeded for freshwater fish that are host fish of the listed mussel glochidia, atrazine may indirectly affect the listed mussels by disrupting the parasitic glochidial life cycle stage of the mussel that is reliant on suitable host fish. In such cases, the dose response relationship from the toxicity study used for calculating the RQ of the surrogate prey item or host fish is analyzed to estimate the probability of acute effects associated with an exposure equivalent to the EEC. The greater the probability that exposures will produce effects on a taxa, the greater the concern for potential indirect effects for listed species dependant upon that taxa (U.S. EPA, 2004).

As an herbicide, indirect effects to the listed mussels from potential effects on primary productivity of aquatic plants are a principle concern. If plant RQs fall between the endangered species and non-endangered species LOCs, a no effect determination for listed species that rely on multiple plant species to successfully complete their life cycle (termed plant dependent species) is determined. If plant RQs are above non-endangered species LOCs, this could be indicative of a potential for adverse effects to those listed species that rely either on a specific plant species (plant species obligate) or multiple plant species (plant dependant) for some important aspect of their life cycle (U.S. EPA, 2004). Based on the information provided in Appendix C, the listed mussels do not rely on a specific plant species (i.e., the listed mussels do not have an obligate relationship with a specific species of aquatic plant).

Direct effects to riparian zone vegetation may also indirectly affect the listed mussels by reducing water quality and available habitat via increased sedimentation. Direct impacts to the terrestrial plant community (i.e., riparian habitat) are evaluated using submitted terrestrial plant toxicity data. If terrestrial plant RQs exceed the Agency's LOC for direct effects to non-endangered plant species, based on EECs derived using EFED's Terrplant model (Version 1.2.1) and submitted guideline terrestrial plant toxicity data, a conclusion that atrazine may affect the listed mussels via potential indirect effects to the riparian habitat (and resulting impacts to habitat due to increased sedimentation) is made. Further analysis of the potential for atrazine to affect the listed mussels via reduction in riparian habitat includes a description of the importance of riparian vegetation to the assessed species and types of riparian vegetation that may potentially be impacted by atrazine use within the action area.

In summary, the potential for indirect effects to the listed mussels was evaluated using methods outlined in U.S. EPA (2004) and described below in Sections 5.1.2.1 through 5.1.2.4.

5.1.2.1 Evaluation of Potential Indirect Effects via Reduction in Food Items (Freshwater Zooplankton and Phytoplankton)

Freshwater mussels are filter-feeders, consuming primarily phytoplankton, but also detritus, zooplantkton, and other microorganisms (Ukeles, 1971; Coker et al., 1921; Churchill and Lewis, 1924; and Fuller, 1974). Data on the relative percentage of each type of food item in the mussel's diet are unavailable. Potential indirect effects from direct effects on plant and animal food items (i.e., phytoplankton and zooplankton) were evaluated by considering the diet of the assessed mussels and the effects data for the most sensitive food item in each taxonomic group (i.e., freshwater algae and midge). The acute RQs used to characterize potential indirect effects to the assessed mussels from direct acute effects on freshwater phytoplankton and zooplankton food sources are provided in Table 5.3. Acute RQs are presented for the atrazine use rates that correspond to agricultural and non-agricultural EECs across the four regions in order to provide a range of possible acute RQ values.

Indirect effects to the listed mussels based on direct acute effects to dietary items may occur for phytoplankton and zooplankton. As shown in Table 5.3, acute LOCs are exceeded for phytoplankton for all labeled uses of atrazine within the action area, with RQs ranging from 2.4 to 109. Acute RQs for zooplankton exceed LOCs for corn, sorghum, fallow/idle land, and forestry (in all geographic regions except the west) uses of atrazine, with values ranging from 0.06 to 0.15, based on the most sensitive freshwater invertebrate acute toxicity endpoint. However, acute RQs based on non-agricultural uses of atrazine on residential, turf, and rights-of-way, as well as forestry uses in the western region of the action area, are less than LOCs for aquatic invertebrates. These risk quotients are further characterized in Section 5.2.1.2.

Table 5.3 Summary of Acute RQs Used to Estimate Indirect Effects to the Listed Mussels via Direct Effects on Dietary Food Items					
Use (appl. method; rate; # appl.; interval between appl.)	**Range of Peak EECs[a]**	**Direct Effects to Phytoplankton**		**Direct Effects to Zooplankton**	
		Acute RQ (non-vascular plant EC_{50} = 1 µg/L)[b]	**LOC Exceedance and Risk Interpretation**	**Acute RQ (midge LC_{50} = 720 µg/L)[c]**	**LOC Exceedance and Risk Interpretation**
Corn (aerial liquid; 2.5 lb ai/A; 2 appl.)	80.1 - 109	80.1 - 109	Yes[d]	**0.11 – 0.15[e]**	Yes[d]
Sorghum (aerial liquid; 2 lb ai/A; 1 appl.)	58.4 – 69.2	58.4 – 69.2	Yes[d]	**0.08 – 0.09**	Yes[d]
Fallow/Idle land (aerial liquid; 2.25 lb ai/A; 1 appl.)	51.6 - 103	51.6 - 103	Yes[d]	**0.07 – 0.14**	Yes[d]
Forestry (aerial liquid; 4 lb ai/A; 1 appl.)	North: 48.5 South: 46.1 East: 44.2 West: 27.4	North: 48.5 South: 46.1 East: 44.2 West: 27.4	Yes[d]	**North: 0.07** **South: 0.06** **East: 0.06** West: 0.04	North: Yes[d] South: Yes[d] East: Yes[d] West: No[f]
All other non-agricultural uses	2.4 - 20	2.4 - 20	Yes[d]	<0.03	No[f]

[a] Peak screening-level EECs include the range of modeled concentrations from all four regions of the action area (Table 3.6).
[b] Based on 1-week EC_{50} value of 1 µg/L for four species of freshwater algae (MRID # 000235-44).
[c] Based on 48-hour LC_{50} value of 720 for the midge (MRID #000243-77). Slope information on the toxicity study that was used to derive the RQ for the midge is not available. Therefore, the probability of an individual effect was calculated using the probit slope of 4.4, which is the only technical grade atrazine value reported in the available freshwater invertebrate studies; 95% confidence intervals could not be calculated based on the available data (MRID # 452029-17; Table A-18).
[d] RQ > LOC (LOC = 1 for aquatic plants and 0.05 for invertebrates). Further evaluation of refined EECs (based on site-specific information and available monitoring data) relative to the threshold concentrations (for community-level effects) is necessary.
[e] Based on an assumed probit dose of 4.4, the range of individual effect probabilities for aquatic invertebrates at acute RQs that exceed LOCs is 1 in 6,930 (for RQ of 0.15) to 1 in 2.6E+07 (for RQ of 0.06).
[f] RQ < acute endangered species LOC of 0.05.

The screening-level methodology for aquatic plants (i.e., phytoplankton) and freshwater inveretebrates (i.e., zooplankton) assumes risks to these taxonomic groups because the RQ values shown in Table 5.3 (based on the most sensitive toxicity data for non-vascular plants and freshwater invertebrates) exceed the Agency's LOCs. Although the listed species LOC is used for freshwater invertebrates, the non-listed species LOC is used for aquatic plants because the assessed mussels do not have an obligate relationship with any one particular type of phytoplankton as a food item. Further evaluation of the potential aquatic community-level effects that may result from atrazine exposure to phytoplankton and zooplankton as food sources for the listed mussels is provided as part of the risk description in Section 5.2.1.2.

The chronic RQs used to characterize potential indirect effects to the assessed mussels from direct acute effects on freshwater zooplankton food sources are provided in Table 5.4. Based on this analysis, LOCs were exceeded for chronic exposures to freshwater invertebrates for corn (all regions), sorghum (south and east regions), and fallow/idle land (west region) uses of atrazine, with chronic RQ values ranging from 1.1 to 1.8. These exceedances are based on screening-level 21-day EECs and the toxicity data from the most sensitive freshwater invertebrate tested. Further analysis of potential chronic effects to aquatic invertebrates, as they relate specifically to zooplankton food items of the assessed mussels, is completed to determine if potential chronic risks to freshwater invertebrates are likely to adversely affect the assessed mussels in Section 5.2.1.2.

In summary, indirect effects based on direct impacts to the food supply "may affect" the seven assessed mussels, because LOCs are exceeded for aquatic plants (i.e., phytoplankton) and freshwater invertebrates (i.e., zooplankton), which are food items of freshwater mussels. Additional analysis is needed to determine if the LOC exceedances, based on the most sensitive aquatic plant and freshwater invertebrate toxicity data and the screening-level EECs, are likely to adversely affect the assessed freshwater mussels.

Table 5.4 Summary of Chronic RQs Used to Estimate Indirect Effects to the Listed Mussels via Direct Effects on Zooplankton as Dietary Food Items

Effect to Listed Mussels	Use (appl. Method; rate; # appl.; interval between appl.)	Range of 21-day EECs (µg/L)[a]	Freshwater Invertebrate Chronic RQ (NOAEC= 60 µg/L)[b]	Chronic LOC Exceeded?
Indirect effects to mussel's diet based on direct chronic toxicity to zooplankton	Corn (aerial liquid; 2 lb ai/A; 2.5 appl.)	79 – 109	**1.3 – 1.8**	Yes (all regions)[c]
	Sorghum (aerial liquid; 2 lb ai/A; 1 appl.)	West: 59 South: 62 East: 68 North: 57	West: 0.98 **South: 1.0** **East: 1.1** North: 0.95	Yes (South and East regions only)[c]
	Fallow/Idle land (aerial liquid; 2.25 lb ai/A; 1 appl.)	West: 103 South: 58 East: 54 North: 51	**West: 1.7** South: 0.97 East: 0.90 North: 0.85	Yes (West region only)[d]
	All other uses	≤47	≤0.29	No

[a] 21-day screening-level EECs include the range of modeled concentrations from all four regions of the action area (Table 3.6). 21-day screening-level EECs from each of the four regions are provided for the sorghum and fallow/idle land scenarios in order to differentiate where RQs exceed LOCs.
[b] Based on a 30-day NOAEC value of 60 µg/L for the scud (MRID #000243-77).
[c] RQ > chronic LOC of 1.0. Further evaluation of the RQs is necessary to determine if atrazine is likely to adversely affect the assessed species.
[d] RQ < chronic LOC of 1.0.

5.*1.2.2 Evaluation of Potential Indirect Effects via Reduction in Host Fish for Mussel Glochidia)*

Freshwater mussels have a unique life cycle that involves a parasitic stage on host fish. Once mussel larvae (glochidia) fully develop, they are released into the water where they attach to the gills and fins of appropriate host fishes, which they parasitize for a short time until they develop into juvenile mussels. Glochidial hosts of the assessed mussel species are summarized in Table 2.3 and include largemouth and smallmouth bass, fathead minnow, different species of shiner, and other warmwater fish. Potential indirect effects from direct effects on freshwater host fish were evaluated by considering the most sensitive freshwater fish effects data. The acute and chronic RQs used to characterize potential indirect effects to the assessed mussels from direct effects on freshwater host fish are provided in Tables 5.5 and 5.6. None of the acute LOCs were exceeded for freshwater fish based on the highest use pattern EECs; however, the chronic LOC of 1.0 was exceeded for chronic exposure for some uses based on the screening-level 60-day EECs and a NOAEC of 65 µg/L. Therefore, indirect effects to the listed mussels via direct chronic effects to host fish may occur; however, acute toxicity to host fish is not expected. These RQs are further characterized in Section 5.2.1.3.

Table 5.5 Summary of Acute RQs Used to Estimate Indirect Effects to the Listed Mussels via Direct Effects on Host Fish

Effect to Listed Mussels	Surrogate Species	Toxicity Value (µg/L)	EEC (µg/L)	RQ	Probability of Individual Effect	LOC Exceedance
Indirect effects to mussels via direct acute effects to host fish	Rainbow trout	LC_{50} = 5,300[a]	Peak = 109[b]	0.02	1 in 5.2E+05 (1 in 249 to 1 in 5.2E+10)[c]	No[d]

[a] Based on a 96-hour LC_{50} value of 5,300 µg/L for the rainbow trout (MRID #000247-16).
[b] Based on peak southern corn screening-level EEC (Table 3.6).
[c] Based on a probit slope value of 2.72 for the rainbow trout with 95% confidence intervals of 1.56 and 3.89 (MRID #000247-16).
[d] RQ < acute endangered species LOC of 0.05.

Table 5.6 Summary of Chronic RQs Used to Estimate Indirect Effects to the Listed Mussels via Direct Effects on Host Fish

Effect to Listed Mussels	Use (appl. Method; rate; # appl.; interval between appl.)	Range of 60-day EECs (µg/L)[a]	Freshwater Fish Chronic RQ (NOAEC= 65 µg/L)[b]	Chronic LOC Exceeded?
Chronic Direct Toxicity	Corn (aerial liquid; 2.5 lb ai/A; 2 appl.)	76 - 105	**1.2 – 1.6**	Yes (all regions)[c]
	Sorghum (aerial liquid; 2 lb ai/A; 1 appl.)	West: 57 South: 60 East: 66 North: 55	0.88 0.92 **1.0** 0.85	Yes (East region only)[c]
	Fallow/Idle land (aerial liquid; 2.25 lb ai/A; 1 appl.)	West: 103 South: 57 East: 54 North: 51	**1.6** 0.87 0.83 0.78	Yes (West region only)[c]
	All other uses	≤45	≤0.69	No[d]

[a] 60-day screening-level EECs include the range of modeled concentrations from all four regions of the action area (Table 3.6). 60-day screening-level EECs from each of the four regions are provided for the sorghum and fallow/idle land scenarios in order to differentiate where chronic RQs exceed LOCs.
[b] Based on a 44-week NOAEC value of 65 µg/L for the brook trout (MRID #000243-77).
[c] RQ > chronic LOC of 1.0. Further evaluation of the RQs is necessary to determine if atrazine is likely to adversely affect the assessed species.
[d] RQ < chronic LOC of 1.0.

Based on the results of this analysis, LOCs were exceeded for chronic exposures to fish for corn (all regions), sorghum (east region), and fallow/idle land (west region). These LOC exceedances were based on screening-level 60-day EECs. Further analysis of the potential effects to freshwater fish, as they relate to host availability for the assessed mussels, is completed to determine if potential chronic risks to fish are likely to adversely affect the assessed mussels in Section 5.2.1.3.

5.1.2.3 Evaluation of Potential Indirect Effects via Reduction in Habitat and/or Primary Productivity (Freshwater Aquatic Plants)

Potential indirect effects to the listed mussels via direct effects to habitat and/or primary production were assessed using RQs from freshwater aquatic vascular and non-vascular plant data as a screen. This screening-level analysis is based on the most sensitive EC_{50} value from all of the available non-vascular and vascular aquatic plant toxicity information. No known obligate relationship exists between the listed mussels and any single freshwater non-vascular or vascular plant species; therefore, endangered species RQs using the NOAEC/EC_{05} values for aquatic plants were not derived. If aquatic plant RQs exceed the Agency's non-endangered species LOC (because the assessed listed mussels rely on multiple plant species), potential community-level effects are evaluated using the threshold concentrations, as described in Section 4.2. RQs used to estimate potential indirect effects to the listed mussels from effects on aquatic plant primary productivity are summarized in Table 5.7.

Based on the results of this analysis, LOCs for direct effects to aquatic non-vascular plants are exceeded for all modeled atrazine use scenarios. LOCs for direct effects to aquatic vascular plants are also exceeded for modeled EECs based on corn, sorghum, fallow/idle land, and forestry (in the northern, southern, and eastern regions of the action area); however, RQs are less than LOCs for use scenarios including forestry in the western region of the action area, residential, turf, and rights-of-way. Therefore, atrazine may indirectly affect the seven listed mussels via effects to non-vascular aquatic plants for all modeled use scenarios and on vascular aquatic plants for the corn, sorghum, fallow/idle land, and forestry use scenarios. Further analysis of the potential effects to aquatic plant communities, as they relate to food availability and primary productivity for the assessed species, is used to determine if potential risks to aquatic plants are likely to adversely affect the assessed mussels. This refined analysis is presented in Section 5.2.1.4.

Table 5.7 Summary of RQs Used to Estimate Indirect Effects to the Listed Mussels via Effects to Aquatic Plants

Indirect Effect to Listed Mussels	Use (appl. Method; rate; # appl.; interval between appl.)	Range of Peak EECs (µg/L)[a]	Non-vascular plant RQ (EC_{50} = 1 µg/L[b])	Vascular plant RQ (EC_{50} = 37 µg/L[c])	LOC Exceedance
Reduced Habitat and/or Primary Productivity via Direct Toxicity to Aquatic Plants	Corn (aerial liquid; 2.5 lb ai/A; 2 appl.)	80.1 - 109	80.1 - 109	2.2 – 2.9	Yes[d]
	Sorghum (aerial liquid; 2 lb ai/A; 1 appl.)	58.4 – 69.2	58.4 – 69.2	1.6 – 1.9	Yes[d]
	Fallow/Idle land (aerial liquid; 2.25 lb ai/A; 1 appl.)	51.6 - 103	51.6 - 103	1.4 – 2.8	Yes[d]

Table 5.7 Summary of RQs Used to Estimate Indirect Effects to the Listed Mussels via Effects to Aquatic Plants					
Indirect Effect to Listed Mussels	**Use (appl. Method; rate; # appl.; interval between appl.)**	**Range of Peak EECs (µg/L)[a]**	**Non-vascular plant RQ (EC_{50} = 1 µg/L[b])**	**Vascular plant RQ (EC_{50} = 37 µg/L[c])**	**LOC Exceedance**
	Forestry (aerial liquid; 4 lb ai/A; 1 appl.)	North: 48.5 South: 46.1 East: 44.2 West: 27.4	27.4 – 48.5	North: 1.3 South: 1.2 East: 1.2 West: 0.7	North: Yes[d] South: Yes[d] East: Yes[d] West: Yes[e]
	Residential (granular; 2 lb ai/A; 2 appl.; 30 d interval) and (liquid; 1 lb ai/A; 1 appl.)	7.6 – 19.9	7.6 – 19.9	0.2 – 0.5	Yes[e]
	Turf (granular; 2 lb ai/A; 2 appl.; 30 d interval) and (liquid; 1 lb ai/A; 1 appl.)	6.6 – 17.9	6.6 – 17.9	0.2 – 0.5	Yes[e]
	Rights-of-Way (liquid; 1 lb ai/A; 1 appl.)	2.4 – 3.8	2.4 – 3.8	0.1	Yes[e]

[a] Peak screening-level EECs include the range of modeled concentrations from all four regions of the action area (Table 3.6). Peak screening-level EECs from each of the four regions are provided for the forestry scenario in order to differentiate where vascular plant RQs exceed LOCs.
[b] Based on 1-week EC_{50} value of 1 µg/L for four species of freshwater algae (MRID # 000235-44).
[c] Based on 14-day EC_{50} value of 37 µg/L for duckweed (MRID # 430748-08).
[d] RQ > non-endangered aquatic plant species LOC of 1.0 for non-vascular and vascular plants; RQ < non-endangered plant species LOC of 1.0 for vascular plants. Direct effects to non-vascular aquatic plants are possible. Further evaluation of the EECs relative to the threshold concentrations (for community-level effects) is necessary.
[e] RQ > non-endangered aquatic plant species LOC of 1.0 for non-vascular plants; RQ < non-endangered plant species LOC of 1.0 for vascular plants. Direct effects to non-vascular aquatic plants are possible. Further evaluation of the EECs relative to the threshold concentrations (for community-level effects) is necessary.

5.1.2.4 Evaluation of Potential Indirect Effects via Reduction in Terrestrial Plant Community (Riparian Habitat)

Potential indirect effects to the listed mussels resulting from direct effects on riparian vegetation were assessed using RQs from terrestrial plant seedling emergence and vegetative vigor EC_{25} data as a screen. Based on the results of the submitted terrestrial plant toxicity tests, it appears that emerging seedlings are more sensitive to atrazine via soil/root uptake than emerged plants via foliar routes of exposure. However, all tested plants, with the exception of corn in the seedling emergence and vegetative vigor tests, and ryegrass in the vegetative vigor test, exhibited adverse effects following exposure to atrazine. The results of these tests indicate that a variety of terrestrial plants that may inhabit riparian zones may be sensitive to atrazine exposure. RQs used to estimate potential indirect effects to the listed mussels from seedling emergence and vegetative vigor effects on terrestrial plants within riparian areas are summarized in Tables 5.8 and 5.9, respectively.

As shown in Table 5.8, terrestrial plant RQs are above the Agency's LOC for all species except corn. For species with LOC exceedances, RQ values based on aerial application of atrazine to forestry at 4.0 lb ai/A range from 1.5 to 93; the maximum RQ value based on an equivalent ground application is 40, approximately a two-fold reduction as compared to aerial applications. Granular application of atrazine to residential lawns at 2.0 lb ai/A is also likely to impact terrestrial plants with RQs ranging from <1 (corn and soybeans) to 13 (carrots). Monocots and dicots show similar sensitivity to atrazine; therefore, RQs are similar across both taxa.

