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Baseline Ecological Risk Assessment For the Arsenic Contaminated Wetland Associated With the C. D. Besadny Fish and Wildlife Area and the Kewaunee River

Bureau of Watershed Management
Water Quality Standards Section

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14.2 Ecological Risk Characterizations

Summary

A baseline ecological risk assessment (BERA) was conducted for the arsenic impacted wetland area of the C. D. Besadny Fish and Wildlife Area located on the Kewaunee River. The principles for conducting the BERA followed current guidelines published by U. S. EPA and other sources. A suite of interrelated study components produced information for use in a weight-of-evidence approach in the ecological risk assessment. The components included analysis for arsenic in site surface waters, soils, groundwater, and biota; conducting toxicity testing on site waters and soils using indigenous and surrogate species; reviewing applicable criteria, guidelines, and literature studies of toxicity to relate to site measurements and toxicity testing results: and conducting site surveys and observations.

The source of the arsenic to the wetland is most likely associated with a railroad car derailment that occurred in the early 1940's. The BERA was principally conducted along with other studies such as groundwater modeling to determine the present and future risks to wildlife, birds, and aquatic resources from exposures to arsenic in the site media following the implementation of an interim remedial action at the site. The interim action included covering an approximately four acre area of highly arsenic contaminated wetland soils and associated surface water with geotextile fabric and organic material and constructing a chain link fence around the perimeter of the 15 acre contaminated site. These actions resulted in the limiting of access and reducing exposure risks from the highly contaminated areas to a number of bird, wildlife, and aquatic organisms that may normally utilize the wetland as part of their daily and seasonal activities. The interim actions also have the affect of limiting human access and exposure to the site. The effects of the remaining contaminated media on the overall site and the potential movement of the contaminated groundwater under the cap to the river were looked at in the BERA.

There are a number of components of wetlands in general including basic life history information and trophic level relationships of site receptors where there is no or only limiting information and data. For example, there is not a lot known about the algal, invertebrate, or microbial flora communities of wetlands, and not a lot of data on arsenic toxicity to the organisms in these communities that may be indigenous to wetlands. This can increase the uncertainties in doing an ecological risk assessment in wetland habitats. Wetlands have natural components that can be toxic to organisms (e.g. un-ionized ammonia, hydrogen sulfide, and low dissolved oxygen) that are in turn affected by the hydrologic regimes of the wetland. The extreme conditions leads to biotic communities that are tolerant to the natural stressors. At times in the hydrologic cycle, water levels will drop below the ground surface eliminating surface waters and therefore the habitat for aquatic organisms which in turn eliminates the food sources for consumers. The end result is elimination or reduction of risk from arsenic exposures to the consumers from the natural water level fluctuations.

A summary of the risk characterizations to the health of receptor groups including humans from exposures to the site is shown in the table on the following page. The characterization of risks looks at exposures to the impacted wetland and the Kewaunee River separately. In regard to critical concentrations of arsenic in groundwater reaching the river for the protection of humans and aquatic life, the GeoTrans groundwater model needs to be reviewed for accuracy in predicting the time lines that increasing concentrations of arsenic will reach the river. Risks in the table are characterized by assigning a qualitative descriptor based on a review of all the integrated data and information for the receptor group. The relative risk descriptors used are "minimal"," low", "moderate" and "high". Based on the outcomes of the risk characterizations, risk management decisions will need to be made for the site.