Table 5.8 Non-target Terrestrial Plant Seedling Emergence RQs

Surrogate Species	**EC_{25} (lbs ai/A)[a]**	**EEC Dry adjacent areas[b]**	**RQ Dry adjacent areas**
Monocot - Corn	> 4.0	Aerial: 0.14 – 0.28 Ground: 0.6 – 0.12 Granular: 0.04	<LOC
Monocot - Oat	0.004	Aerial: 0.14 – 0.28 Ground: 0.06 – 0.12 Granular: 0.04	**Aerial: 35 - 70** **Ground: 15 - 30** **Granular: 10**
Monocot - Onion	0.009	Aerial: 0.14 – 0.28 Ground: 0.06 – 0.12 Granular: 0.04	**Aerial: 16 - 31** **Ground: 7 - 13** **Granular: 4.4**
Monocot - Ryegrass	0.004	Aerial: 0.14 – 0.28 Ground: 0.06 – 0.12 Granular: 0.04	**Aerial: 35 -70** **Ground: 15 - 30** **Granular: 10**
Dicot - Carrot	0.003	Aerial: 0.14 – 0.28 Ground: 0.06 – 0.12 Granular: 0.04	**Aerial: 47 - 93** **Ground: 20 - 40** **Granular: 13**
Dicot - Soybean	0.19	Aerial: 0.14 – 0.28 Ground: 0.06 – 0.12 Granular: 0.04	**Aerial: <LOC – 1.5** Ground: <LOC Granular: <LOC
Dicot - Lettuce	0.005	Aerial: 0.14 – 0.28 Ground: 0.06 – 0.12 Granular: 0.04	**Aerial: 28 - 56** **Ground: 12 - 24** **Granular: 8**
Dicot - Cabbage	0.014	Aerial: 0.14 – 0.28 Ground: 0.06 – 0.12 Granular: 0.04	**Aerial: 10 - 20** **Ground: 4 - 9** **Granular: 2.9**
Dicot - Tomato	0.034	Aerial: 0.14 – 0.28 Ground: 0.06 – 0.12 Granular: 0.04	**Aerial: 4 - 8** **Ground: 2 - 4** **Granular: 1.2**
Dicot - Cucumber	0.013	Aerial: 0.14 – 0.28 Ground: 0.06 – 0.12 Granular: 0.04	**Aerial: 11 - 22** **Ground: 5 - 9** **Granular: 3.1**

[a] From Chetram (1989); MRID 420414-03.
[b] Range of EECs based on use scenarios presented in Table 3.12 (i.e., aerial and ground: forestry, fallow/idle land, corn, sorghum; and granular residential).

Vegetative vigor studies indicate that terrestrial plants are generally less sensitive to foliar exposure of atrazine as compared to soil/root uptake. As shown in Table 5.9, vegetative vigor RQs exceed the Agency's LOC for only three dicot species (soybeans, cabbage, and cucumber), based on aerial application of atrazine at 2 to 4 lbs ai/A, with RQs ranging from 4 to 25. For ground applications, LOCs are exceeded for two dicot species, cabbage and cucumber, at application rates of 2 lbs ai/A with RQs ranging from 1.4 to 2.5. At higher ground application rates of 4 lbs ai/A, LOCs are also exceeded for soybeans, in addition to cabbage and cucumbers. Vegetative vigor RQs do not exceed LOCs for any of the tested monocot species.

Table 5.9 Non-target Terrestrial Plant Vegetative Vigor Toxicity RQs			
Surrogate Species	EC_{25} (lbs ai/A)[a]	Drift EEC (lbs ai/A)[b]	Drift RQ
Monocot - Corn	> 4.0	Aerial: 0.1 – 0.2 Ground: 0.02 – 0.04	<LOC
Monocot - Oat	2.4	Aerial: 0.1 – 0.2 Ground: 0.02 – 0.04	<LOC
Monocot - Onion	0.61	Aerial: 0.1 – 0.2 Ground: 0.02 – 0.04	<LOC
Monocot - Ryegrass	> 4.0	Aerial: 0.1 – 0.2 Ground: 0.02 – 0.04	<LOC
Dicot - Carrot	1.7	Aerial: 0.1 – 0.2 Ground: 0.02 – 0.04	<LOC
Dicot - Soybean	0.026	Aerial: 0.1 – 0.2 Ground: 0.02 – 0.04	**Aerial: 4 - 8** **Ground: <LOC - 2**
Dicot - Lettuce	0.33	Aerial: 0.1 – 0.2 Ground: 0.02 – 0.04	<LOC
Dicot - Cabbage	0.014	Aerial: 0.1 – 0.2 Ground: 0.02 – 0.04	**Aerial: 7 - 14** **Ground: 1 - 3**
Dicot - Tomato	0.72	Aerial: 0.1 – 0.2 Ground: 0.02 – 0.04	<LOC
Dicot - Cucumber	0.008	Aerial: 0.1 – 0.2 Ground: 0.02 – 0.04	**Aerial: 13 - 25** **Ground: 3 - 5**

[a] From Chetram (1989); MRID 420414-03.
[b] Range of EECs based on use scenarios presented in Table 3.12 (i.e., aerial and ground: forestry, fallow/idle land, corn, and sorghum).

Further analysis of the potential for atrazine to affect the listed mussels via reduction in riparian habitat, including a description of the importance of riparian vegetation to the assessed species and types of riparian vegetation that may potentially be impacted by atrazine use within the action area, is discussed in Section 5.2.1.5.

5.1.3 Adverse Modification to Designated Critical Habitat

Critical habitat was designated for the ovate clubshell and southern clubshell mussels by the USFWS on August 2, 2004 (USFWS, 2004). Designated critical habitat receives special protection under Section 7 of the ESA including prohibition of destruction or adverse modification of critical habitat with regard to Federal actions, such as use of pesticides registered under FIFRA. Critical habitat designations identify, to the extent known using the best scientific and commercial data available, habitat areas that provide essential life cycle needs of the species (i.e., areas on which the PCEs are found, as defined in 50 CFR 414.12(b)). Activities that may destroy or adversely modify critical habitat are those that alter the PCEs and jeopardize the continued existence of the species. Evaluation of actions related to atrazine use that may alter the PCEs of the ovate and southern clubshell mussel's critical habitat form the basis of the critical habitat impact analysis. As previously discussed in Section 2.8 of the problem formulation, PCEs that are identified as assessment endpoints are limited to those that are of biological nature and those PCEs for which atrazine effects data are available. For the purposes of this critical habitat impact analysis, PCEs selected as assessment endpoints for the ovate and southern clubshell mussels are grouped according to the measures of ecological effect that are used to determine whether the assessment endpoint (i.e. PCE) may be adversely modified. For example, all PCEs that may be adversely impacted by atrazine-related effects to riparian vegetation are evaluated together. As such, groupings of PCEs and the measures of ecological effect used in this critical habitat impact analysis are identified in Table 5.10.

Table 5.10 PCE Groupings for Critical Habitat Impact Analysis

PCE[a]	Measure of Ecological Effect
Chemical characteristics necessary for normal behavior, growth, and viability of all life stages of mussels related to: - Fish hosts with adequate living and foraging areas	- Acute and chronic freshwater fish and invertebrate data
- Characteristics necessary for normal behavior, growth, and viability of all mussel life stages related to: (1) Sand, gravel, and cobble substrates with low to moderate amounts of filamentous algae; (2) Water quality including oxygen content; and (3) Suitable habitat for fish hosts	- Acute vascular and non-vascular aquatic plant data - community-level threshold concentrations
- Characteristics necessary for normal behavior, growth, and viability of all mussel life stages related to: (1) Stream and river bank stability; (2) Water quality including temperature and turbidity; and (3) Substrates with low amount of sedimentation necessary for viability of listed mussels and suitability of spawning habitat for fish hosts	- Terrestrial plant seedling emergence and vegetative vigor data
Chemical characteristics necessary for normal behavior, growth, and viability of all life stages of mussels	- Acute freshwater mussel and chronic freshwater invertebrate data - Acute and chronic zooplankton and non-vascular plant data - community-level threshold concentrations

[a] PCEs involving flow regime and presence of predacious non-native species are not evaluated because there is no perceived link between the risk assessment biotic endpoints and abiotic PCEs, and no data are available to differentiate native versus non-native sensitivity to atrazine.

As shown in the critical habitat maps for the ovate clubshell and southern clubshell mussels (see Figures C.1 and C.2 in Appendix C), designated critical habitat for both species occurs in the Tombigbee River Basin within Alabama, Mississippi, Georgia, and Tennessee. Because designated critical habitat for ovate and southern clubshell mussels occurs in states that comprise the southern geographic region of the defined action area for all listed mussels evaluated in this assessment, screening-level surface water EECs from the southern region (Table 3.5) are used to derive RQs for the critical habitat impact analysis. Risk estimates of potential adverse modification to the PCEs identified in Table 5.10 are provided in Sections 5.1.3.1 through 5.1.3.4.

5.1.3.1 Adverse Modification to Designated Critical Habitat via Direct Effects to Host Fish and Food Items

Adverse modification of designated critical habitat via actions that may directly impact the host fish (and food items of the host fish) of the ovate and southern clubshell mussels are evaluated by considering the most sensitive acute and chronic freshwater fish and invertebrate toxicity data. Specific host fish species for the southern clubshell mussel include the Alabama shiner and tricolor shiner (Table 2.3); however, specific host fish species for the ovate southern clubshell are unknown.

Direct effects to fish hosts have been evaluated as part of the indirect effect analysis for the listed mussels in Section 5.1.2.2. Based on the analysis provided in Table 5.5, no acute LOCs were exceeded for freshwater fish, based on the highest atrazine use patterns in the southern region of the action area where critical habitat has been designated for the ovate and southern clubshell mussels. Therefore, atrazine use is not expected to adversely modify the critical habitat PCE associated with direct acute impacts to host fish. However, as shown in Table 5.6, chronic LOCs were exceeded for freshwater fish, based on screening-level 60-day EECs representative of atrazine use on corn in the southern region. Further analysis of refined EECs is completed to determine whether chronic effects to freshwater fish would likely result in adverse modification to critical habitat via reduction in host fish for the ovate and southern clubshell mussels.

Further evaluation of potential adverse modification to critical habitat foraging areas for the host fish is completed by considering indirect effects to the host fish based on direct effects to dietary food items. Acute and chronic RQs for each atrazine use pattern and corresponding screening-level EECs from the southern region were derived, based on the most sensitive freshwater aquatic invertebrate toxicity data. As shown in Tables 5.3 and 5.4, acute and chronic LOCs for freshwater invertebrates are exceeded for modeled atrazine use scenarios including corn, sorghum, and fallow/idle land. In addition, acute LOCs are also exceeded for forestry uses of atrazine. Based on this screening-level analysis, atrazine use may adversely modify the critical habitat via impacts to the aquatic invertebrate food base required to maintain foraging areas for host fish of the ovate and southern clubshell mussels. Further evaluation of the potential for adverse habitat modification based on refined EECs, the probability of an individual effect to host fish food items, and toxicity data for other potential food items of host fish is included in Section 5.2.2.1.

5.1.3.2 Adverse Modification to Designated Critical Habitat via Direct Effects to Aquatic Plants

Adverse modification of designated critical habitat via actions that may directly impact aquatic vascular and non-vascular plants are associated with those characteristics necessary for normal behavior, growth, and viability of all ovate and southern clubshell life stages. These characteristics include the following PCEs: (1) substrates with low to moderate amounts of filamentous algae; (2) maintenance of water quality parameters including oxygen content; and (3) suitable habitat for fish hosts.

In the watersheds within the action area for the listed mussels, primary productivity is essential for supporting their growth and abundance. In addition, freshwater vascular and non-vascular plant data are used to evaluate a number of the PCEs associated with the critical habitat impact analysis. Specifically, non-vascular plant data are used to determine whether adverse modification to critical habitat may occur via changes in the amount of attached filamentous algae on substrates. In addition, both vascular and non-vascular aquatic plant data are used in the critical habitat impact analysis to determine whether water quality parameters including oxygen content, and suitable habitat for host fish of the ovate and southern clubshell mussels may be adversely modified.

Indirect effects to the habitat of listed mussels via direct effects to freshwater aquatic plants (i.e., primary production) have been assessed in Section 5.1.2.3. Based on the results of the this analysis, shown in Table 5.7, LOCs for direct effects to aquatic non-vascular plants are exceeded for all modeled use scenarios in the southern geographic region where designated critical habitat occurs. In addition, vascular plant LOCs are also exceeded for modeled EECs based on corn, sorghum, fallow/idle land, and forestry uses of atrazine in the southern geographic region. Therefore, atrazine may adversely modify designated critical habitat for the ovate and southern clubshell mussels via direct effects on non-vascular aquatic plants for all modeled use scenarios and on vascular plants for corn, sorghum, fallow/idle land, and forestry uses of atrazine. Further analysis of the 14-, 30-, 60-, and 90-day time-weighted EECs relative to their respective threshold concentrations for use patterns of concern in the southern geographic region is completed in Section 5.2.2.2 to determine whether effects to individual aquatic plants would likely result in adverse modification to critical habitat via community-level effects to the aquatic community including the ovate and southern clubshell mussels and their host fish.

Based on the available freshwater non-vascular aquatic plant toxicity data, atrazine is expected to result in an alteration of the presence of filamentous algae on sand, gravel, and cobble substrates within designated critical habitat for the ovate and southern clubshell mussels. Therefore, the preliminary effects determination for this endpoint is "may affect'. However, this alteration is expected to reduce the presence of filamentous algae on substrates. Substrates with low to moderate amounts of filamentous algae are necessary to provide suitable habitat for these listed mussels. In summary, atrazine "may affect" the presence of filamentous algae on mussel substrates; however, this impact is not expected to adversely modify critical habitat of the ovate and southern clubshell mussels necessary for normal growth and viability of these mussel species.

Direct effects of atrazine on primary production, plant biomass, and community composition can be expected to cause indirect changes in water quality. Many water quality parameters are strongly influenced by photosynthesis and nutrient uptake. Dissolved oxygen is a very sensitive indicator of changes in photosynthetic rate, and reductions in oxygen concentrations are expected to accompany-atrazine induced reductions in primary productivity in standing water. Therefore, atrazine may adversely modify critical habitat of the ovate and southern clubshell mussels by reducing the oxygen content of the surface water. Further analysis of this “may affect” determination, including consideration of the refined analysis for direct effects to aquatic plants via primary productivity within the streams and rivers of the designated critical habitat for the ovate and southern clubshell mussels is completed as part of the risk description in Section 5.2.2.2.

5.1.3.3 Adverse Modification to Designated Critical Habitat via Direct Effects to Riparian Vegetation

Adverse modification of designated critical habitat via actions that may directly impact terrestrial plants (i.e., riparian vegetation) are associated with those characteristics necessary for normal behavior, growth, and viability of all ovate and southern clubshell life stages. These characteristics include the following PCEs: (1) geomorphically stable stream and river channels; (2) maintenance of water quality parameters including turbidity and temperature; and (3) substrates with low amount of sedimentation necessary for viability of the listed mussels and spawning habitat for fish hosts.

Indirect effects to the listed mussels resulting from direct effects on riparian vegetation were assessed in Section 5.1.2.4. This evaluation is also applicable to the critical habitat impact analysis as similar EECs and terrestrial plant seedling emergence and vegetative vigor toxicity endpoints (see Tables 5.8 and 5.9) are used for both types of analyses. Based on the evaluation of potential indirect effects associated with reduction in riparian vegetation, terrestrial plant RQs for seedling emergence are above the Agency’s LOCs for all terrestrial plants, with the exception of corn. Although vegetative vigor studies indicate that terrestrial plants are generally less sensitive to foliar exposure of atrazine as compared to soil/root uptake, vegetative vigor RQs also exceed LOCs for three dicot species (soybeans, cabbage, and cucumber). The results of this analysis indicate that a variety of terrestrial plants that may inhabit riparian zones may be sensitive to atrazine exposure; therefore, atrazine use may adversely affect critical habitat of the ovate and southern clubshell mussels via direct impacts to riparian vegetation. Further evaluation of the PCEs associated with adverse modification to critical habitat via streambank stability, water quality parameters (i.e., turbidity and temperature), and sedimentation are addressed as part of the risk description in Section 5.2.2.3.

5.1.3.4 Adverse Modification to Designated Critical Habitat via Effects to Chemical Characteristics Necessary for Normal Behavior, Growth, and Viability of All Mussel Life Stages

Chemical characteristics necessary for normal behavior, growth, and viability of all life stages of the ovate and southern clubshell mussels are assessed using inference from other LOC exceedances. For example, if LOCs are exceeded for direct effects and indirect effects related to reduction in the listed mussel’s food items, the chemical environment is presumed to be such that normal behavior, growth, and viability of the ovate and southern clubshell mussel’s critical habitat may be adversely modified. Other indirect effects to the ovate and southern clubshell mussel (i.e., host fish living, foraging and spawning areas; water quality, stream bank stability, and silt-free substrate with low amounts of filamentous algae) are assessed via specified PCEs for their designated critical habitat. Based on the screening-level direct effect analysis in Section 5.1.1, acute RQs for the listed mussels were below LOCs; however, chronic LOCs were exceeded based on atrazine use on corn, sorghum, and fallow-idle land in the southern region where designated critical habitat for the ovate and southern clubshell mussels occurs. In addition, LOCs were exceeded for phytoplankton (based on all modeled atrazine uses) and zooplankton (based on corn, sorghum, fallow/idle land, and forestry uses of atrazine) as a food source for the listed mussels (Section 5.1.2.1; Tables 5.3 and 5.4). Therefore, the initial screen suggests that critical habitat may be adversely modified via effects to chemical characteristics necessary for normal behavior, growth, and viability of all listed mussel life stages. Additional refinements of risk and their impacts to this effects determination are presented in Section 5.2.2.4 of this assessment.

5.2 Risk Description

The risk description synthesizes an overall conclusion regarding the likelihood of adverse impacts and/or modification leading to an effects determination (i.e., “no effect,” “may affect, but not likely to adversely affect,” or “likely to adversely affect”) for the seven listed mussels and designated critical habitat for the ovate and southern clubshell mussels.

If the RQs presented in the Risk Estimation (Section 5.1) show no direct or indirect effects for individual listed mussels, and no adverse modification to PCEs of the ovate and southern clubshell mussel’s designated critical habitat (RQs < LOC), a “no effect” determination is made, based on screening-level modeled EECs of atrazine’s use within the action area. If, however, direct or indirect effects to the individual listed mussels are anticipated and/or effects may adversely modify the PCEs of the ovate and southern clubshell mussel’s designated critical habitat (RQs > LOC), the Agency concludes a preliminary “may affect” determination for the FIFRA regulatory action regarding atrazine. A summary of the results of the risk estimation (i.e., “no effect” or “may affect” finding) presented in Sections 5.1.1 through 5.1.3 is provided in Table 5.11 for direct and indirect effects to the listed mussels as well as adverse modification to PCEs of the ovate and southern clubshell mussel’s designated critical habitat. Conclusions of “may effect” based on RQs presented in Section 5.1 are further evaluated to distinguish actions that are likely to adversely affect (“LAA”) from those that are not likely to adversely affect (“NLAA”) the assessed mussel species and their designated critical habitat.

Table 5.11 Preliminary Effects Determination Summary for the Assessed Listed Mussels and Critical Habitat Impact Analysis Based on Risk Estimation		
Direct and Indirect Effects to Listed Mussels[a]		
Assessment Endpoint	**Preliminary Effects Determination**	**Basis for Preliminary Determination**[b]
1. Survival, growth, and reproduction of assessed mussel individuals via direct effects	Acute direct effects: No effect	No acute LOCs are exceeded (Table 5.1).
	Chronic direct effects: May affect	Chronic LOCs are exceeded for corn, sorghum and fallow/idle land use of atrazine, based on available chronic toxicity data from surrogate freshwater invertebrates (Table 5.2).
2. Indirect effects to assessed mussel individuals via reduction in food items (i.e., freshwater phytoplankton and zooplankton)	Phytoplankton: May affect	LOCs for phytoplankton are exceeded for all labeled uses of atrazine (Table 5.3).
	Zooplankton: May effect	Acute and chronic LOCs are exceeded for corn, sorghum, and fallow/idle land uses; acute LOCs are also exceeded for forestry uses (Tables 5.3 and 5.4).
3. Indirect effects to assessed mussel individuals via reduction in host fish for mussel glochidia	Acute indirect effects: No effect	Acute RQs for freshwater fish are less than LOCs (Table 5.5).
	Chronic indirect effects: May affect	Chronic LOCs are exceeded for corn, sorghum and fallow/idle land use of atrazine (Table 5.6)
4. Indirect effects to assessed mussel individuals via reduction of habitat and/or primary productivity	May affect	LOCs are exceeded for non-vascular aquatic plants for all modeled atrazine use scenarios (Table 5.7). LOCs are exceeded for vascular plants for the corn, sorghum, fallow/idle land, and forestry use scenarios (Table 5.7).
5. Indirect effects to assessed mussel individuals via reduction of terrestrial vegetation (i.e., riparian habitat) required to maintain acceptable water quality and habitat	May affect	LOCs are exceeded for all tested species except corn based on seedling emergence (Table 5.8). LOCs are exceeded for soybeans, cabbage, and cucumbers based on vegetative vigor (Table 5.9).
Adverse Modification to Designated Critical Habitat via PCE Analysis		
6. Fish hosts with adequate living, foraging, and spawning areas	Acute effects to host fish: No effect	Acute RQs for freshwater fish are less than LOCs (Table 5.5).
	Chronic effects to host fish: May affect	Chronic LOCs are exceeded for corn, sorghum and fallow/idle land use of atrazine (Table 5.6)
	Direct effects to host fish food items: May affect	Acute and chronic LOCs for freshwater invertebrates are exceeded for modeled atrazine use scenarios including corn, sorghum, and fallow/idle land. Acute LOCs are also exceeded for forestry uses of atrazine (Tables 5.3 and 5.4).
	Direct effects to host fish spawning habitat: May affect	LOCs are exceeded for aquatic plants and terrestrial plants (Tables 5.7, 5.8, and 5.9).
7. Water quality necessary for normal behavior, growth and viability of all mussel life stages	Temperature and turbidity: May affect	LOCs are exceeded for terrestrial plants (Tables 5.8 and 5.9).
	Oxygen content: May affect	LOCs are exceeded for aquatic plants (Table 5.7).
8. Substrates with low to	Filamentous algae: May affect	LOCs are exceeded for aquatic plants (Table 5.7).

Table 5.11 Preliminary Effects Determination Summary for the Assessed Listed Mussels and Critical Habitat Impact Analysis Based on Risk Estimation

Direct and Indirect Effects to Listed Mussels[a]		
Assessment Endpoint	**Preliminary Effects Determination**	**Basis for Preliminary Determination[b]**
moderate amounts of filamentous algae and low sedimentation	Sedimentation: May affect	LOCs are exceeded for terrestrial plants (Tables 5.8 and 5.9).
9. Stream and river bank stability	May affect	LOCs are exceeded for terrestrial plants (Tables 5.8 and 5.9).
10. Chemical characteristics necessary for normal behavior, growth, and viability of all life stages of mussels	Acute direct effects: No effect	No acute LOCs are exceeded (Table 5.1).
	Chronic direct effects: May affect	Chronic LOCs are exceeded for corn, sorghum and fallow/idle land use of atrazine, based on available chronic toxicity data from surrogate freshwater invertebrates (Table 5.2).
	Indirect food source-Phytoplankton: May affect	LOCs for phytoplankton are exceeded for all labeled uses of atrazine (Table 5.3).
	Indirect food source – Zooplankton: May affect	Acute and chronic RQs for zooplankton exceed LOCs (Tables 5.3 and 5.4).

[a] The direct and indirect effects determination for the stirrupshell mussel is "no effect" because this species is presumed to be extinct (Hartfield, 2006). Therefore, the direct/indirect effects determinations apply to the other seven mussels (i.e., pink pearly mucket, rough pigtoe, shiny pigtoe, fine-rayed pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell) included in this assessment.
[b] All screening-level EECs for the preliminary effects determination are based on modeled scenarios for surface water (Table 3.6) and terrestrial plants (Table 3.16); toxicity values are based on the most sensitive endpoint summarized in Table 4.3.

Following a "may affect" determination, additional information is considered to refine the potential for exposure at the predicted levels based on additional modeling and monitoring data, the life history characteristics (i.e., habitat range, feeding preferences, etc.) of the seven listed mussels, and potential community-level effects to aquatic plants.

Two separate refined analyses are conducted for listed mussels and designated critical habitat that are present within and outside the boundaries of vulnerable watersheds, as discussed in Section 3.2.6.1. Vulnerable watersheds, defined by WARP, include watersheds that are predominantly second and third order streams and are located in areas of high atrazine use on corn and sorghum. The principle factors that are used to differentiate those watersheds within "vulnerable" areas include high atrazine use, propensity for runoff, soil type, and rainfall. Based on the analysis conducted in Section 3.2.6.1, the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels inhabit streams that are at least partially located within the boundary of vulnerable watersheds. The other assessed listed species, including the shiny pigtoe, heavy pigtoe, ovate clubshell and southern clubshell mussels, as well as all designated critical habitat for the ovate and southern clubshell mussels, are located outside of the boundary of vulnerable watersheds, as defined by WARP. For listed mussels that occur within the boundary of vulnerable watersheds (i.e., the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe), the screening-level modeled EECs representative of potential atrazine exposure are refined based on the available targeted ecological monitoring data presented in Section 3.2.6.1 and Appendix D. As previously discussed, the 40 selected sampling locations included in the Ecological Monitoring Program were chosen to be representative of the upper 20th percentile of watersheds (i.e., 1,172 watersheds) that are highly vulnerable to atrazine runoff. An analysis of the representativeness of the monitoring data to other sites within the vulnerable watershed boundary is not available; therefore, it is conservatively assumed that all of ecological monitoring data may be representative of atrazine exposure in vulnerable waters where the listed mussels are known to occur within the boundaries of the 1,172 highly vulnerable watersheds.

The other listed mussels (i.e., shiny pigtoe, heavy pigtoe, ovate clubshell and southern clubshell mussels) and designated critical habitat for the ovate and southern clubshell mussels are located in watersheds entirely outside of the boundary of the 1,172 highly vulnerable watersheds. Therefore, EECs based on the ecological monitoring data taken from second and third order streams in highly vulnerable watersheds were not considered to be representative of exposure outside of the boundary of highly vulnerable watersheds because they are likely to over-predict exposure. For mussels and designated critical habitat located outside of the highly vulnerable watersheds, EECs were based on the PRZM/EXAMS flow-adjusted modeling discussed in Section 3.2.5 and presented in Table 3.7, as well as ancillary non-targeted monitoring data. Targeted in this context means that the monitoring data are from studies designed to detect peak and longer term atrazine concentrations in highly vulnerable watersheds as defined by WARP. Ancillary monitoring data includes consideration of peak values from NAWQA data within the action area (Section 3.2.6.2) and other non-targeted monitoring data from watersheds with sufficient sampling frequency to derive 14 through 90-day rolling average exposure concentrations (i.e., Heidelberg data; see Section 3.2.6.4) for comparative purposes. Flow-adjusted EECs are used to refine exposure in occupied streams and designated habitat outside of the vulnerable watershed boundary because these values are based on site-specific flow data from streams in which the mussels are known to occur. Comparison of the variability in flow rates between occupied streams and watersheds sampled as part of the targeted ecological monitoring data shows that flow rates from monitored locations are less than the 5th percentile of the flow rate for occupied streams. Given that flow is one of the major factors contributing to atrazine exposure levels (i.e., low flow streams are likely to have higher detected concentrations of atrazine than streams with higher flow rates), use of targeted monitoring data from streams with generally lower flow rates would be expected to over-predict EECs for occupied streams, where the flow rate is generally higher. In addition, approximately 60 to 70% of the targeted ecological monitoring data show atrazine concentrations that are similar to, or lower than, the flow-adjusted EECs based on modeling. Atrazine concentrations from the targeted ecological monitoring data that do exceed the flow-adjusted modeled EECs are within a factor of 2 to 3 times higher. Given that the targeted monitoring data is representative of the most vulnerable watersheds to atrazine runoff with flow rates that are less than the 5th percentile of occupied streams, and relatively close agreement exists between the flow-adjusted modeled EECs and the monitoring data from highly vulnerable watersheds, it is unlikely that atrazine exposure outside the boundary of vulnerable watersheds is greater than the refined flow-adjusted modeled EECs.

Based on the best available information, the Agency uses the refined evaluations to distinguish those actions that "may affect, but are not likely to adversely affect" ("NLAA") from those actions that are "likely to adversely affect" ("LAA") the seven listed mussels (within and outside the boundary of vulnerable watersheds) and designated critical habitat for the ovate and southern clubshell mussels (outside the boundaries of vulnerable areas).

The criteria used to make determinations that the effects of an action are "not likely to adversely affect" the seven listed mussels and designated critical for the ovate and southern clubshell mussels include the following:

- <u>Significance of Effect</u>: Insignificant effects are those that cannot be meaningfully measured, detected, or evaluated in the context of a level of effect where "take" occurs for even a single individual. "Take" in this context means to harass or harm, defined as the following:

    - Harm includes significant habitat modification or degradation that results in death or injury to listed species by significantly impairing behavioral patterns such as breeding, feeding, or sheltering.

    - Harass is defined as actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding, or sheltering.

- <u>Likelihood of the Effect Occurring</u>: Discountable effects are those that are extremely unlikely to occur. For example, use of dose-response information to estimate the likelihood of effects can inform the evaluation of some discountable effects.

- <u>Adverse Nature of Effect:</u> Effects that are wholly beneficial without any adverse effects are not considered adverse.

A description of the risk and effects determination for each of the established direct and indirect assessment endpoints for the seven listed mussels in occupied streams within and outside the boundaries of vulnerable watersheds is provided in Section 5.2.1. A description of the risk and effects determination for the critical habitat impact analysis in less vulnerable watersheds is provided in Section 5.2.2.

5.2.1 Direct and Indirect Effects to the Listed Mussels

5.2.1.1 Direct Effects to the Listed Mussels

The acute RQ of 0.003 (based on the peak EEC of 109 µg/L from the southern corn scenario) is well below the Agency's endangered species LOC for all modeled uses of atrazine within the action area. In addition, non-targeted NAWQA monitoring data (Section 3.2.6.2; Table 3.9) were also considered to provide context to the peak screening-level modeled EECs. The NAWQA data show that atrazine was detected at a peak concentration as high as 201 µg/L in Bogue Chitto Creek. This watershed is located within the action area, but outside the boundary of vulnerable watersheds defined by WARP. Bogue Chitto Creek is currently occupied by the southern clubshell mussel and designated as critical habitat for this species. The peak NAWQA atrazine concentration of 210 µg/L for Bogue Chitto Creek was detected in 1999; however, more recent data from 2001 – 2004 show that concentrations have decreased in this watershed with detections ranging from approximately 5 to 25 µg/L (Table 3.9). In addition, further analysis of the NAWQC monitoring data shows that the 99.9^{th} percentile of all peak atrazine detections (from over 20,000 samples) is 61 µg/L (Table 3.15). Therefore, the 1999 Bogue Chitto Creek peak concentration of 210 µg/L is likely to overestimate current peak exposures of atrazine within this watershed. However, use of the peak value of 210 µg/L, would yield an acute RQ value of <0.005 (EEC = 210 µg/L/mussel LC_{50} = >36,000), which is also below the acute endangered species LOC of 0.05. The Agency, consistent with the Overview Document (U.S. EPA, 2004) and the alternative consultation agreement with the Services (USFWS/NMFS, 2004b), interprets RQs below the endangered species LOC to be consistent with a finding of no effect for direct effects on the listed species for the taxa being assessed. To provide additional information, the probability of an individual mortality to the assessed mussels was calculated for acute RQs < 0.005. A probit slope value for the acute mussel toxicity test is not available; therefore, the effect probability was calculated based on a default slope assumption of 4.5 with upper and lower bounds of 2 and 9 (Urban and Cook, 1986). Based on the default dose response curve slope of 4.5, the corresponding estimated chance of an individual acute mortality to the listed mussels at an RQ level of <0.005 (based on the acute toxic endpoint for freshwater mussels) is 1 in 5.0E+24. It is recognized that extrapolation of very low probability events is associated with considerable uncertainty in the resulting estimates. In order to explore the possible bounds to such estimates, the upper and lower default bounds (2 to 9) were used to calculate upper and lower estimates of the effects probability associated with the acute RQ. The respective lower and upper effects probability estimates are 1 in 478,000 (2.1E-04%) and 1 in 7.0E+94 (~1.4E-93%). In summary, the Agency concludes a "no effect" determination for acute direct effects to the four listed mussels (i.e., shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussels) that occur in watersheds outside of the vulnerable watershed boundary, via acute mortality, based on all available lines of evidence.

In order to characterize potential acute direct effects to pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels that occur within the boundary of vulnerable watersheds, peak concentrations from the ecological monitoring data were considered as an upper bound of exposure. Based on the monitoring data discussed in Section 3.2.6.1 and Table D-3 of Appendix D, atrazine was detected at a maximum peak concentration of 209 µg/L at sampling location IN 11. Atrazine was also detected at peak concentrations exceeding the PRZM/EXAMS pond screening-level EEC of 109 µg/L at an additional two locations including MO 01 (183 µg/L) and NE 07 (112 µg/L); however, peak concentrations from the remaining 37 watersheds were less than 109 µg/L with values ranging from 0.13 to 83 µg/L. Refinement of the peak EEC from 109 µg/L to 209 µg/L, based on the maximum detected peak concentration of atrazine from the ecological monitoring data, would result in an acute RQ value of <0.006 (refined EEC: 209 µg/L/freshwater mussel LC_{50}: >36,000 µg/L). The acute RQ of < 0.006 is also well below the Agency's LOC. Therefore, the Agency also concludes a "no effect" determination for acute direct effects to the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels that occur within the vulnerable watershed boundary.

Chronic toxicity data for freshwater mussels are not available; therefore, the most sensitive NOAEC value from the available freshwater invertebrate data was used as a surrogate. Chronic RQs, based on modeled screening-level EECs from Table 3.6 and the surrogate chronic freshwater invertebrate endpoint value for the scud (NOAEC = 60 µg/L), exceed the Agency's LOCs with RQ values ranging from 1 to 1.8. However, chronic RQs are likely to be overestimated given the available acute toxicity data for freshwater unionid mussels, which shows that mussels are less sensitive to atrazine than freshwater invertebrates routinely used in aquatic toxicity testing (i.e., cladocerons and amphipods). Available chronic data from the open literature on freshwater mollusks suggests that growth effects may occur at concentrations between 125 µg/L and 1,000 µg/L (NOAEC of 125 µg/L reported in Baturo, 1995; LOAEC of 1000 µg/L reported in Streit and Peter, 1978). Although these studies were not considered appropriate for use in RQ calculations due to limitations in the study design and the lack of definitive NOAEC values (see Table A-21b of Appendix A), they suggest that chronic effects to freshwater mollusks may occur at concentrations between 125 µg/L and 1,000 µg/L.

Alternatively, potential use of an acute to chronic ratio (ACR) to estimate a chronic NOAEC for freshwater mussels was considered. ACRs were calculated for all freshwater invertebrate species where data allowed. However, there is a high degree of uncertainty in this analysis because acute and chronic studies conducted on the same species within the same laboratory were not available. Also, some acute studies reported non-discrete (i.e., "greater than") LC_{50} values. Non-discrete values were used only if they resulted in the highest (most conservative) ACR. The highest ACR across all freshwater invertebrate taxa is >300 (midge LC_{50} of >33,000 µg/L / midge NOAEC of 110 µg/L). If the ACR value of >300 was applied to the acute LC_{50} in freshwater mussels (>36,000 µg/L), the resulting estimated NOAEC would be approximately 120 µg/L. Therefore, use of the midge NOAEC of 60 µg/L is more conservative than the ACR-estimated NOAEC of 120 µg/L for freshwater mussels.

In addition, screening-level chronic EECs derived from the standard ecological water body are not likely to be representative of actual exposure concentrations in flowing water bodies outside of vulnerable areas where the shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussels occur. These listed mussel's inhabit flowing water bodies, which are subject to extensive mixing and dilution. In contrast, the standard ecological water body is assumed to be static.

As described in Section 3.2.5, additional modeling was completed to characterize the potential effect of flow on the screening-level EECs and provide refined chronic exposures for listed mussels that occupy streams and rivers outside the boundary of vulnerable watersheds. Based on this analysis, flow-adjusted 21-day EECs are approximately 81 to 95% lower than 21-day EECs modeled using the static water body. This analysis suggests that screening-level EECs derived using the standard ecological water body may over-estimate exposure in less vulnerable water bodies with flowing water, including those where the shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussels occur. As shown in Table 3.7, 21-day flow-adjusted EECs (for the scenario yielding the highest screening-level EEC within each of the four geographic regions) range from 5 to 12 µg/L. Refined chronic RQ values based on the 21-day flow-adjusted EECs and most sensitive NOAEC of 65 µg/L range from 0.08 to 0.2, well below the Agency's LOC of 1.0 for chronic risk to aquatic invertebrates. Although predicted 21-day atrazine concentrations from the non-targeted monitoring data (21 µg/L; see Table 3.11) are approximately 2 times higher than the maximum predicted based on flow-adjusted modeled EECs, consideration of the non-targeted monitoring data would also result in chronic RQs less than LOCs. Furthermore, consideration of the available open literature on freshwater mollusks indicates that potential chronic effects do not occur at estimated atrazine chronic exposure concentrations.

Although RQs exceed LOCs based on modeled screening-level chronic exposures and the most sensitive surrogate aquatic invertebrate across all species tested, atrazine's use within the less vulnerable watersheds of the action area is not likely to adversely affect the listed shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussels because refined flow-adjusted EECs and available non-targeted Heidelberg monitoring data indicate that chronic (21-day) exposure concentrations are well below the levels shown to cause adverse effects in mollusks. Therefore, the effects determination for the assessment endpoint of direct chronic effects to the listed shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussels occupying watersheds outside of the vulnerable area is "may affect, but not likely to adversely affect or NLAA." This finding is based on insignificance of effects (i.e., chronic exposure to atrazine is not likely to result in a "take" of a single shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussel in watersheds outside of vulnerable areas).

Consideration of the available targeted monitoring data from vulnerable watersheds confirms that longer-term screening-level EECs are likely to be overestimated by the static water body scenario. However, the highest flow-adjusted 21-day EECs of 12 µg/L may under-represent actual chronic exposure concentrations of atrazine in highly vulnerable areas based on the available monitoring data, which show a range of 21-day concentrations from 0.11 to 62 µg/L. Use of the maximum 21-day averaging monitoring concentration of 62 µg/L would result in a refined chronic RQ value that exceeds the LOC (EEC of 62 µg/L / NOAEC of 60 µg/L = 1.03; LOC = 1.0). Further review of the monitoring data shows that atrazine was detected at a concentration exceeding the freshwater invertebrate NOAEC (60 µg/L) in only one out of 40 sampled watersheds at NE 07. The range of 21-day average monitoring concentrations from the remaining 39 watersheds (excluding NE 07) is 0.11 to 44 µg/L. In addition, as discussed above, RQs for direct chronic effects to freshwater mussels based on the most sensitive aquatic invertebrate (freshwater scud) NOAEC value of 60 µg/L across all taxa are likely to be overestimated. The available chronic data from the open literature on freshwater mollusks suggest that direct effects may occur at concentrations between 125 and 1,000 µg/L, approximately two-fold higher than the maximum 21-day monitoring concentration of atrazine from highly vulnerable watersheds.

Therefore, atrazine's use within the vulnerable watersheds of the action area is not likely to adversely affect the listed pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels because the available monitoring data considered to be representative of potentially vulnerable watersheds in which these mussels reside indicate that chronic (21-day monitoring) exposure concentrations are expected to be less than levels shown to cause adverse chronic effects in mollusks. Therefore, the effects determination for the assessment endpoint of direct chronic effects to the listed pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels occupying watersheds within vulnerable areas is "may affect, but not likely to adversely affect or NLAA." This finding is based on insignificance of effects (i.e., chronic exposure to atrazine is not likely to result in a "take" of a single pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussel within vulnerable watersheds of the action area).

5*.2.1.2 Indirect Effects via Reduction in Food Items (Freshwater Zooplankton and Phytoplankton)*

Although data on the relative percentages of each type of food item in the listed mussel's diet are unavailable, freshwater mussels primarily consume phytoplankton, as well as detritus, zooplankton, and other microorganisms (Ukeles, 1971; Coker et al., 1921; Churchill and Lewis, 1924; and Fuller, 1974). Based on the screening-level analysis, LOCs are exceeded for phytoplankton for all labeled uses of atrazine within the action area. In addition, both screening-level acute and chronic RQs for zooplankton exceed their respective LOCs for all of the agricultural uses of atrazine. A description of the refined analysis for potential indirect effects to the listed mussels via reduction in zooplankton and phytoplankton as food items is provided below.

Zooplankton

With respect to zooplankton, screening-level acute RQs were based on the lowest LC_{50} value of 720 µg/L for the midge (*Chironomus* spp.). Consideration of all acute toxicity data for the midge shows a wide range of sensitivity within and between species of the same genus (2 orders of magnitude) with values ranging from 720 to >33,000 µg/L. Although effects data for the midge was used as a surrogate for dietary zooplankton, given that its lowest LC_{50} value is the most sensitive value for freshwater invertebrates, this species is generally not considered as zooplankton and is, therefore, unlikely to be consumed by the listed mussels. Freshwater zooplankton are dominated by four major groups of animals: protozoa, rotifers, and two subclasses of the Crustacea including the cladocerans and copepods. Out of the four major groups of animals considered as zooplankton, toxicity data for atrazine is available for cladocerons (*Daphnia*) only. As previously discussed in Section 4.1.3.1, acute atrazine LC_{50} values for *Daphnia* range from 3,500 to >30,000 µg/L. The acute RQ value for zooplankton, based on the maximum screening-level modeled EEC of 109 µg/L and the most sensitive *Daphnia* LC_{50} value of 3,500 µg/L is 0.03, less than the acute LOC. As previously discussed in Section 5.2.1.1, the available non-targeted NAWQA monitoring data from Bogue Chitto Creek show that atrazine was detected at a maximum peak concentration of 201 µg/L in 1999, approximately two-fold higher than the modeled peak screening-level EEC of 109 µg/L. However, it is unlikely that the NAWQA peak value from 1999 is representative of current peak exposures, given more recent 2000-2004 data from Bogue Chitto Creek, which show detected concentrations $\leq$ 25 µg/L, and the 99.9th percentile of 61 µg/L from all peak NAWQA data. Based on the peak monitoring concentration of 201 µg/L, the revised acute RQ for zooplankton of 0.06 (refined EEC: 201 µg/L/*Daphnia* LC_{50}: 3,500 µg/L) exceeds the acute LOC value of 0.05; however, LOCs are not exceeded based on the 99.9th percentile of all peak NAWQA monitoring data or recent data from 2000-2004 that is specific for Bogue Chitto Creek within the action area. Slope information on the toxicity study used to derive the RQ for zooplankton is not available. Therefore, the probability of an individual effect was calculated using the probit slope of 2.43 from an acute *Daphnia* study on the formulated product (80% ai) of atrazine; 95% confidence intervals could not be calculated based on the available data (MRID #420414-01). Based on the probit dose response curve slope of 2.42, the corresponding estimated chance of an individual effect to zooplankton at an RQ level of 0.06 is 1 in 644 (0.2%). Given that zooplankton are not the primary food source for listed mussels, the probability of an individual effect to zooplankton is low (i.e., 0.2%), and acute LOCs are not exceeded based on peak 99.9th percentile NAWQA data and 2000-2004 data specific for Bogue Chitto Creek), the effects determination for indirect effects to the shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussel via direct acute effects on zooplankton as dietary food items in less vulnerable watersheds is "may affect, but not likely to adversely affect or NLAA". This finding is based on insignificance of effects (i.e., effects to zooplankton in less vulnerable watersheds are not likely to be extensive over the suite of possible food items to result in "take" of a single listed shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussel).

The screening-level chronic RQ for zooplankton in less vulnerable watersheds, based on the highest modeled 21-day screening-level EEC of 107 µg/L and the most sensitive chronic freshwater invertebrate NOAEC of 60 µg/L for the scud, exceeds the Agency's LOC (see Table 5.4). However, as previously discussed, freshwater invertebrates including the scud are not considered as zooplankton; therefore, the effects data were refined to consider the available chronic atrazine toxicity data for cladocerons. The lowest NOAEC value for *Daphnia magna*, based on a reduction in the survival of F_0 young/female at 250 µg/L, is 140 µg/L. This NOAEC value of 140 µg/L is greater than the highest modeled 21-day screening-level EEC of 107 µg/L, as well as the highest refined 21-day flow-adjusted EEC of 12 µg/L. In addition, consideration of the 21-day atrazine concentrations from the non-targeted monitoring data (21 µg/L; see Table 3.11), although approximately 2 times higher than the maximum predicted based on flow-adjusted modeled EECs, would also result in chronic RQs less than LOCs. Given that all refined measures of exposure (i.e., 21-day flow adjusted EECs and non-targeted monitoring data) are well below levels that produced chronic effects in cladocerons, the effects determination for the shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussel in watersheds outside of vulnerable areas via direct chronic effects on zooplankton as dietary food items is “may affect, but not likely to adversely affect or NLAA”. This finding is based on insignificance of effects (i.e., chronic exposure to atrazine is not likely to result in a “take” of a single listed shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussel via a reduction in zooplankton as food items).

Refined analysis of potential acute and chronic impacts to zooplankton in vulnerable watersheds where the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels occur is based on the ecological monitoring data discussed in Section 3.2.6.1 and summarized in Appendix D. As previously discussed, the available ecological monitoring data show that atrazine was detected at a maximum peak concentration of 209 µg/L at sampling location IN 11, approximately two-fold higher than the modeled peak screening-level EEC of 109 µg/L. Based on the peak monitoring concentration of 209 µg/L, the revised acute RQ for zooplankton of 0.06 (refined EEC: 209 µg/L/*Daphnia* LC_{50}: 3,500 µg/L) exceeds the acute LOC value of 0.05. This refined analysis for potential acute effects to zooplankton in less vulnerable watersheds is also applicable to highly vulnerable watersheds because the refined EECs based on targeted monitoring data within vulnerable watersheds (209 µg/L) and non-targeted monitoring data outside the boundary of vulnerable watersheds (201 µg/L) are similar and result in identical refined acute RQ values of 0.06. Therefore, the refined analysis for potential acute direct effects to zooplankton in vulnerable watersheds suggests that acute exposure to atrazine “may affect” the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels via a reduction in food items. However, based on the probit dose response curve slope of 2.42, the corresponding estimated chance of an individual effect to zooplankton at an RQ level of 0.06 is 1 in 644 (0.2%). Given that zooplankton are not the primary food source for the listed freshwater mussels and there is a low probability of an individual effect to zooplankton food items, the effects determination for indirect effects to the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels via direct acute effects on zooplankton as prey in vulnerable watersheds is “may affect, but not likely to adversely affect or NLAA”. This finding is based on insignificance of effects (i.e., effects to zooplankton in vulnerable watersheds are not likely to be extensive over the suite of possible food items to result in “take” of a single listed pearly mucket, rough pigtoe, and fine-rayed pigtoe mussel).

As noted above, the refined analysis of potential chronic effects to zooplankton and resulting indirect effects to the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels in vulnerable watersheds is based on the targeted ecological monitoring data. The targeted monitoring data shows that the maximum 21-day average concentration for atrazine is 62 µg/L, approximately two-fold lower the corresponding 21-day screening-level EEC predicted by modeling. Use of the maximum 21-day averaging monitoring concentration of 62 µg/L would result in a refined chronic RQ value that exceeds the LOC (based on the most sensitive freshwater invertebrate NOAEC value of 60 µg/L). However, refined chronic effects data specific to cladocerons, which are considered to be representative of zooplankton, indicate that they are less sensitive to atrazine than the most sensitive freshwater invertebrate, with a corresponding NOAEC value of 140 µg/L.

Therefore, chronic effects to zooplankton are not expected to occur at 21-day monitored concentrations of 62 µg/L. The effects determination for the assessment endpoint of indirect effects to the listed pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels occupying watersheds within vulnerable areas via direct chronic effects to zooplankton food items is "may affect, but not likely to adversely affect or NLAA." This finding is based on insignificance of effects (i.e., chronic exposure to atrazine is not likely to result in a "take" of a single pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussel within vulnerable watersheds of the action area via a reduction in zooplankton as food items).

Phytoplankton

As shown in Table 5.3, direct adverse effects to non-vascular aquatic plants (i.e., phytoplankton), which are the primary component of the listed mussel's diet, are possible, based on all screening-level modeled atrazine uses. Direct effects to non-vascular plants are expected both within and outside the boundary of vulnerable watersheds, based on peak detected concentrations of atrazine in the ecological monitoring data (209 µg/L), the non-targeted NAWQA data from Bogue Chitto Creek (209 µg/L), as well as peak refined flow-adjusted EECs (120 µg/L). Based on these potential effects, atrazine may indirectly affect the seven listed mussels (within and outside the vulnerable watershed boundary) via a reduction in food items required for growth and viability of juvenile and adult stages. In order to determine whether potential effects to individual plant species would likely result in community-level effects to the listed mussels, the time-weighted screening-level EECs (for 14-, 30-, 60-, and 90-day averages from Table 3.6) were compared to their respective time-weighted threshold concentrations. As discussed in Section 4.2, concentrations of atrazine from the exposure profile at a particular use site and/or action area that exceed any of the following time-weighted threshold concentrations indicate that changes in the aquatic plant community structure (including food items for the mussels) could be affected:

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

A comparison of the range of the screening-level 14-, 30-, 60-, and 90-day EECs for the listed mussels with the atrazine threshold concentrations representing potential aquatic community-level effects is provided in Table 5.12.

Table 5.12 Summary of Modeled Scenario Time-Weighted Screening-Level EECs with Threshold Concentrations for Potential Community-Level Effects

Use Scenario	14-day		30-day		60-day		90-day	
	EECs (µg/L) [a]	**Threshold Conc. (µg/L)**	**EECs (µg/L)** [a]	**Threshold Conc. (µg/L)**	**EECs (µg/L)** [a]	**Threshold Conc. (µg/L)**	**EECs (µg/L)** [a]	**Threshold Conc. (µg/L)**
Corn	**79 - 108**		**78 - 107**		**77 - 105**		**74 - 102**	
Sorghum	**58 - 68**		**56 - 68**		**55 - 66**		**53 - 64**	
Fallow / idle land	**54 - 103**		**54 - 103**		**54 - 103**		**54 - 103**	
Forestry	27 - **48**	38	26 - **47**	27	**26 - 45**	18	**25 - 44**	12
Residential	8 - 14		8 - 14		8 - 14		8 - **13**	
Turf	7 - 18		7 - 18		7 - **18**		7 - **17**	
Rights-of-Way	2 - 4		2 - 4		2 - 4		2 - 4	

[a] Screening-level EECs from Table 3.6.

Based on the results of this comparison, predicted screening-level 14-, 30-, 60-, and 90-day EECs for corn, sorghum, fallow/idle land, and forestry modeled uses exceed their respective threshold concentrations for community level effects. In addition, predicted 60- and 90-day EECs for turf and 90-day EECs for residential uses of atrazine exceed their respective threshold concentrations. These screening-level EECs were estimated using PRZM/EXAMS and the non-flowing standard water body scenario, which is intended to be representative of exposures in headwater streams. As previously discussed, these chronic screening-level EECs are expected to over-estimate exposure in both vulnerable and less vulnerable water bodies with flowing water, where the listed mussels are known to occur. All of the listed mussels included in this assessment require moderate to swift currents in deep waters over relatively stable sand, gravel, cobble substrates for normal feeding, growth, and viability of all life stages; therefore, chronic EECs based on a non-flowing water body are expected to over-estimate actual exposure concentrations of atrazine for the assessed mussels in their expected range. Additional information on the impact of flowing water on the modeled EECs and non-targeted monitoring data for less vulnerable areas, and available monitoring data for vulnerable areas, was used to refine exposure concentrations of atrazine for the seven assessed mussels, relative to those presented for the standard water body scenario. Analyses of flow-adjusted EECs and relevant monitoring data are presented in detail in Sections 3.2.5 and 3.2.6.1, respectively, and summarized below.

In order to characterize the potential impact of flowing water on the longer-term exposures (i.e., 14 through 90-days) in less vulnerable watersheds, further modeling was conducted to provide a general sense of the relative reduction in long term exposure that might occur in water bodies where flow is higher than small headwater streams. The impact of various flow rates was characterized using the Index Reservoir (IR) as the receiving water body and USGS mean seasonal flow data from streams where the shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussels are known to occur in less vulnerable watersheds. The results of this analysis show that use of seasonal flow rates from streams where these listed mussels are known to occur yields longer-term EECs that are significantly reduced as compared to screening-level EECs derived using the standard water body. A comparison of the maximum flow-adjusted 14-, 30-, 60-, and 90-day EECs for two atrazine use scenarios that yield the highest EECs (i.e., corn in the southern region and fallow/idle land in the western region) with the atrazine threshold concentrations representing potential aquatic community-level effects is provided in Table 5.13.

Table 5.13 Summary of Flow-Adjusted EECs with Threshold Concentrations for Potential Community-Level Effects in Less Vulnerable Watersheds

Use Scenario	14-day		30-day		60-day		90-day	
	EECs (µg/L)	Threshold Conc. (µg/L)	EECs (µg/L)	Threshold Conc. (µg/L)	EECs (µg/L)	Threshold Conc. (µg/L)	EECs (µg/L)	Threshold Conc. (µg/L)
Corn[a]	15	38	7	27	4	18	2	12
Fallow / idle land[b]	8		4		2		1	

[a] Flow-adjusted EECs for corn are based on the percentage decrease in maximum screening-level EECs using USGS mean seasonal flow data for the southern region (Table 3.7).
[b] Flow-adjusted EECs for fallow/idle land are based on the percentage decrease in maximum screening-level EECs using USGS mean seasonal flow data for the western region (Table 3.7).

As shown in Table 5.13, refined flow-adjusted 14-, 30-, 60- and 90-day EECs based on atrazine use patterns that yield the highest screening-level EECs (i.e., corn in the southern region and fallow/idle land in the western region), are well below their respective threshold concentrations. Although monitoring data from non-targeted areas shows that longer-term concentrations of atrazine exceed the maximum flow-adjusted EECs by approximately a factor of 2, consideration of similar duration exposures from non-targeted monitoring data (Table 3.11) confirm that all long-term atrazine concentrations are also less than their respective threshold concentrations. It should be noted that the non-targeted data was collected from the Sadusky watershed, which is located within the boundary of vulnerable watersheds; therefore, use of this data is considered as a conservative estimate of exposure in less vulnerable watersheds. The flow-adjusted 14- through 90-day EECs would have to increase by a factor of approximately three to four to exceed the threshold concentrations. However, it is unlikely that flow-adjusted EECs underpredict atrazine exposure in streams and rivers that are outside the boundary of vulnerable watersheds.

Although atrazine use may indirectly affect individual aquatic non-vascular plants that comprise the majority of the listed mussel's diet, its use within less vulnerable watersheds of the action area is not likely to indirectly affect the shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussels via a reduction in phytoplankton food items. This finding is based on insignificance of effects (i.e., community-level effects to non-vascular plants are not likely to result in "take" of a single shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussel). Therefore, the effects determination for the assessment endpoint of indirect effects on the shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussels via direct effects on prey (i.e., phytoplankton) in less vulnerable watersheds is "may affect, but not likely to adversely affect or NLAA."

In addition to the modeling exercises, the Agency used existing targeted monitoring data to further characterize atrazine concentrations in the vulnerable watersheds of the action area where the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels are known to occur. Consideration of the available targeted monitoring data from vulnerable watersheds in Section 3.2.6.1 and Appendix D confirms that longer-term screening-level EECs are likely to be overestimated by the static water body scenario. However, the flow-adjusted 14-, 30-, 60-, and 90-day EECs presented in Table 5.13 appear to under-represent actual chronic exposure concentrations of atrazine in vulnerable areas under some conditions based on the available monitoring data. As shown in Tables D-3 and D-4 of Appendix D, the flow-adjusted chronic EECs are less than their corresponding rolling averages from the available monitoring data in approximately 25 to 43% of the sampled watersheds. Therefore, the ecological monitoring data rolling averages are used to determine whether community-level effects may occur for aquatic non-vascular plants in vulnerable areas that are occupied by the listed mussels. Comparison of the range of rolling averages from the ecological monitoring data with their corresponding threshold concentrations is provided in Table 5.14.

Table 5.14 Summary of Ecological Monitoring Data Rolling Averages with Threshold Concentrations for Potential Community-Level Effects in Vulnerable Watersheds							
14-day		**30-day**		**60-day**		**90-day**	
Range of EECs (µg/L)[a]	**Threshold Conc. (µg/L)**	**Range of EECs (µg/L)[a]**	**Threshold Conc. (µg/L)**	**Range of EECs (µg/L)[a]**	**Threshold Conc. (µg/L)**	**Range of EECs (µg/L)[a]**	**Threshold Conc. (µg/L)**
0.11 – **80**[a] (7.5%)[e]	38	0.10 – **62**[b] (12.5%)[e]	27	0.10 – **26**[c] (5%)[e]	18	0.10 – **18**[d] (5%)[e]	12

[a] Range of 14-day rolling averages from the ecological monitoring data in Table D-3 of Appendix D. Maximum 14-day average concentrations exceed the threshold concentration of 38 µg/L at the following locations: IN 11 (65 µg/L), MO 01 (40-78 µg/L), and NE 07 (80 µg/L).
[b] Range of 30-day rolling averages from the ecological monitoring data in Table D-3 of Appendix D. Maximum 30-day average concentrations exceed the threshold concentration of 27 µg/L at the following locations: IN 11 (32 µg/L), MO 01 (29-43 µg/L), MO 02 (27-32 µg/L), NE 04 (27 µg/L) and NE 07 (45 µg/L).
[c] Range of 60-day rolling averages from the ecological monitoring data in Table D-3 of Appendix D. Maximum 60-day average concentrations exceed the threshold concentration of 18 µg/L at the following locations: MO 01 (19-26 µg/L)and NE 07 (23 µg/L).
[d] Range of 90-day rolling averages from the ecological monitoring data in Table D-3 of Appendix D. Maximum 90-day average concentrations exceed the threshold concentration of 12 µg/L at the following locations: MO 01 (12-18 µg/L) and MO 02 (12 µg/L).
[e] Percentage of watersheds (N = 40) that exceed the corresponding threshold concentration.

As shown in Table 5.14, 14-, 30-, 60- and 90-day rolling averages based on the ecological monitoring data exceed their respective threshold concentrations for a small number of watersheds ranging from approximately 5 to 12.5 percent of the total. Data from the following sites exceeded at least one of the threshold concentrations: IN 11, MO 01, MO 02, NE 04, and NE 07. Although it is uncertain if these sites are representative of the streams and rivers where the listed mussels occur, it is assumed, until further analysis is available, that data from these watersheds may be representative of chronic atrazine exposure conditions in vulnerable watersheds within the action area. Therefore, community-level effects are possible for non-vascular plants within vulnerable watersheds of the action area where the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe feed on phytoplankton. The effects determination for the assessment endpoint of indirect effects on listed mussels via direct effects on phytoplankton as food is “may affect and likely to adversely affect or LAA” for the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels in highly vulnerable watersheds of the action area.

5.2.1.3 Indirect Effects via Reduction in Host Fish

The highest RQ based on the highest PRZM/EXAMS screening-level EEC (southern corn scenario) and the lowest freshwater fish LC_{50} value is 0.02, which is less than the acute LOC of 0.05. As previously discussed, recent targeted and non-targeted monitoring from highly vulnerable and less vulnerable watersheds reported peak EECs that are approximately 2-fold higher than the highest peak screening-level EEC used to calculate RQs. Based on the highest peak EEC reported in the recent targeted monitoring studies in vulnerable watersheds, the acute RQ would be 0.04 (EEC of 209 µg/L / LC_{50} of 5,300 µg/L = RQ of 0.04), which is also below the acute LOC. Therefore, based on the lack of LOC exceedance in vulnerable and less vulnerable watersheds, a conclusion of "no effect" to the seven listed mussels via direct acute effects on freshwater host fish necessary for the mussel glochidia was made.

Chronic RQs, which are based on modeled screening-level 60-day EECs and the surrogate freshwater fish chronic endpoint value for brook trout (NOAEC = 65 µg/L), exceed the Agency's LOCs for corn, sorghum, and fallow/idle land uses with RQ values ranging from 1 to 1.6 (see Table 5.6). However, as previously discussed, chronic RQs based on screening-level EECs (derived using the PRZM/EXAMS pond scenario) are likely to be overestimated given that freshwater mussels are known to occur in flowing water bodies, where chronic atrazine exposures are expected to be significantly lower than 60-day exposure concentrations in a static pond. Based on the analysis conducted in Section 3.2.5, flow-adjusted 60-day EECs are approximately 93 to 98% lower than 60-day EECs modeled using the static water body. As shown in Table 3.7, 60-day flow-adjusted EECs (for the scenarios yielding the highest screening-level EEC from within each of the four geographic regions) range from 2 to 4 µg/L. In addition, the previously discussed non-targeted and targeted vulnerable watershed monitoring data report maximum 60-day rolling averages of 21 and 26 µg/L, respectively. All of the 60-day flow-adjusted and monitoring data EECs are lower than the most sensitive full life-cycle NOAEC of 65 µg/L by roughly a factor of three. The refined chronic RQ value based on the 60-day flow-adjusted EEC is 0.02, and the chronic RQs based on the 60-day EECs from non-targeted and vulnerable watershed monitoring data are <0.4. Therefore, all RQ values are below the Agency's LOC of 1.0 for chronic risk to freshwater fish.

As discussed in Section 4.1.2.3, several open literature studies raise questions about sublethal effects of atrazine on plasma steroid levels, behavior modifications, gill physiology, and endocrine-mediated functions in freshwater fish and anadromous fish. Consideration of the sublethal data indicates that effects associated with alteration of gill physiology and endocrine-mediated olfactory functions may occur in anadromous fish including salmon at atrazine concentrations as low as 1 µg/L (Waring and Moore, 2004; Moore and Lower, 2001). However, there are a number of limitations in the design of these studies, which are addressed in detail in Sections A.2.4 of Appendix A, that preclude quantitative use of the data in this risk assessment. For example, Moore and Lower (2001) exposed epithelial tissue (after removal of skin and cartilage) and not intact fish to atrazine, and potential solvent effects could not be reconciled (i.e., no negative control was tested). Furthermore, no quantitative relationship is established between reduced olfactory response (measured as electrophysiological response) of male epithelial tissue to the female priming hormone in the laboratory and reduction in salmon reproduction (i.e., the ability of male salmon to recognize and mate with ovulating females). In addition, the relevance of sublethal anadromous fish data to this freshwater mussel assessment is questionable. Other sublethal effects observed in fish studies have included behavioral modifications, alterations of plasma steroid levels, and changes in kidney histology at atrazine concentrations ranging from 5 to 35 µg/L (see Section 4.1.2.3). However, a number of uncertainties were also identified with each of the studies, which are discussed in Section A.2.4 of Appendix A.

In summary, it is not possible to quantitatively link the sublethal effects to the selected assessment endpoints for the listed mussels (i.e., survival, growth, and reproduction of individuals). Also, effects to reproduction, growth, and survival were not observed in the four submitted fish life-cycle studies at levels that produced the reported sublethal effects (Appendix A). In addition, there are a number of limitations in the design of these studies, which are addressed in detail in Sections A.2.4a and A.2.4b of Appendix A, that preclude quantitative use of the data in risk assessment.

Although atrazine RQs based on the static water body EECs and a NOAEC of 65 µg/L exceed the chronic LOC of 1.0, its use within the action area is not likely to adversely affect the listed mussels via reduction in available fish hosts because flow-adjusted EECs and available monitoring data indicate that atrazine concentrations are expected to be lower than concentrations that would result in LOC exceedances. Therefore, the effects determination for the assessment endpoint of indirect effects to the listed mussels via direct chronic effects to host fish is "may affect, but not likely to adversely affect or NLAA." This finding is based on insignificance of effects (i.e., chronic exposure to atrazine is not likely to result in a "take" of a single listed mussel via direct chronic effects to host fish).

5*.2.1.4 Indirect Effects via Reduction in Habitat and/or Primary Productivity (Freshwater Aquatic Plants)*

Based on the static pond scenario, the non-vascular aquatic plant LOC of 1.0 was exceeded for all modeled uses. In addition, vascular plant RQs also exceeded the LOC of 1.0 for corn, sorghum, fallow/idle land, and forestry uses of atrazine. Direct effects to vascular and non-vascular plants are expected in both vulnerable and less vulnerable watersheds of the action area, based on peak detected concentrations of atrazine in the ecological monitoring data and non-targeted NAWQA data, which are up to two-fold higher than predicted peak modeled EECs. Based on these potential screening-level direct effects to aquatic plants, atrazine may indirectly affect the seven listed mussels by reducing food supply and primary productivity. Therefore, screening-level time-weighted EECs (for 14-day, 30-day, 60-day, and 90-day averages) were compared to their respective community level effects threshold concentrations to determine whether potential effects to individual plant species are likely to result in community level effects.

A comparison of the screening-level 14-, 30-, 60-, and 90-day EECs for the listed mussels with the atrazine threshold concentrations representing potential aquatic community-level effects is provided in Table 5.12 as part of the risk description for indirect effects to listed mussels based on a reduction of dietary phytoplankton. The results of this analysis (Section 5.2.1.2) show that screening-level EECs exceed threshold concentrations indicative of community-level effects for all durations and modeled atrazine uses with the exception of rights-of-ways. The screening-level EECs were refined by considering site-specific flow data and non-targeted monitoring data (for less vulnerable watersheds) and available targeted ecological monitoring data (for vulnerable watersheds) because screening-level EECs are expected to over-estimate exposure in flowing water bodies where the listed mussels occur.

Comparison of the refined flow-adjusted EECs for less vulnerable watersheds with respective threshold concentrations is shown in Table 5.13 and also discussed in Section 5.2.1.2. The results of this comparison show that flow-adjusted EECs for all atrazine uses and available non-targeted monitoring data are well below threshold concentrations (all durations) for community level effects; therefore, atrazine use in the less vulnerable watersheds of the action area is not likely to adversely affect the shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussels via community-level effects to aquatic vegetation. As previously discussed, the flow-adjusted 14- through 90-day EECs would have to underpredict exposures by a factor of approximately three to four to result in exceedance of the threshold concentrations. However, it is unlikely that flow-adjusted EECs underpredict longer-term atrazine exposure in streams and rivers located in less vulnerable watersheds for reasons previously discussed. Therefore, the effects determination for the assessment endpoint of indirect effects on the listed mussels via direct effects on habitat and/or primary productivity of aquatic plants is “may affect, but not likely to adversely affect or NLAA” for the shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussels in less vulnerable watersheds of the action area. This finding is based on insignificance of effects (i.e., community-level effects to aquatic plants are not likely to result in “take” of a single shiny pigtoe, heavy pigtoe, ovate clubshell, and southern clubshell mussel).

Existing monitoring data were also used to further characterize atrazine concentrations in vulnerable watersheds of the action area where the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe listed mussels are known to occur. As shown in Table 5.14, 14-, 30-, 60-, and 90-day rolling averages based on the ecological monitoring data from vulnerable watersheds exceed their respective threshold concentrations. Therefore, community-level effects are possible for aquatic plants within vulnerable watersheds of the action area where the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe occur. The effects determination for the assessment endpoint of indirect effects on listed mussels via direct effects on habitat and/or primary productivity of aquatic plants is “may affect and likely to adversely affect or LAA” for the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels in vulnerable watersheds of the action area.

5*.2.1.5 Indirect Effects via Alteration in Terrestrial Plant Community (Riparian Habitat)*

As shown in Tables 5.8 and 5.9, seedling emergence and vegetative vigor RQs exceed LOCs for a number of the tested plant species. Based on exceedance of the seedling emergence LOCs for all species tested except corn, the following general conclusions can be made with respect to potential harm to riparian habitat via runoff exposures:

- Atrazine may enter riparian areas via runoff where it may be taken up through the root system of sensitive plants.

- Comparison of seedling emergence EC_{25} values to EECs estimated using TERRPLANT suggests that inhibition of new growth may occur. Inhibition of new growth could result in degradation of high quality riparian habitat over time because as older growth dies from natural or anthropogenic causes, plant biomass may be prevented from being replenished in the riparian area. Inhibition of new growth may also slow the recovery of degraded riparian areas that function poorly due to sparse vegetation because atrazine deposition onto bare soil would be expected to inhibit the growth of new vegetation.

- Because LOCs were exceeded for most species tested (9/10) in the seedling emergence studies, it is likely that many species of herbaceous plants may be potentially affected by exposure to atrazine in runoff.

A number of dicots in riparian habitats may also be impacted via foliar exposure from atrazine in spray drift as evidenced by vegetative vigor LOC exceedances in three dicots. Therefore, riparian habitats comprised of herbaceous plants sensitive to atrazine may be adversely affected by spray drift. However, comparison of the seedling emergence and vegetative vigor RQs indicates that runoff, and not spray drift, is a larger contributor to potential risk for riparian vegetation. Vegetative vigor risk quotients were not exceeded for monocots; therefore, drift would not be anticipated to affect riparian zones comprised primarily of monocot species such as grasses.

Because RQs for terrestrial plants are above the Agency's LOCs, atrazine use is considered to have the potential to directly impact plants in riparian areas, potentially resulting in degradation of stream water quality via sedimentation and alteration of the listed mussel's habitat. Therefore, an analysis of the potential for habitat degradation to affect the listed mussels is necessary.

Riparian plants beneficially affect water and stream quality in a number of ways (discussed below) in both adjacent river reaches and areas downstream of the riparian zone. Atrazine use in the action area, which is inclusive of the listed mussels range, may potentially affect these species by impacting riparian vegetation and subsequently causing sedimentation that results in degraded water quality and alteration of available habitat. In order to characterize the potential indirect effects caused by atrazine-related impacts to riparian vegetation, a general discussion of riparian habitat and its relevance to the listed mussels and a description of the types of riparian zones that may be potentially impacted by atrazine use in the action area for the listed mussels are discussed below.

Importance of Riparian Habitat to the Listed Mussels

Riparian vegetation provides a number of important functions in the stream/river ecosystem, including the following:

- serves as an energy source;
- provides organic matter to the watershed;
- provides shading, which ensures thermal stability of the stream; and
- serves as a buffer, filtering out sediment, nutrients, and contaminants before they reach the stream.

The specific characteristics of a riparian zone that are optimal for the listed mussels are expected to vary with developmental stage, the use of the reach adjacent to the riparian zone, and the hydrology of the watershed. Criteria developed by Fleming et al. (2001) have been used to assess the health of riparian zones and their ability to support habitat for aquatic communities. These criteria, which include the width of vegetated area (i.e. distance from cropped area to water), structural diversity of vegetation, and canopy shading, are summarized in Table 5.15.

Table 5.15 Criteria for Assessing the Health of Riparian Areas to Support Aquatic Habitats (adapted from Fleming et al. 2001)

Criteria	Quality			
	Excellent	**Good**	**Fair**	**Poor**
Buffer width	>18m	12 - 18m	6 - 12m	<6m
Vegetation diversity	>20 species	15 - 20 species	5 - 14 species	<5 species
Structural diversity	3 height classes grass/shrub/tree	2 height classes	1 height class	sparse vegetation
Canopy shading	mixed sun/shade	sparse shade	90% sun	no shade

To maintain at least “good” water quality for aquatic habitats in general, riparian areas should contain at least a 12 m (~40 feet) wide vegetated area, 15 plant species, vegetation of at least two height classes, and provide at least sparse shade (>10% shade). In general, higher quality riparian zones (wider vegetated areas with greater plant diversity) are expected to have a lower probability of being significantly affected by atrazine than poor quality riparian areas (narrower areas with less vegetation and little diversity).

The following three attributes of riparian vegetation habitat quality were evaluated for this assessment: stream bank stability, sedimentation, and thermal stability. Each of these attributes and their relative importance with respect to the listed mussels is discussed briefly below.

<u>Stream and river bank stabilization:</u> Riparian vegetation typically consists of three distinct height classes of plants, which include a groundcover of grasses and forbs, an understory of shrubs and young trees, and an overstory of mature trees. These plants serve as structural components for streams, with the root systems helping to maintain stream stability, and the large woody debris from the mature trees providing instream cover. Riparian vegetation has been shown to be essential to maintenance of a stable

stream (Rosgen, 1996). Destabilization of the stream can have a severe impact on aquatic habitat quality. In fact, geomorphically stable stream and river channels and banks are identified as PCEs for designated critical habitat of the ovate and southern clubshell mussels. Any action that would significantly alter channel morphology or geometry to a degree that would appreciably reduce the value of the critical habitat for both the long-term survival and recovery of the species is considered as part of the critical habitat impact analysis in Section 5.2.2.3.

Following a disturbance in the watershed bank, the stream may widen, releasing sediment from the stream banks and scouring the stream bed. Changes in depth and or the width/depth ratio via physical modification to the stability of stream and river banks may also affect light penetration and the flow regime of the listed mussel's habitat. Destabilization of the stream can have severe effects on aquatic habitat quality by increasing sedimentation within the watershed. The effects of sedimentation are summarized below.

Sedimentation: Sedimentation refers to the deposition of particles of inorganic and organic matter from the water column. Increased sedimentation is caused primarily by disturbances to river bottoms and streambeds and by soil erosion. Riparian vegetation is important in moderating the amount of sediment loading from upland sources. The roots and stems of riparian vegetation can intercept eroding upland soil (USDA NRCS, 2000), and riparian plant foliage can reduce erosion from within the riparian zone by covering the soil and reducing the impact energy of raindrops onto soil (Bennett, 1939).

Freshwater mussels require fast flowing, silt free streams and rivers in order to survive. Therefore, they are susceptible to adverse effects caused by sedimentation in waterways. Specific biological impacts on mussels from excessive sediments include reduced feeding and respiratory efficiency from clogged gills, disrupted metabolic processes, reduced growth rates, increased substrata instability, limited burrowing activity and physical smothering (Ellis, 1936; Stansbery, 1971; Markings and Bills, 1979; Kat, 1982; Vannote and Minshall, 1982; Aldridge et al., 1987; and Waters, 1995). Physical effects of sediment on the listed mussels appear to be multifold, and include changes in suspended and bed material load; alteration in bed sediment composition; changes in channel form, position, and degree of stability; alteration of light penetration via turbidity; active aggrading (filling) or degrading (scouring) of channels; and changes in channel position that may reduce suitable habitat for mussels (Vannote and Minshall, 1982; Kanehl and Lyons, 1992; Brim Box and Mossa, 1999).

Interstitial spaces in the substrate also provide crucial habitat for juvenile mussels. When clogged due to sedimentation, interstitial flow rates and spaces become reduced (Brim Box and Mossa, 1999), thus reducing juvenile mussel habitat. Sediments also act as a means of transport for delivering contaminants such as nutrients and pesticides to streams. Juveniles can readily ingest contaminants adsorbed to silt particles or in interstitial pore water during normal feeding activities (Yeager et al., 1994; Newton, 2003).

According to the USFWS *Recovery Plan of the Mobile River Basin Aquatic Ecosystem* (USFWS, 2000b), which addresses specific threats to four of the assessed mussel species included in this assessment (heavy pigtoe, ovate clubshell, southern clubshell, and stirrupshell mussels), sedimentation is considered the greatest factor threatening the aquatic ecosystems across the Mobile River Basin. Sedimentation is also cited as a primary cause for the decline of freshwater mussels in the USFWS recovery plans for the other species included in this assessment (pink pearly mucket (USFWS, 1985), rough pigtoe (USFWS, 1984a), shiny pigtoe (USFWS, 1984b), and the fine-rayed pigtoe (USFWS, 1984c). Excessive sediments deposited on stream bottoms can smother and kill relatively immobile bottom-dwelling species such as freshwater mussels and can eliminate more mobile aquatic species (such as host fish) by making their habitat unsuitable for feeding or reproduction (Brookes, 1994; National Research Council, 1992; Waters, 1995; Hartfield and Hartfield, 1996). Increased sedimentation may affect the spawning habitat of host fish by settling on spawning gravel and reducing flow of water and dissolved oxygen to the eggs and fry (Everest et al., 1987). In addition, fine particles settling on the streambed can also disrupt the food chain by reducing habitat quality for aquatic invertebrates, and adversely affect groundwater-surface water interchange (Nelson et al., 1991). Increased turbidity from sediment loading may also reduce light transmission, potentially affecting aquatic plants (Cloern, 1987; Weissing and Huisman, 1994) that are important source of food for the listed mussels.

The critical habitat impact analysis (Section 5.2.2.3) also considers potential adverse modification to designated critical habitat for the ovate and southern clubshell mussels via sedimentation for the following PCEs: alteration of host fish spawning areas, water quality parameters including turbidity, and silt-free substrates.

<u>Thermal stability</u>. Riparian habitat including mature woody trees provides stream shading resulting in thermal stability. Although the sensitivity of the listed mussels to fluctuations in water temperature are unknown, stream shading has been shown to be positively correlated with freshwater unionid mussel species richness and density (Arbuckle and Downing, in press; obtained from http://limnology.eeob.iastat.edu/Studies/MusselStudies/FinalReport/Chapter4.htm; January 25, 2007).

Water quality parameters including temperature that may be impacted by direct effects to forested riparian areas are also considered as part of the critical habitat impact analysis in Section 5.2.2.3.

Sensitivity of Forested Riparian Zones to Atrazine

As previously summarized in Table 5.15, the parameters used to assess riparian quality include buffer width, vegetation diversity, vegetation cover, structural diversity, and canopy shading. Buffer width, vegetation cover, and/or canopy shading may be reduced if atrazine exposure impacts plants in the riparian zone or prevents new growth from emerging. Plant species diversity and structural diversity may also be affected if only sensitive plants are impacted (Jobin et al., 1997; Kleijn and Snoeijing, 1997), leaving non-sensitive plants in place. Atrazine may also affect the long term health of high quality riparian habitats by affecting seed germination. Thus, if atrazine exposure impacted these riparian parameters, water quality within the action area for the listed mussels could be affected.

Because the majority of woody plants (i.e., shrubs and trees) are not sensitive to environmentally-relevant atrazine concentrations (MRID #46870400-01), effects on shading, streambank stabilization, and structural diversity (in terms of height classes) of woody forested vegetation are not expected. Effects are expected to be limited to herbaceous (non-woody) plants (e.g., grasses), which are not generally associated with shading.

The riparian health criteria described in Fleming et al. (2001; Table 5.15) and the characteristics associated with effective vegetative buffer strips suggest that healthier riparian zones would be less sensitive to the impacts of atrazine runoff than poorer riparian zones. Although riparian zones rich in species diversity and woody species may contain sensitive species, it is unlikely that they would consist of a high proportion of very sensitive plants. Wider buffers have more potential to reduce atrazine residues over a larger area, resulting in lower loading levels. According to Fleming et al. (2001), buffer distances of >18 m (approximately 60 feet) are characterized as “excellent” in supporting aquatic habitats. It should be noted that the label requirements for atrazine specify no use within 66 feet of intermittent and perennial streams. While this “buffer” area was established to decrease atrazine loading to waterbodies resulting from drift, if maintained with other good to excellect (Table 5.15) riparian habitat attributes, it is likely to reduce atrazine runoff to adjacent waterbodies. In addition, trees and woody plants in a healthy riparian area act to filter spray drift (Koch et al., 2003) and push spray drift plumes over the riparian zone (Davis et al., 1994), thus reducing exposure to lower height classes of plants (i.e., grassy and non-woody vegetation), which tend to be more sensitive. Therefore, higher quality riparian zones are expected to be less sensitive to atrazine than riparian zones that are narrow, low in species diversity, and comprised of young herbaceous plants or unvegetated areas. The available data suggest that riparian zones comprised of herbaceous plants and grasses would likely be most sensitive to atrazine effects. Bare ground riparian areas and areas with sparse vegetation could also be adversely affected by prevention of new growth of grass, which can be an important component of riparian vegetation for maintaining water quality.

Based on the low sensitivity of forested areas containing woody shrubs and trees to atrazine, it is unlikely that atrazine will adversely affect these types of riparian vegetation adjacent to use sites and watersheds within the action area of the listed mussels.

Potential for Atrazine to Indirectly Affect the Listed Mussels via Effects on Riparian Vegetation

It is difficult to estimate the magnitude of potential impacts of atrazine use on riparian habitat and the magnitude of potential effects on stream water quality from such impacts as they relate to survival, growth, and reproduction of the listed mussels. The level of exposure and any resulting magnitude of effect on riparian vegetation are expected to be highly variable and dependent on many factors. The extent of runoff and/or drift into stream corridor areas is affected by the distance the atrazine use site is offset from the stream, local geography, weather conditions, and quality of the riparian buffer itself. The sensitivity of the riparian vegetation is dependent on the susceptibility of the plant species present to atrazine and composition of the riparian zone (e.g. vegetation density, species richness, height of vegetation, width of riparian area).

Quantification of risk to the listed mussels from potential effects to riparian areas is precluded by the following factors:

- The relationship between distance of soil input into the watershed and sediment deposition in areas critical to survival, reproduction, and growth of the listed mussels is not known;
- Riparian areas within the action area are highly variable in their composition and location with respect to atrazine use; therefore, their sensitivity to potential damage is also variable; and
- The action area for the listed mussels is a large geographic area, encompassing 10 states.

In addition, even if plant community structure was quantifiably correlated with riparian function, it may not be possible to discern the effects of atrazine on species composition separate from other agricultural actions or determine if atrazine is a significant factor in altering community structure. Plant community composition in agricultural field margins is likely to be modified by many agricultural management practices. Vehicular impact and mowing of field margins and off-target movement of fertilizer and herbicides are all likely to cause changes in plant community structure of riparian areas adjacent to agricultural fields (Jobin et al., 1997; Kleijn and Snoeijing, 1997; Schippers and Joenje, 2002). Although herbicides are commonly identified as a contributing factor to changes in plant communities adjacent to agricultural fields, some studies identify fertilizer use as the most important factor affecting plant community structure near agricultural fields (e.g. Schippers and Joenje, 2002) and community structure is expected to be affected by a number of other factors (de Blois et al., 2002). Specifically, the alteration and destruction of stream habitat due to impoundments for flood control, navigation, hydroelectric power, and recreation are critical factors that may impact water quality for the listed mussels within the defined action area (USFWS 1985, 1984a, 1984b, 1984c, 1989, 2000). Thus, the effect of atrazine alone on riparian community structure is complicated by other multiple stressors likely to occur within the action area for the listed mussels. Although the data do not allow for a quantitative estimation of risk from potential riparian habitat alteration, a qualitative discussion is presented below.

In summary, terrestrial plant RQs are above LOCs for all uses; therefore, riparian vegetation may be affected by use of atrazine. As previously discussed, the potential for atrazine to affect the listed mussels via impacts on riparian vegetation depends primarily on the extent of potentially sensitive (herbaceous and grassy) riparian areas and their impact on water quality in the streams and rivers where the listed mussels are known to occur. Because woody plants are generally not sensitive to atrazine at expected exposure concentrations, riparian areas which have predominantly forested vegetation containing woody shrubs and trees are not likely to be impacted by atrazine use. Therefore, atrazine is not likely to adversely affect populations of listed mussels in watersheds with predominantly forested riparian areas.

Conversely, atrazine may affect grassy and herbaceous riparian vegetation, resulting in increased sedimentation which could impact the listed mussels in ways previously described. However, the extent to which herbaceous or grassy riparian area versus forested riparian areas are present within the action area surrounding the listed mussel's range is unknown.

Therefore, there are separate effects determinations for indirect effects to the listed mussels via direct atrazine effects on riparian vegetation, depending on the presence of forested (woody shrubs and trees) versus herbaceous (grassy and non-woody) riparian vegetation adjacent to the streams and rivers within the listed mussel's action area. For areas where the riparian habitat is predominantly forested with shrubs and trees, the effects determination is "may affect, but not likely to adversely affect or NLAA". This finding is based on insignificance of effects (i.e., effects to forested riparian vegetation in the action area encompassing the range of the listed mussels are not likely to result in "take" of a single listed mussel). For habitats of the assessed mussels that are in close proximity to potential atrazine use sites and where the riparian vegetation is comprised of grasses and non-woody plants, the effects determination is "may affect and likely to adversely affect or LAA". A graphic representation of the effects determination for this assessment endpoint, based on evaluation of the sedimentation, streambank stability, and thermal stability attributes for riparian vegetation is provided in Figure 5.1.

Given the "LAA" finding for areas where herbaceous and grassy riparian vegetation is present, the Agency has completed a summary of the environmental baseline and cumulative effects for the listed mussel species included in this assessment in Appendix H. The environmental baseline is defined as the effects of past and ongoing human induced and natural factors leading to the status of the species, its habitat, and ecosystem, within the action area. The baseline information provides a snapshot of the assessed mussel's status at this time. A summary of all USFWS biological opinions that are relevant to the listed mussels that have been made available to EPA included in this assessment is also provided as part of the baseline status. Cumulative effects include the effects of future state, tribal, local, private, or other non-federal entity activities on endangered and threatened species and their critical habitat that are reasonably expected to occur in the action area.

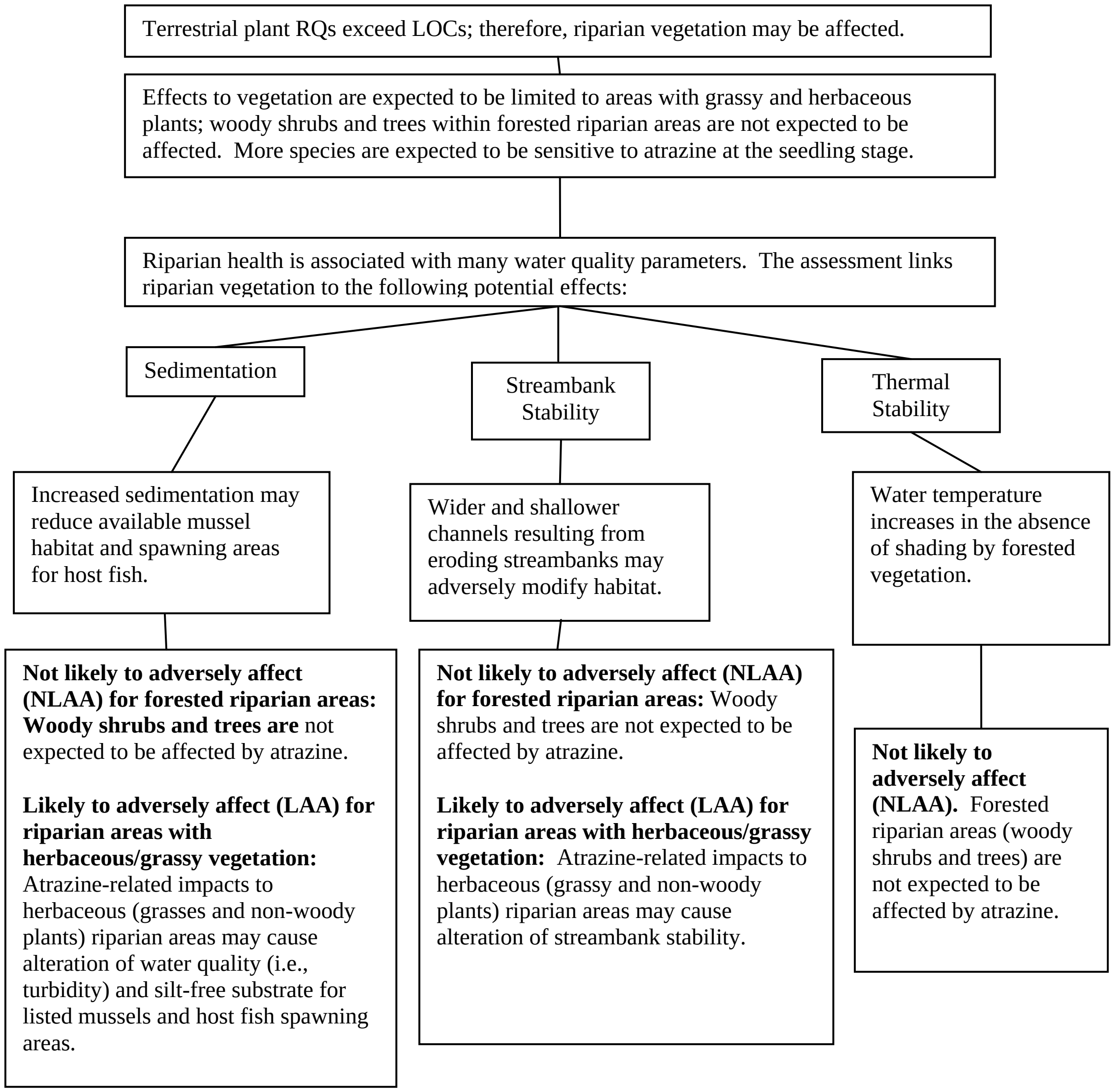

Figure 5.1 Summary of the Potential of Atrazine to Affect the Listed Mussels via Riparian Habitat Effects

5.2.2 Adverse Modification to Designated Critical Habitat

As previously discussed, designated critical habitat for the ovate and southern clubshell mussels are located in watersheds considered to be less vulnerable than those identified by WARP. Therefore, EECs derived using the ecological monitoring data located in highly vulnerable watersheds were not considered to be representative of atrazine exposure for designated critical habitat located outside of the highly vulnerable watershed boundaries. For designated critical habitat located outside of the highly vulnerable watersheds, refined EECs were based on the PRZM/EXAMS flow-adjusted modeling discussed in Section 3.2.5 (Table 3.7) and available non-targeted monitoring data including peak values from NAWQA data (Section 3.2.6.2) and Heidelberg College data (Section 3.2.6.4) with sufficient sampling frequency to derive 14- through 90-day rolling average exposure concentrations for comparative purposes.

5.2.2.1 Adverse Modification to Designated Critical Habitat via Direct Effects to Host Fish and Food Items)

Adverse modification of critical habitat via alteration of living and foraging areas for host fish is assessed by considering direct effects to host fish and their food items in the southern geographic region of the action area where critical habitat has been designated for the ovate and southern clubshell mussels. Known host fish for the southern clubshell include two species of shiners, and host fish for the ovate southern clubshell are unknown. Therefore, the most sensitive acute and chronic toxicity data for freshwater fish and invertebrates were considered in order to be protective to unidentified species of freshwater fish hosts for these listed mussel species.

Direct acute and chronic effects to host fish were evaluated as part of the indirect effects analysis for listed mussels in Section 5.2.1.3. Acute RQs are less the Agency's LOCs; therefore, the effects determination for adverse modification to designated critical habitat via acute direct effects of atrazine to host fish is "no effect". Chronic RQs exceed LOCs based on screening-level 60-day EECs; however refined RQs based on flow-adjusted EECs and non-targeted monitoring data are well below the chronic LOC. Therefore, the effects determination for the critical habitat impact analysis PCE associated with adequate living areas for host fish via direct chronic effects is "may affect, but not likely to adversely affect or NLAA". This finding is based on insignificance of effects (i.e., chronic effects to the living areas for host fish are not likely to adversely modify the critical habitat for the ovate and southern clubshell mussels).

Potential adverse modification to critical habitat foraging areas for host fish of the ovate and southern clubshell mussels was addressed at the screening-level in Section 5.1.3.1 by considering indirect effects to the host fish based on direct effect to prey items (i.e., freshwater invertebrates). Based on this screening-level analysis, acute and chronic LOCs were exceeded. Further evaluation of direct effects to prey items of host fish was conducted by considering the flow-adjusted EECs for the southern region and non-targeted monitoring data. Acute RQs for atrazine use in the southern geographic portion of the action area exceed LOCs based on flow-adjusted peak EECs and available non-targeted peak monitoring data from NAWQA. The NAWQA data from Bogue Chitto Creek is particularly relevant because this watershed is designated as critical habitat for the southern clubshell mussel. However, the Bogue Chitto Creek peak concentration of 201 µg/L was detected in 1999, and more recent data from 2001-2004 indicate that peak atrazine concentrations in this watershed have decreased over time to $\leq$ 25 µg/L. Although the 1999 Bogue Chitto Creek data are likely to overestimate current peak atrazine exposure in the watersheds of the southern region of the action area, flow-adjusted EECs indicate that peak values may exceed 100 µg/L. In addition, further consideration of the 99.9th percentile of all peak monitoring data from NAWQA (61 µg/L) would result in acute RQs that exceed the aquatic invertebrate LOC of 0.05. Although acute LOCs are exceeded, acute RQs for aquatic invertebrates are based on the lowest LC_{50} value of 720 µg/L for the midge (*Chironomus* spp.). However, as discussed in Section 4.1.3.1, the available acute toxicity data for freshwater invertebrates show high variability, ranging from 720 to >33,000 µg/L. With the exception of the midge, reported acute toxicity values for other freshwater invertebrates that may be food items for host fish are 3,500 µg/L and higher. The corresponding acute RQ based an acute LC_{50} value of 3,500 µg/L and the maximum flow-adjusted EEC (120 µg/L) would be 0.03, less than the acute LOC. Although use of the 1999 peak concentration from Bogue Chitto Creek would result in acute RQs above the Agency's LOC, this concentration is likely to overestimate exposure given more recent NAWQA data in this watershed, as well as consideration of all the peak atrazine data available from NAWQA. Furthermore, the probability of an individual effect to the most sensitive aquatic invertebrate food item (i.e., the midge), based on the dose-response probit slope of 4.4 and an RQ of 0.16 is 1 in 4,300 (0.02%). Chronic RQs based on refined flow-adjusted 21-day EECs and non-targeted monitoring data (21-day EEC = 21 µg/L) are less than the Agency's LOCs.

Table 5.16 Summary of Acute and Chronic RQs (Based on Flow-adjusted and Non-Targeted EECs) Used to Estimate Adverse Modification to Critical Habitat via Direct Effects on Dietary Items of Host Fish

Peak and 21-day EECs (µg/L)	Freshwater Invertebrate Acute RQ (EC_{50} = 720 µg/L[a])	Acute LOC Exceedance and Risk Interpretation	Freshwater Invertebrate Chronic RQ (NOAEC= 60 µg/L[b])	Chronic LOC Exceedance and Risk Interpretation
Flow-Adjusted Modeled EECs[c] Peak = 120 21-D = 10	0.16	Yes[d]	0.16	No[e]
Non-targeted Monitoring EECs[f] Peak = 201 21-D = 21	0.28	Yes[d]	0.35	No[e]

[a] Based on 48-hour EC_{50} value of 720 µg/L for the midge (MRID # 000243-77). Slope information for this study is not available. Therefore, the probability of an individual effect was calculated using a probit slope of 4.4, which is the only technical grade atrazine value reported in the available freshwater invertebrate acute studies; 95% confidence intervals could not be calculated based on the available data (Table A-18).
[b] Based on 30-day NOAEC value of 60 µg/L for the scud (MRID # 000243-77).
[c] Flow-adjusted peak and 21-day EECs from the southern region (Table 3.7)
[d] RQ > acute endangered species LOC of 0.05. The probability of an individual effect to aquatic invertebrates at the acute RQ of 0.16 is 1 in 4,300 (0.02%); at an RQ of 0.28, the probability of an individual effect to aquatic invertebrates is 1 in 133 (0.75%).
[e] RQ < chronic LOC of 1.0.
[f] Peak non-targeted monitoring EEC is based on NAWQA data from 1999 for Bogue Chitto Creek (Table 3.9). The 21-day value is based on Heidelberg College monitoring data from the Sadusky watershed (Table 3.11).

Given that most of the freshwater fish identified as host fish feed non-selectively, coupled with the low magnitude of anticipated individual effects to the most sensitive food item (i.e., the midge), atrazine is not likely to affect the host fish for the ovate and southern clubshell mussels via a reduction in freshwater invertebrate food items. This finding is based on insignificance of effects (i.e., effects to freshwater invertebrates cannot be meaningfully measured, detected, or evaluated in the context of a level of effects where adverse modification to critical habitat via changes in the foraging habitat of host fish would occur). Therefore, the effects determination for the critical habitat impact analysis

PCE associated with adequate foraging areas for host fish is "may affect, but not likely to adversely affect or NLAA".

5*.2.2.2 Adverse Modification to Designated Critical Habitat via Direct Effects to Aquatic Plants*

The following PCEs are evaluated in order to determine whether adverse modification of designated critical habitat for the ovate and southern clubshell mussels may occur via actions that directly effect aquatic vascular and non-vascular plants: (1) substrates with low to moderate amounts of filamentous algae; (2) maintenance of water quality parameters such as oxygen content; and (3) suitable habitat for fish hosts.

As an herbicide, any potential effects on filamentous algae are expected to be reductions in algal mass. Given that the PCE is associated with low levels of filamentous algae, atrazine is not expected to adversely modify critical habitat of the ovate and southern clubshell mussels by increasing the amount of filamentous algae on substrate necessary for normal growth and viability of these mussel species. The effects determination for the PCE associated with low to moderate amounts of filamentous algae on substrates is "may affect, but not likely to adversely effect or NLAA". This determination is based on insignificance of effects (i.e., atrazine is not expected to adversely modify the critical habitat because its use is likely to reduce the amount of filamentous algae on substrates).

As previously discussed in Section 5.1.3.2, reductions in oxygen levels could be impacted by atrazine if concentrations reach levels that negatively impact the aquatic plant community and reduce primary productivity. However, all of the rivers and streams designated as critical habitat for the ovate and southern clubshell mussels are located in flowing water bodies, where the oxygen content is less likely to be influenced by temporary variability in plant biomass as a result of atrazine exposure. In addition, comparison of the refined modeling considering flow and the non-targeted monitoring data suggest that atrazine concentrations in less vulnerable watersheds are well below the 14-, 30-, 60-, and 90-day threshold concentrations for aquatic community-level effects. Therefore, atrazine use within the less vulnerable watersheds of the action area where critical habitat occurs is not expected to result in direct effects on primary productivity of aquatic plants and resulting alteration of oxygen content. The effects determination for the critical habitat impact analysis PCE associated with water quality parameters including oxygen content is "may affect, but not likely to adversely affect or NLAA". This finding is based on insignificance of effects (i.e., effects to water quality cannot be meaningfully measured, detected, or evaluated in the context of a level of effects where adverse modification to critical habitat (via changes to primary productivity and resulting oxygen content) would occur.

Based on the screening-level analysis of indirect effects to the habitat of listed mussels via direct effects to aquatic plants (Section 5.1.2.3), atrazine may adversely modify designated critical habitat of the ovate and southern clubshell mussels. Further analysis of the flow-adjusted 14-, 30-, 60-, and 90-day EECs from the southern geographic region and similar durations of exposure from the available non-targeted monitoring data relative to their respective threshold concentrations was completed to determine whether effects to individual aquatic plants would result in adverse modification to critical habitat via community-level effects to the ovate and southern clubshell mussels. The results of this analysis are used as a basis to determine whether similar habitat modification may occur for host fish. As shown in Table 5.13, refined flow-adjusted 14-, 30-, 60-, and 90-day EECs, based on atrazine use on corn in the southern region, are well below their respective threshold concentrations. In addition, similar durations of exposure from the non-targeted monitoring data are also less than their respective threshold concentrations.

Although atrazine may indirectly affect individual aquatic plants, its use within the southern region of the action area (outside the boundary of vulnerable watersheds) is not likely to adversely modify designated critical habitat for host fish of the ovate and southern clubshell mussels, based on community-level effects to the aquatic community. The effects determination for the critical habitat impact analysis PCE associated with suitable habitat for host fish of the ovate and southern clubshell mussels is “may affect, but not likely to adversely affect or NLAA”. This finding is based on insignificance of effects (i.e., effects to suitable habitat for host fish cannot be meaningfully measured, detected, or evaluated in the context of a level of effects where adverse modification to critical habitat via alteration to the structure and function of the aquatic plant community would occur).

5.*2.2.3 Adverse Modification to Designated Critical Habitat via Direct Effects to Riparian Vegetation*

Reduction in riparian vegetation could impact the following PCEs: (1) presence/maintenance of geomorphically stable stream and river channels; (2) maintenance of water quality parameters including temperature and turbidity; and (3) presence/maintenance of silt-free substrates necessary for viability of the ovate and southern clubshell mussels and spawning habitat for their fish hosts.

The potential for atrazine to affect riparian vegetation was evaluated as an indirect effect to the assessed listed mussels and is presented in Section 5.2.1.5. Conclusions from the analysis presented in Section 5.2.1.5 are also applicable to the evaluation of riparian vegetation as it relates to adverse modification of designated critical habitat and include the following:

- Riparian areas comprised of predominantly grassy, herbaceous, and/or sparse vegetation in close proximity to atrazine use may be affected such that their ability to maintain water quality could be reduced.

- Riparian areas comprised of predominantly forested vegetation (i.e., woody shrubs and trees) are not likely to be affected by use of atrazine because of the low sensitivity of woody plants to atrazine.

The results of the screening-level evaluation indicate that atrazine use may adversely affect the critical habitat of the ovate and southern clubshell mussels via direct impacts to herbaceous and grassy riparian vegetation. However, critical habitat with riparian areas comprised of predominantly forested vegetation such as trees and woody shrubs would not likely be adversely affected by use of atrazine. Therefore, for areas where forested riparian vegetation including woody shrubs and trees is present, the effects determination is “may affect, but not likely to adversely affect or NLAA”. This finding is based on insignificance of effects (i.e., potential effects to forested vegetation cannot be meaningfully measured, detected, or evaluated in the context of a level of effect where adverse modification to critical habitat via changes in streambank stability, water temperature, turbidity, and sedimentation may occur).

Atrazine may affect herbaceous and grassy riparian vegetation, resulting in streambank instability, sedimentation, reduction in water quality (via increased turbidity), and modification to substrates necessary for the ovate and southern clubshell and spawning habitat for their host fish. Therefore, for areas where herbaceous and grassy riparian vegetation is present, the aforementioned PCEs of critical habitat may be adversely modified, and the effects determination is “may affect and likely to adversely affect or LAA”. Appendix H contains information relevant to environmental baseline and cumulative effects for the critical habitat impact analysis, given this determination.

5*.2.2.4 Adverse Modification to Designated Critical Habitat via Effects to Chemical Characteristics Necessary for Normal Behavior, Growth, and Viability of All Mussel Life Stages*

The critical habitat impact analysis associated with chemical characteristics necessary for normal behavior, growth, and viability of all life stages of the ovate and southern clubshell mussels is based on the direct effects to listed mussels (Section 5.2.1.1) and indirect effects to listed mussels via reduction in food items (Section 5.2.1.2). Other indirect effects to the ovate and southern clubshell mussel (via alteration to host fish living, foraging and spawning areas; water quality, stream bank stability, and silt-free substrates with low amounts of filamentous algae) are assessed via other specified PCEs for their designated critical habitat. If LOCs are exceeded for direct effects and for indirect effects based on a reduction in food items, then the chemical environment is presumed to be such that normal behavior, growth, and viability of the ovate and southern clubshell mussel’s critical habitat may be adversely modified. Potential direct and indirect effects were previously evaluated. Results of those analyses are summarized below.

With respect to direct effects, acute effects are not likely for listed mussels because acute RQs are well below LOCs. Therefore, adverse modification to designated critical habitat based on direct acute effects to listed mussels is not likely to occur. The effects determination for this endpoint is “no effect”. Based on the screening-level analysis, direct chronic effects to the listed mussels may occur. However, refinement based on flow-adjusted 21-day EECs and consideration of non-targeted monitoring data yields chronic RQs that are also below LOCs. Therefore, adverse modification to critical habitat based on chronic direct effects to the ovate and southern clubshell mussels is not expected to occur, and the effects determination is “may affect, but not likely to adversely affect or NLAA". This finding is based on insignificance of effects (i.e., chronic exposure to atrazine is not likely to adversely modify the chemical environment presumed to be essential for normal behavior, growth, and viability of all mussel life stages).

Indirect effects to listed mussels based on a reduction in zooplankton as a food source may occur because acute and chronic RQs are above LOCs (based on screening-level EECs and toxicity data for the most sensitive freshwater invertebrate). As shown in Section 5.2.1.2, the refined acute and chronic analyses for zooplankton include consideration of flow-adjusted EECs and non-targeted monitoring data in addition to effects data that are specific to zooplankton. Based on the refined analysis, the effects determination for adverse habitat modification via direct acute effects to zooplankton is "may affect, but not likely to adversely affect or NLAA" because zooplankton are not the primary food source for the ovate and southern clubshell mussels, the probability of an individual effect to zooplankton is low (i.e., 0.2%), and the refined RQ based on peak NAWQA 2000-2004 monitoring data specific for a designated critical habitat watershed (i.e., Bogue Chitto Creek) is well below the acute LOC for aquatic invertebrates. The chronic effects determination is also "may affect, but not likely to adversely affect or NLAA" because all refined measures of chronic exposure (i.e., 21-day flow-adjusted EECs and similar durations of exposure from non-targeted monitoring data) are well below chronic effect levels in zooplankton. Both "NLAA" effects determinations for adverse modification to critical habitat via reduction in zooplankton as a food source to the ovate and southern clubshell mussels are based on insignificance of effects (i.e., acute and chronic effects to zooplankton as food items are not likely to result in adverse modification of critical habitat for the ovate and southern clubshell mussels).

Phytoplankton is the primary component of the listed mussel's diet. Screening-level RQs for phytoplankton (i.e., non-vascular aquatic plants) exceed LOCs for all labeled uses of atrazine. As shown in Section 5.2.1.2 and Table 5.13, refined flow-adjusted 14-, 30-, 60- and 90-day EECs, based on the southern corn scenario, are well below their respective threshold concentrations representative of community-level effects. In addition, 14-, 30-, 60-, and 90-day EECs based on available non-targeted monitoring data are also less than their respective threshold concentrations. Therefore, adverse modification to critical habitat based on reduction in phytoplankton as food for the ovate and southern clubshell mussels is not expected to occur, and the effects determination is "may affect, but not likely to adversely affect or NLAA". This finding is based on insignificance of effects (i.e., reduction in phytoplankton due to chronic exposure to atrazine is not likely to adversely modify the chemical environment presumed to be essential for normal behavior, growth, and viability of all mussel life stages).

6. Uncertainties

6.2 Exposure Assessment Uncertainties

While peak exposures in available monitoring data are within a factor of two of modeling, longer term concentrations (e.g. 30-day averages) are generally higher in screening-level modeling than in monitoring data. Conversely, refined modeling using flow through the Index Reservoir water body (typically used for drinking water assessments) are similar when comparing peak concentrations, but are lower than the longer term concentrations seen in a subset of monitoring sites in the most vulnerable watersheds. However, the majority of atrazine concentrations from monitored sites that are greater than modeled EECs are within 2 to 3 times of the refined flow-adjusted modeled EECs. Viewed in the context of exposure for all atrazine use areas, the refined modeling is likely to represent a reasonable approximation of high end atrazine exposure.

The primary factor that may result in over-estimation of exposure in the screening-level modeling is the assumption of no flow in the modeled water body. Factors that may account for under-estimation of exposure in the refined modeling relative to the most vulnerable watersheds may include differences between reservoir volume, watershed size, and flow dynamics relative to stream characteristics, as well as differences in the flow rates used in the refined modeling (taken from occupied streams generally at 4th order and higher) compared to flow rates in the 2nd and 3rd order streams represented by most of the vulnerable watershed sites. Furthermore, the impact of setbacks on runoff estimates has not been quantified, although well-vegetated setbacks are likely to result in significant reduction in runoff loading of atrazine.

Overall, analysis indicates that increasing flow will result in significant reduction in exposure relative to screening level model estimates, particularly for longer-term durations of exposure (14-day, 30-day, etc.).

6.1.1 Modeling Assumptions

Overall, the uncertainties addressed in this assessment cannot be quantitatively characterized. Given the available data and use of conservative modeling assumptions, it is expected that the screening-level modeled EECs over-predict exposure for longer-term durations, but are within a factor of two as compared with peak monitored concentrations. However, refined flow-adjusted EECs are likely to be conservative for all but a subset of watersheds most vulnerable to atrazine runoff.

In general, the simplifying assumptions used in this assessment appear from the characterization in Section 3.2.7 to be reasonable given the analysis completed and the available monitoring data. There are also a number of assumptions that tend to result in over-estimation of exposure. Although these assumptions cannot be quantified, they are qualitatively described. For instance, modeling in this assessment for each atrazine use assumes that all applications have occurred concurrently on the same day at the exact same application rate. This is unlikely to occur in reality, but is a reasonable conservative assumption in lieu of actual data.

6.1.2 Impact of Vegetative Setbacks on Runoff

Unlike spray drift, tools are currently not available to evaluate the effectiveness of a vegetative setback on runoff and loadings. The effectiveness of vegetative setbacks is highly dependent on the condition of the vegetative strip. For example, a well-established, healthy vegetative setback can be a very effective means of reducing runoff and erosion from agricultural fields. Alternatively, a setback of poor vegetative quality or a setback that is channelized can be ineffective at reducing loadings. Until such time as a quantitative method to estimate the effect of vegetative setbacks on various conditions on pesticide loadings becomes available, the aquatic exposure predictions are likely to overestimate exposure where healthy vegetative setbacks exist and underestimate exposure where poorly developed, channelized, or bare setbacks exist.

6.1.3 PRZM Modeling Inputs and Predicted Aquatic Concentrations

In general, the linked PRZM/EXAMS model produces estimated aquatic concentrations that are expected to be exceeded once within a ten-year period. The Pesticide Root Zone Model (PRZM) is a process or "simulation" model that calculates what happens to a pesticide in a farmer's field on a day-to-day basis. It considers factors such as rainfall and plant transpiration of water, as well as how and when the pesticide is applied. It has two major components: hydrology and chemical transport. Water movement is simulated by the use of generalized soil parameters, including field capacity, wilting point, and saturation water content. The chemical transport component can simulate pesticide application on the soil or on the plant foliage. Dissolved, adsorbed, and vapor-phase concentrations in the soil are estimated by simultaneously considering the processes of pesticide uptake by plants, surface runoff, erosion, decay, volatilization, foliar wash-off, advection, dispersion, and retardation.

Uncertainties associated with each of these individual components add to the overall uncertainty of the modeled concentrations. Additionally, model inputs from the environmental fate degradation studies are chosen to represent the upper confidence bound on the mean, values that are not expected to be exceeded in the environment 90 percent of the time. Mobility input values are chosen to be representative of conditions in the environment. The natural variation in soils adds to the uncertainty of modeled values. Factors such as application date, crop emergence date, and canopy cover can also affect estimated concentrations, adding to the uncertainty of modeled values. Factors within the ambient environment such as soil temperatures, sunlight intensity, antecedent soil moisture, and surface water temperatures can cause actual aquatic concentrations to differ for the modeled values.

Additionally, the rate at which atrazine is applied and the percent of crops that are actually treated with atrazine may be lower than the Agency’s default assumption of the maximum allowable application rate being used and the entire crop being treated. The geometry of a watershed and limited meteorological data sets also add to the uncertainty of estimated aquatic concentrations.

6.2 Effects Assessment Uncertainties

6.2.1 Age Class and Sensitivity of Effects Thresholds

It is generally recognized that test organism age may have a significant impact on the observed sensitivity to a toxicant. The acute toxicity data for fish are collected on juvenile fish between 0.1 and 5 grams. Aquatic invertebrate acute testing is performed on recommended immature age classes (e.g., first instar for daphnids, second instar for amphipods, stoneflies, mayflies, and third instar for midges).

Testing of juveniles may overestimate toxicity at older age classes for pesticidal active ingredients, such as atrazine, that act directly (without metabolic transformation) because younger age classes may not have the enzymatic systems associated with detoxifying xenobiotics. In so far as the available toxicity data may provide ranges of sensitivity information with respect to age class, this assessment uses the most sensitive life-stage information as measures of effect for surrogate aquatic animals, and is therefore, considered as protective of freshwater mussels and their host fish.

6.2.2 Use of Acute Freshwater Invertebrate Toxicity Data for the Midge

The initial acute risk estimate for freshwater invertebrates was based on the lowest toxicity value from *Chironomus* studies, which showed a wide range of sensitivity within and between species of the same genus (2 orders of magnitude). Therefore, screening-level acute RQs based on the most sensitive toxicity endpoint for freshwater invertebrates may represent an overestimation of potential indirect effects to the host fish of the ovate and southern clubshell mussels via a reduction in available food.

6.2.3 Impact of Multiple Stressors on the Effects Determination

The influence of length of exposure and concurrent environmental stressors to the listed mussels (i.e., construction of dams and locks, fragmentation of habitat, change in flow regimes, increased sedimentation, degradation of quantity and quality of water in the watersheds of the action area, predators, etc.) will likely affect the species' response to atrazine. Additional environmental stressors may increase the listed mussel's sensitivity to the herbicide, although there is the possibility of additive/synergistic reactions. Timing, peak concentration, and duration of exposure are critical in terms of evaluating effects, and these factors are expected to vary both temporally and spatially within the action area. Overall, the effect of this variability may result in either an overestimation or underestimation of risk. However, as previously discussed, the Agency's LOCs are set to be protective given the wide range of possible uncertainties.

6.2.4 Use of Threshold Concentrations for Community-Level Endpoints

For the purposes of this ESA, threshold concentrations are used to predict potential indirect effects to the listed mussels and adverse modification to designated critical habitat (via aquatic plant community structural change). The conceptual aquatic ecosystem model used to develop the threshold concentrations is intended to simulate the ecological production dynamics in a 2nd or 3rd order Midwestern stream; however, the model has been correlated to the micro- and mesocosm studies, which were derived from a wide range of experimental studies (i.e., jar studies to large enclosures in lentic and lotic systems), that represent the best available information for atrazine-related community-level endpoints.

The threshold concentrations are intended to be predictive of potential atrazine-related community-level effects in aquatic ecosystems, such as those that occur in known locations for the listed mussels and their designated critical habitat, where the species composition may differ from those included in the micro- and mesocosm studies. Although it is not possible to determine how well the responses observed in the micro- and mesocosm studies reflect the action area watersheds for the listed mussels, estimated chronic atrazine exposure concentrations in less vulnerable watersheds of the action area (from modeled EECs assuming flow) are predicted to be between 5 to 12 times lower than the community-level threshold concentrations, depending on the modeled atrazine use and averaging period. However, an evaluation of targeted monitoring data from vulnerable watersheds suggests that chronic exposure concentrations of atrazine exceed these threshold concentrations in a number of areas. Given that threshold concentrations were derived based on the best available information from available community-level data for atrazine, these values are intended to be protective of the aquatic community, including the listed mussels and their designated critical habitat. Additional uncertainties associated with use of the thresholds to estimate community-level effects are discussed in Section B.8 of Appendix B.

6.2.5. Sublethal Effects

The assessment endpoints used in ecological risk assessment include potential effects on survival, growth, and reproduction of the assessed mussels and organisms on which mussels depend for survival such as fish. A number of studies were located that evaluated potential sublethal effects to fish from exposure to atrazine. Although many of these studies reported toxicity values that were less sensitive than the submitted studies, they were not considered for use in risk estimation. In particular, fish studies were located in the open literature that reported effects on endpoints other than survival, growth, or reproduction at concentrations that were considerably lower than the most sensitive endpoint from submitted studies.

Upon evaluation of the available studies, however, the most sensitive NOAEC from the submitted full life-cycle studies was considered to be the most appropriate chronic endpoint for use in risk assessment. In the full life cycle study, fish are exposed to atrazine from one stage of the life cycle to at least the same stage of the next generation (e.g. egg to egg). Therefore, exposure occurs during the most sensitive life stages and during the entire reproduction cycle. Four life cycle studies have been submitted in support of atrazine registration. Species tested include brook trout, bluegill sunfish, and fathead minnows. The most sensitive NOAEC from these studies was 65 µg/L.

Reported sublethal effects including changes in hormone levels, behavioral effects, kidney pathology, gill physiology, and potential olfaction effects have been observed at concentrations lower than 65 µg/L (see Appendix A and Section 4.1.2.). These studies were not considered appropriate for risk estimation in place of the life cycle studies because quantitative relationships between these effects and the ability of fish to survive, grow, and reproduce has not been established. The magnitude of the reported sublethal effect associated with reduced survival or reproduction has not been established; therefore it is not possible to quantitatively link sublethal effects to the selected assessment endpoints for this ESA. In addition, in the fish life cycle studies, no effects were observed to survival, reproduction, and/or growth at levels associated with the sublethal effects. Also, there were limitations to the studies that reported sublethal effects that preclude their quantitative use in risk assessment (see Appendix A and Section 4.2.1). Nonetheless, if future studies establish a quantitative link between the reported sublethal effects and fish survival, growth, or reproduction, the conclusions with respect to potential effects to host fish may need to be revisited.

6.2.6. Exposure to Pesticide Mixtures

This assessment considered only the single active ingredient of atrazine. However, the assessed species and their environments may be exposed to multiple pesticides simultaneously. Interactions of other toxic agents with atrazine could result in additive effects ($1/LC50_{mix} = 1/LC50_{Pesticide\_A} + 1/LC50_{Pesticide\_B}\ldots$), synergistic effects ($1/LC50_{mix} = 1/LC50_{Pesticide\_A} + 1/LC50_{Pesticide\_B}\ldots x\ Y$; where $Y > 1$) or antagonistic effects ($1/LC50_{mix} = 1/LC50_{Pesticide\_A} + 1/LC50_{Pesticide\_B}\ldots\ x\ Y$; where $Y < 1$). Conceptually, the combined effect of the mixture is equal to the sum of the effects of each stressor (1 + 1 = 2) for additive toxicity. Synergistic effects occur when the combined effect of the mixture is greater than the sum of each stressor (1 + 1 >2), and antagonistic effects occur when the combined effect of the mixture is less than the sum of each stressor (1 + 1 <2).

The available data suggest that pesticide mixtures involving atrazine may produce either synergistic, additive, or antagonistic effects. Mixtures that have been studied include atrazine with insecticides such as organophosphates and carbamates or with herbicides including alachlor and metolachlor. Additive or synergistic effects have been reported in several taxa including fish, amphibians, invertebrates, and plants.

As previously discussed, evaluation of pesticide mixtures is beyond the scope of this assessment because of the myriad of factors that cannot be quantified based on the available data. Those factors include identification of other possible co-contaminants and their concentrations, differences in the pattern and duration of exposure among contaminants, and the differential effects of other physical/chemical characteristics of the receiving waters (e.g. organic matter present in sediment and suspended water). Evaluation of factors that could influence additivity/synergism is beyond the scope of this assessment and is beyond the capabilities of the available data to allow for an evaluation. However, it is acknowledged that not considering mixtures could over- or under-estimate risks depending on the type of interaction and factors discussed above.

6.3 Assumptions Associated with the Acute LOCs

The risk characterization section of this endangered species assessment includes an evaluation of the potential for individual effects. The individual effects probability associated with the acute RQ is based on the mean estimate of the slope and an assumption of a probit dose response relationship for the effects study corresponding to the taxonomic group for which the LOCs are exceeded.

Sufficient dose-response information was not available to estimate the probability of an individual effect on the midge (one of the dietary food items of the host fish). Acute ecotoxicity data from the midge were used to derive RQs for freshwater invertebrates. Based on a lack of dose-response information for the midge, the probability of an individual effect was calculated using the only probit dose response curve slope value reported in available freshwater invertebrate ecotoxicity data for technical grade atrazine. Therefore, a probit slope value of 4.4 for the amphipod was used to estimate the probability of an individual effect on the freshwater invertebrates. It is unclear whether the probability of an individual effect for freshwater invertebrates other than amphipods would be higher or lower, given a lack of dose-response information for other freshwater invertebrate species. However, the assumed probit dose response slope for freshwater invertebrates of 4.4 would have to decrease to approximately 1 to 2 to cause an effect probability ranging between 1 in 10 and 1 in 100, respectively, for freshwater invertebrates.

6.4. Uncertainty in the Potential Effect to Riparian Vegetation vs. Increased Sedimentation

Effects to riparian vegetation were evaluated using submitted guideline seedling emergence and vegetative vigor studies and non-guideline woody plant effects data. LOCs were exceeded for seedling emergence and vegetative vigor endpoints with the seedling emergence endpoint being considerably more sensitive. Based on LOC exceedances and the lack of readily available information to allow for characterization of riparian areas of the listed mussels, it was concluded that atrazine use is likely to adversely affect the assessed listed mussels via potential impacts on grassy/herbaceous riparian vegetation resulting in increased sedimentation. However, soil retention/sediment loading is dependent on a number of factors including land management and tillage practices. Use of herbicides (including atrazine) may be incorporated into a soil conservation plan. Therefore, although this assessment concludes that atrazine is likely to adversely affect the assessed listed species and its designated critical habitat by potentially impacting sensitive herbaceous riparian areas, it is possible that adverse impacts on sediment loading may not occur in areas where soil retention strategies are used.

7. Summary of Direct and Indirect Effects to the Listed Mussels and Adverse Modification to Designated Critical Habitat for the Ovate and Southern Clubshell Mussels

In fulfilling its obligations under Section 7(a)(2) of the Endangered Species Act, the information presented in this ESA represents the best data currently available to assess the potential risks of atrazine to the eight listed mussels and their designated critical habitat. A summary of the risk conclusions and effects determination for the eight listed mussels and designated critical habitat for the ovate and southern clubshell mussels, given the uncertainties discussed in Section 6, by assessment endpoint, is presented in Tables 7.1 and 7.2. Table 7.3 provides a summary of the direct and indirect effects determinations for each of the eight assessed listed mussels.

The direct and indirect effects determination for the stirrupshell mussel is "no effect" because this species is presumed to be extinct (Hartfield, 2006). With the exception of "LAA" determinations for indirect effects to listed mussels via community-level effects to aquatic plants in vulnerable watersheds, and habitat impacts via atrazine-related alteration grassy/herbaceous riparian vegetation in all portions of the action area, all other effects determinations for the direct and indirect assessment endpoints are "no effect" or "NLAA". An "LAA" determination was concluded for indirect prey and habitat effects to the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels that occur in vulnerable watersheds of the action area, based on potential direct aquatic plant community-level effects. The "LAA" determination is based on the results of recently submitted atrazine monitoring data from vulnerable watersheds; however, the degree to which this targeted monitoring data represents exposures in occupied streams (for the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe) that co-occur with vulnerable watersheds has not been determined. For the purposes of this assessment, it is conservatively assumed that detected concentrations of atrazine from the monitoring data may be representative of exposures in vulnerable watersheds of the action area. However, if further analysis reveals that the monitoring data are not representative of atrazine concentrations in vulnerable watersheds where the pink pearly mucket, rough pigtoe, and fine-rayed pigtoe mussels occur, the "LAA" effects determination will be revisited and could be changed to "NLAA" for this particular assessment endpoint. An "LAA" determination was also concluded for the seven listed mussels based on indirect effects to habitat and water quality via direct effects to herbaceous/grassy riparian vegetation. However, atrazine is not likely to adversely affect listed mussels in watersheds with predominantly forested riparian areas because woody shrubs and trees are generally not sensitive to environmentally-relevant concentrations of atrazine.

In the critical habitat impact analysis, "LAA" effects determinations were concluded for the following PCEs associated with potential adverse modification to critical habitat via atrazine-related impacts to grassy/herbaceous riparian vegetation: alteration of host fish spawning habitat, increase in sedimentation and resulting impact on silt-free substrates and turbidity-related water quality parameters, and alteration of streambank stability. All other PCEs evaluated as part of the critical habitat impact analysis were determined to be either "no effect" or "NLAA".

Table 7.1. Effects Determination Summary for the Assessed Listed Mussels (by Assessment Endpoint)				
Direct and Indirect Effects to Listed Mussels[a]				
Assessment Endpoint	**Effects Determination and Basis for Less Vulnerable Watersheds (applicable to shiny pigtoe [SP], heavy pigtoe [HP], ovate clubshell [OC], and southern clubshell [SC] listed mussels)**		**Effects Determination and Basis for Highly Vulnerable Watersheds (applicable to pink pearly mucket [PPM], rough pigtoe [RP], and fine-rayed pigtoe [FRP] listed mussels)**	
	Effects Determination[b]	**Basis**	**Effects Determination[b]**	**Basis**
1. Survival, growth, and reproduction of assessed mussel individuals via direct acute or chronic effects	Acute direct effects: NE	No acute LOCs are exceeded.	Acute direct effects: NE	No acute LOCs are exceeded.
	Chronic direct effects: NLAA	Chronic LOCs are exceeded based on screening-level EECs; however, RQs based on flow-adjusted EECs and non-targeted monitoring data are less than concentrations shown to cause adverse effects in freshwater mollusks. This finding is based on insignificance of effects (i.e., chronic exposure to atrazine is not likely to result in "take" of a single SP, HP, OC, and SC mussel in less vulnerable watersheds).	Chronic direct effects: NLAA	Chronic LOCs are exceeded based on screening-level EECs; however detected concentrations of atrazine in monitoring data from vulnerable watersheds are less than those shown to cause adverse effects in freshwater mollusks. This finding is based on insignificance of effects (i.e., chronic exposure to atrazine is not likely to result in "take" of a single PPM, RP, and FRP mussel in vulnerable watersheds).
2. Indirect effects to assessed mussel individuals via reduction in food items (i.e., freshwater phytoplankton and zooplankton)	Phytoplankton: NLAA	Individual aquatic plant species within less vulnerable watersheds of the action area may be affected. However, refined 14-, 30-, 60- and 90-day EECs, which consider the impact of flow and non-targeted monitoring data, are less than the threshold concentrations representing community-level effects. This finding is based on insignificance of effects (i.e., community-level effects to aquatic plants are not likely to result in "take" of a single SP, HP, OC, and SC mussel in less vulnerable watersheds via a reduction in food items).	Phytoplankton: LAA[c]	Individual aquatic plant species within vulnerable watersheds of the action area may be affected. 14-, 30-, 60-, and 90- day rolling averages based on the ecological monitoring data exceed their respective threshold concentrations for 5 to 12.5% of the sampled vulnerable watersheds. Therefore, community-level effects are possible for phytoplankton, resulting in indirect effects to the food supply of the PPM, RP, and FRP mussels, within vulnerable watersheds of the action area.
	Acute direct effects	Acute LOCs are exceeded based on	Acute direct effects	Acute LOCs are exceeded based on the

Table 7.1. Effects Determination Summary for the Assessed Listed Mussels (by Assessment Endpoint)				
Direct and Indirect Effects to Listed Mussels[a]				
Assessment Endpoint	**Effects Determination and Basis for Less Vulnerable Watersheds (applicable to shiny pigtoe [SP], heavy pigtoe [HP], ovate clubshell [OC], and southern clubshell [SC] listed mussels)**		**Effects Determination and Basis for Highly Vulnerable Watersheds (applicable to pink pearly mucket [PPM], rough pigtoe [RP], and fine-rayed pigtoe [FRP] listed mussels)**	
	Effects Determination[b]	**Basis**	**Effects Determination[b]**	**Basis**
	to zooplankton: NLAA	screening-level EECs and the most sensitive freshwater invertebrate toxicity data. Based on the refined analysis, which considered flow-adjusted EECs, non-targeted monitoring data, and effects data specific to zooplankton, acute effects to zooplankton are not likely to result in indirect effects to the SP, HP, OC, and SC mussels via reduction in food items because zooplankton are not the primary food source for these listed mussels, the probability of an individual effect to zooplankton is low (i.e., 0.2%), and the refined RQ based on peak NAWQA 2000-2004 monitoring data specific for a watershed within the action area is well below the acute LOC. This finding is based on insignificance of effects (i.e., effects to zooplankton in less vulnerable watersheds are not likely to be extensive over the suite of possible food items to result in "take" of a single listed SP, HP, OC, and SC mussel).	to zooplankton: NLAA	maximum peak atrazine concentration from the monitoring data. However, zooplankton are not the primary food source for listed mussels and there is a low probability of an individual effect to zooplankton. Therefore, direct acute effects to zooplankton are not likely to result in indirect effects to the listed mussels via a reduction in food items. This finding is based on insignificance of effects (i.e., effects to zooplankton in vulnerable watersheds are not likely to be extensive over the suite of possible food items to result in "take" of a single PPM, RP, and FRP mussel).
	Chronic direct effects to zooplankton: NLAA	Chronic LOCs are exceeded based on screening-level EECs and the most sensitive freshwater invertebrate toxicity data. However, all refined measures of exposure (21-day flow-adjusted EECs	Chronic direct effects to zooplankton: NLAA	Chronic LOCs are exceeded based on screening-level EECs and the most sensitive freshwater invertebrate toxicity data. However, 21-day rolling averages based on the ecological monitoring data are well below

Table 7.1. Effects Determination Summary for the Assessed Listed Mussels (by Assessment Endpoint)				
Direct and Indirect Effects to Listed Mussels[a]				
Assessment Endpoint	**Effects Determination and Basis for Less Vulnerable Watersheds (applicable to shiny pigtoe [SP], heavy pigtoe [HP], ovate clubshell [OC], and southern clubshell [SC] listed mussels)**		**Effects Determination and Basis for Highly Vulnerable Watersheds (applicable to pink pearly mucket [PPM], rough pigtoe [RP], and fine-rayed pigtoe [FRP] listed mussels)**	
	Effects Determination[b]	**Basis**	**Effects Determination[b]**	**Basis**
		and non-targeted monitoring data) are well below levels of chronic effects in cladocerons. This finding is based on insignificance of effects (i.e., chronic exposure to atrazine in less vulnerable watersheds is not likely to result in a "take" of a single SP, HP, OC, and SC mussel via a reduction in zooplankton as food items).		levels of chronic effects in cladocerons. This finding is based on insignificance of effects (i.e., chronic exposure to atrazine in highly vulnerable watersheds is not likely to result in a "take" of a single PPM, RP, and RFP mussel via a reduction in zooplankton as food items).
3. Indirect effects to assessed mussel individuals via reduction in host fish for mussel glochidia	Acute direct effects to host fish: NE	No acute LOCs are exceeded.	Acute direct effects to host fish: NE	No acute LOCs are exceeded.
	Chronic direct effects to host fish: NLAA	Chronic LOCs are exceeded based on screening-level EECs; however RQs based on flow-adjusted EECs and non-targeted monitoring data are less than chronic LOCs. This finding is based on insignificance of effects (i.e., chronic exposure to atrazine is not likely to result in "take" of a single SP, HP, OC, and SC mussel via direct effects to host fish in less vulnerable watersheds).	Chronic direct effects to host fish: NLAA	Chronic LOCs are exceeded based on screening-level EECs; however, detected concentrations of atrazine in monitoring data from vulnerable watersheds are less than those that would result in LOC exceedances for freshwater fish. This finding is based on insignificance of effects (i.e., chronic exposure to atrazine is not likely to result in "take" of a single PPM, RP, and FRP mussel via direct effects to host fish in vulnerable watersheds).
4. Indirect effects to assessed mussel individuals via direct effects to aquatic plants (i.e., reduction of habitat	Direct effects to aquatic plants: NLAA	Individual aquatic plant species within less vulnerable watersheds may be affected. However, flow-adjusted 14-, 30-, 60-, and 90-day EECs and similar durations of exposure based on non-targeted monitoring data, are less than	Direct effects to aquatic plants: LAA[c]	Individual aquatic plant species within vulnerable watersheds of the action area may be affected. 14-, 30-, 60-, and 90- day rolling averages based on the ecological monitoring data from vulnerable watersheds exceed their respective threshold concentrations for a small

Table 7.1. Effects Determination Summary for the Assessed Listed Mussels (by Assessment Endpoint)				
Direct and Indirect Effects to Listed Mussels[a]				
Assessment Endpoint	**Effects Determination and Basis for Less Vulnerable Watersheds (applicable to shiny pigtoe [SP], heavy pigtoe [HP], ovate clubshell [OC], and southern clubshell [SC] listed mussels)**		**Effects Determination and Basis for Highly Vulnerable Watersheds (applicable to pink pearly mucket [PPM], rough pigtoe [RP], and fine-rayed pigtoe [FRP] listed mussels)**	
	Effects Determination[b]	**Basis**	**Effects Determination[b]**	**Basis**
and/or primary productivity)		the threshold concentrations representing community-level effects. This finding is based on insignificance of effects (i.e., community-level effects to aquatic plants are not likely to result in "take" of a single SP, HP, OC, and SC mussel via direct effects on habitat and primary productivity in less vulnerable watersheds).		percentage of the data set. Therefore, community-level effects are possible for phytoplankton, resulting in indirect effects to the PPM, RP, and FRP mussels, via direct effects on habitat and primary productivity, within vulnerable watersheds of the action area.
5. Indirect effects to assessed mussel individuals via reduction of terrestrial vegetation (i.e., riparian habitat) required to maintain acceptable water quality and habitat[d]	Direct effects to forested riparian vegetation: NLAA	Riparian vegetation may be affected because terrestrial plant RQs are above LOCs. However, woody shrubs and trees are generally not sensitive to atrazine; therefore, listed mussels in watersheds with predominantly forested riparian vegetation (i.e., woody shrubs and trees) are not likely to adversely affected. This finding is based on insignificance of effects (i.e., effects to forested riparian vegetation in the action area are not likely to result in "take" of a single listed mussel).	Direct effects grassy/herbaceous riparian vegetation: LAA	Riparian vegetation may be affected because terrestrial plant RQs are above LOCs. The LAA effects determination for listed mussels that are in close proximity to grassy/herbaceous riparian areas is based on the sensitivity of herbaceous vegetation to atrazine.

[a] The direct and indirect effects determination for the stirrupshell mussel is "no effect" because this species is presumed to be extinct (Hartfield, 2006). The following direct/indirect effects determinations apply to the other seven listed mussels included in this assessment.
[b] NE = "no effect"; NLAA ' "may affect, but not likely to adversely affect"; and LAA = "may affect and likely to adversely affect".
[c] Further analysis of the ecological monitoring data is required to determine the representativeness of the data to other watersheds within vulnerable areas where the listed mussel species occur. If the analysis suggests that the monitoring data are representative of atrazine concentrations in vulnerable watersheds where the listed mussels occur, the effects determination will remain as "LAA." However, if further analysis reveals that the monitoring data are not representative of atrazine concentrations in vulnerable watersheds where the listed mussels occur, the effects determination will be revised to "NLAA".

Table 7.1. Effects Determination Summary for the Assessed Listed Mussels (by Assessment Endpoint)				
Direct and Indirect Effects to Listed Mussels[a]				
Assessment Endpoint	Effects Determination and Basis for Less Vulnerable Watersheds (applicable to shiny pigtoe [SP], heavy pigtoe [HP], ovate clubshell [OC], and southern clubshell [SC] listed mussels)		Effects Determination and Basis for Highly Vulnerable Watersheds (applicable to pink pearly mucket [PPM], rough pigtoe [RP], and fine-rayed pigtoe [FRP] listed mussels)	
	Effects Determination[b]	Basis	Effects Determination[b]	Basis

[d] The effects determinations for indirect effects to the listed mussels based on direct impacts to riparian habitat is applicable to the entire action area including riparian areas adjacent to both vulnerable and less vulnerable watersheds. Separate effects determinations are based on the presence of forested or herbaceous/grassy riparian vegetation adjacent to the streams and rivers within the listed mussel's action area.

Table 7.2. Effects Determination Summary for the Critical Habitat Impact Analysis[a]

Assessment Endpoint	Effects Determination[b]	Basis
1. Fish hosts with adequate living, foraging, and spawning areas	Acute direct effects to host fish: NE	No acute LOCs are exceeded.
	Chronic direct effects to host fish: NLAA	Chronic LOCs are exceeded based on screening-level EECs; however, RQs based on flow-adjusted EECs and non-targeted monitoring data are less than chronic LOCs. This finding is based on insignificance of effects (i.e., chronic effects to the living areas for host fish are not likely to adversely modify designated critical habitat for the ovate and southern clubshell mussels).
	Acute direct effects to host fish food items: NLAA	Acute LOCs for freshwater invertebrates are exceeded based on screening-level EECs and the most sensitive ecotoxicity value for the midge. However, refined RQs based on flow-adjusted EECs, recent non-targeted monitoring data, and toxicity data for other freshwater invertebrate food items of host fish are less than LOCs. Based on the non-selective feeding nature of host fish and the low magnitude of anticipated individual effects to prey items, atrazine is not likely to affect host fish of the ovate and southern clubshell mussels via an acute reduction in freshwater invertebrate food items. This finding is based on an insignificance of effects (i.e., acute effects to freshwater invertebrates are not likely to adversely modify critical habitat foraging areas for host fish of the ovate and southern clubshell mussels).
	Chronic direct effects to host fish food items: NLAA	Chronic LOCs for freshwater invertebrates are exceeded based on screening-level EECs. However, chronic RQs based on flow-adjusted EECs and non-targeted monitoring data are less their respective chronic LOCs. This finding is based on an insignificance of effects (i.e., chronic effects to freshwater invertebrates are not likely to adversely modify critical habitat foraging areas for host fish of the ovate and southern clubshell mussels).
	Direct effects to host fish	LOCs are exceeded for aquatic and terrestrial plants based on screening-level EECs. Further analysis

Table 7.2. Effects Determination Summary for the Critical Habitat Impact Analysis[a]		
Assessment Endpoint	**Effects Determination[b]**	**Basis**
	spawning areas: LAA for herbaceous/grassy riparian areas and NLAA for forested riparian areas	of potential impacts to host fish spawning habitat via community-level effects to aquatic plants was completed by comparing flow-adjusted 14-, 30-, 60-, and 90-day EECs and similar durations of exposure from non-targeted monitoring data to their respective threshold concentrations. All flow-adjusted EECs and non-targeted monitoring data are well below threshold concentrations; therefore, host fish spawning habitat is not likely to be adversely affected via community-level effects to aquatic plants. In addition, critical habitat spawning areas for host fish that are in close proximity to forested riparian vegetation are not expected to adversely modified because woody shrubs and treess are generally not sensitive to atrazine. However, critical habitat spawning areas for host fish that are in close proximity to grassy/herbaceous riparian areas may be adversely modified based on the sensitivity of herbaceous vegetation to atrazine.
2. Water quality necessary for normal behavior, growth and viability of all mussel life stages	Temperature: NLAA	Riparian vegetation may be affected because terrestrial plant RQs are above LOCs. However, water quality related to temperature within forested riparian areas is not likely to be impacted because mature woody shrubs and trees, which provide stream shading and thermal stability, are generally not sensitive to atrazine. This finding is based on insignificance of effects (i.e., atrazine is not likely to adversely modify temperature-related water quality within designated critical habitat for the ovate and southern clubshell mussels).
	Turbidity: LAA for herbaceous/grassy riparian areas and NLAA for forested riparian areas	Riparian vegetation may be affected because terrestrial plant RQs are above LOCs. Water quality related to turbidity via increased sedimentation may be impacted within designated critical habitats that are adjacent to grassy/herbaceous riparian vegetation. Therefore, atrazine may adversely modify critical habitat for the ovate and southern clubshell mussels in areas where grassy/herbaceous riparian vegetation is present. Adverse modification to designated critical habitat via turbidity-related water quality impact is not expected in areas where forested riparian vegetation is present.
	Oxygen content: NLAA	Individual aquatic plant species may be affected based on LOC exceedances. Oxygen levels may also be impacted if the atrazine negatively affects the aquatic plant community and primary productivity. However, flow-adjusted 14-, 30-, 60-, and 90-day EECs and similar durations of exposure from non-targeted monitoring data are less than their respective threshold concentrations representative of aquatic plant community-level effects. Therefore, atrazine may affect, but is not likely to adversely modify the oxygen content of the designated critical habitat for the ovate and southern clubshell mussels. This finding is based on insignificance of effects (i.e., atrazine is not likely to adversely modify oxygen content-related water quality via aquatic plant community-level impacts within designated critical habitat for the ovate and southern clubshell mussels).
3. Substrates with low to moderate amounts of	Filamentous algae: NLAA	Atrazine is expected to reduce algal mass and the presence of filamentous algae on substrate necessary for normal growth and viability of listed mussels. Therefore, atrazine is not expected to adversely

Table 7.2. Effects Determination Summary for the Critical Habitat Impact Analysis[a]		
Assessment Endpoint	**Effects Determination[b]**	**Basis**
filamentous algae and low sedimentation		modify critical habitat of the ovate and southern clubshell mussels by increasing the amount of filamentous algae on substrate. This determination is based on insignificance of effects (i.e., adverse modification to critical habitat is not expected because atrazine use is likely to reduce the amount of filamentous algae).
	Sedimentation: LAA for herbaceous/grassy riparian areas and NLAA for forested riparian areas	Riparian vegetation may be affected because terrestrial plant RQs are above LOCs. Sedimentation may be impact silt-free substrates necessary for normal growth and viability of listed mussels within designated critical habitats that are adjacent to grassy/herbaceous riparian vegetation. Therefore, atrazine may adversely modify critical habitat for the ovate and southern clubshell mussels via sedimentation in areas where grassy/herbaceous riparian vegetation is present. Adverse modification to designated critical habitat via sedimentation is not expected in areas where forested riparian vegetation is present.
4. Stream and river bank stability	LAA for herbaceous/grassy riparian areas and NLAA for forested riparian areas	Riparian vegetation may be affected because terrestrial plant RQs are above LOCs. Streambank stability may be impacted within designated critical habitats that are adjacent to grassy/herbaceous riparian vegetation. Therefore, atrazine may adversely modify critical habitat for the ovate and southern clubshell mussels via reduction in streambank stability in areas where grassy/herbaceous riparian vegetation is present. Adverse modification to designated critical habitat via streambank stability is not expected in areas where forested riparian vegetation is present.
5. Chemical characteristics necessary for normal behavior, growth, and viability of all life stages of mussels	Acute direct effects: NE	No acute LOCs are exceeded.
	Chronic direct effects: NLAA	Chronic LOCs are exceeded based on screening-level EECs; however RQs based on flow-adjusted EECs and non-targeted monitoring data are less than concentrations shown cause adverse effects in freshwater mollusks. This finding is based on insignificant of effects (i.e., chronic exposure to atrazine is not likely to result in adverse modification of critical habitat for the ovate and southern clubshell mussels).
	Indirect food source of phytoplankton: NLAA	Individual aquatic plant species of the action area may be affected. However, refined 14-, 30-, 60- and 90-day EECs, which consider the impact of flow and the non-targeted monitoring data, are well below the threshold concentrations representing community-level effects. This finding is based on insignificance of effects (i.e., community-level effects to phytoplankton as a food source are not likely to adversely modify designated critical habitat for the ovate and southern clubshell mussels).
	Acute and chronic indirect food source of zooplankton: NLAA	Acute and chronic LOCs are exceeded based on screening-level EECs and the most sensitive freshwater invertebrate toxicity data. Based on the refined acute analysis for zooplankton (which consider flow-adjusted EECs, non-targeted monitoring data, and effects data specific for zooplankton).adverse modification to critical habitat is not likely because zooplankton are not the primary food source, the probability of an individual effect to zooplankton is low (i.e., 0.2%), and the

Table 7.2. Effects Determination Summary for the Critical Habitat Impact Analysis[a]		
Assessment Endpoint	**Effects Determination[b]**	**Basis**
		refined RQ based on peak NAWQA 2000-2004 monitoring data specific for a designated critical habitat watershed (i.e., Bogue Chitto Creek) is well below the acute LOC. Chronic effects to zooplankton and resulting adverse modification to critical habitat via reduction in food items is also not expected to occur because all refined measures of chronic exposure (i.e., 21-day flow-adjusted EECs and similar durations of exposure from non-targeted monitoring data) are well below chronic effect levels in zooplankton. Both NLAA effects determinations for adverse modification to critical habitat via reduction in zooplankton as a food source to the ovate and southern clubshell mussels are based on insignificance of effects (i.e., acute and chronic effects to zooplankton as food items are not likely to result in adverse modification of critical habitat for the ovate and southern clubshell mussels).

[a] All designated critical habitat for the ovate and southern clubshell mussel occurs in watersheds that are outside of the vulnerable watershed boundary; therefore, the effects determination for the critical habitat impact analysis is conducted for less vulnerable watersheds only.
[b] NE = "no effect"; NLAA ' "may affect, but not likely to adversely affect"; and LAA = "may affect and likely to adversely affect".

Table 7.3. Effects Determination Summary for Each of the Eight Assessed Listed Mussels[a]

Assessed Mussel Species	Direct Effects		Indirect Effects						
	Acute	Chronic	Food Items		Host Fish		Aquatic Habitat: community-level effects	Riparian Vegetation	
			Phytoplankton	Zooplankton	Acute	Chronic		Herbaceous/Grassy Vegetation	Forested Vegetation
Pink pearly mucket	NE	NLAA	**LAA[b]**	NLAA	NE	NLAA	**LAA[b]**	**LAA**	NLAA
Rough pigtoe	NE	NLAA	**LAA[b]**	NLAA	NE	NLAA	**LAA[b]**	**LAA**	NLAA
Shiny pigtoe	NE	NLAA	NLAA	NLAA	NE	NLAA	NLAA	**LAA**	NLAA
Fine-rayed pigtoe	NE	NLAA	**LAA[b]**	NLAA	NE	NLAA	**LAA[b]**	**LAA**	NLAA
Heavy pigtoe	NE	NLAA	NLAA	NLAA	NE	NLAA	NLAA	**LAA**	NLAA
Ovate clubshell	NE	NLAA	NLAA	NLAA	NE	NLAA	NLAA	**LAA**	NLAA
Southern clubshell	NE	NLAA	NLAA	NLAA	NE	NLAA	NLAA	**LAA**	NLAA
Stirrup shell	NE	NE	NE	NE	NE	NE	NE	NE	NE

[a] NE = "no effect"; NLAA ' "may affect, but not likely to adversely affect"; and LAA = "may affect and likely to adversely affect". See Table 7.1 for the basis of the effects determinations for each of the assessed mussel species.
[b] Further analysis of the ecological monitoring data is required to determine the representativeness of the data to other watersheds within vulnerable areas where the listed mussel species occur. If the analysis suggests that the monitoring data are representative of atrazine concentrations in vulnerable watersheds where the listed mussels occur, the effects determination will remain as "LAA." However, if further analysis reveals that the monitoring data are not representative of atrazine concentrations in vulnerable watersheds where the listed mussels occur, the effects determination will be revised to "NLAA".

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