Receptor Group	Risk Characterization To Health of Receptor Group Or Species In Group		Potential Impacts to Structure and/or Functioning of the Wetland or River Ecosystem	Degree of Certainty of Impacts / Comments
Human Health Based On NR 105 (Conceptually treat groundwater as a point source discharge to river)	River	Low -near term High - far term	Yes, human health risk	Arsenic contaminated groundwater will reach river in excess of "effluent" limits. GeoTrans predicts approx. 1,900 yrs. Model prediction needs review.
	Wetland	Low	None anticipated now or future	Security fence excludes access
Plant Community				
Emergent Marsh and Sedge	Wetland	Low	Monotypic communities of tolerant cattail and sedge dominate	Uncertain if some nontolerant low density species impacted. Low residual levels of arsenic outside of fence
Algal - Phyto- and Periphyton	Wetland	Low	No?	Lot of unknowns about algal communities in wetlands
Fish Community	River	Minimal	No	Some arsenic bioaccumulation. Source uncertain
	Wetland	Low	No	Chronic effects in some portions of wetland
Reptiles and Amphibians	Wetland	Moderate to High	Yes, if a number of amphibians & reptiles impacted	Unknowns and uncertainty. Based on one literature value for toxicity.
Surface Water and Benthic Macroinvertebrates				
Benthic Macroinvertebrates	River	Minimal	No	Slight rise of As in river sediment
	Wetland	Low to Moderate	Benthic community likely dominated by tolerant species	Uncertain if low density species impacted. More investigation needed on impacts of arsenic on wetland macroinvertebrate community
Surface Water Macroinvertebrates	River	Minimal	No	Arsenic levels in river near site low
	Wetland	Low	No	Chronic effects in some areas
Mammals and Birds				
Large Mammals	Wetland	Minimal	No	Excluded by security fence
Small Mammals	Wetland	Low	No?	Conflicting outcomes of food chain model compared to possible NR 105 Criteria. Between the two. **Risks put at oderate**
Birds	Wetland	Low	No	
Possible NR 105 Criteria Wildlife & Domestic Animals	Wetland	High	Yes, if enough species impacted	
Microbial Community - Decomposers and Detrivores	Wetland	Low - Moderate	No ?	Unknowns and limited data about Arsenic effects
Aquatic Life Based On NR 105 (Conceptually treat groundwater as point source discharge to river)	River	High - near term	Yes	Impacts possible within decade. GeoTrans groundwater model needs resolution.
	Wetland	Low	No	Chronic effects in some areas

1.0 Introduction

Arsenic contamination of the soils and surface waters of a portion of wetland associated with the C.D. Besadny Fish and Wildlife Area was initially identified by the Wisconsin Department of Natural Resources (WDNR) in 1993. Subsequent investigations by WDNR and the Fox Valley & Western Railroad have made initial determinations of the degree and extent of the contamination both on the wetland and in the adjacent Kewaunee River. Based on the data collected by both parties, WDNR undertook a baseline ecological risk assessment (BERA) to characterize the potential ecological risks associated with the soils, surface waters, and groundwaters of the site following the implementation of interim remedial actions for the site in early 1996. This report consists of the BERA conducted by the Department for the site. An ecological risk assessment is a qualitative and / or quantitative appraisal of the actual or potential impacts of contaminants from a hazardous waste site on plants and animals other than humans and domesticated species. However, potential impacts to human health and domestic animals from the arsenic impacted wetland are discussed in this report to give an overview of all types of exposure to the site.

A BERA can play an important role in the decision making process for a site that includes use during the following stages - site investigation, feasibility studies for remedy selection, and evaluating the effectiveness of remedy implementation. The BERA followed the basic guidelines of U.S. EPA's (1995) *Ecological Risk Assessment Guidance For Superfund* and WDNR's (1992) *Guidance For Assessing Ecological Impacts and Threats From Contaminated Sediments*. U. S. EPA's (1998) more recent *Guidelines for Ecological Risk Assessment* as published in the Federal Register and Pascoe (1993) were also reviewed.

1.1 Site History and Contamination Source

In August of 1993, the WDNR became aware of wetland areas either devoid of vegetation or having stressed vegetation in a portion of the state-owned C.D. Besadny Fish and Wildlife Area to the north of the railroad grade currently owned by the Fox Valley & Western, Ltd. Railroad (FVWR). Initial investigations indicated high levels of arsenic on the slope of the railroad grade (up to 68,000 mg/kg) and that the dead areas out in the marsh were possibly related to these arsenic levels. The Kewaunee River is approximately 1,200 feet to the east. It is believed that in observations made along the base of the slope of the grade in early 1995, that arsenic granules were still present. Observations of the site in early 1995 also showed a number of goose and waterfowl carcasses on the mudflats of the devegetated area.

The source of the site contamination is believed to be a 1943 railroad car derailment and spill of a powder/granular arsenic compound (Reyburn, 1994). Arsenic was commonly used in the period for applications as a herbicide and pesticide. Historical information obtained by WDNR suggested sodium arsenite may have been used in the region for grasshopper control. STS (1994), the railroads consultant, indicated that based on their March 1994 round of sampling, a large release of arsenic, possibly sodium arsenite, had occurred at the site.

Based on the initial findings, a potential responsible party letter was sent to FVWR on February 2, 1994. FVWR hired STS Consultants of Green Bay who subsequently performed assessments of the impacted marsh to determine the extent of marsh soil (March 1994 and February 1995)), marsh surface water (November 1994), and river water and "pore water" (February 1995) arsenic contamination (STS, 1994; 1995).

The results of the site investigation indicated that approximately 15 acres of the marsh soils, surface water, and groundwater on the north side of the tracks contained elevated levels of arsenic with the highest levels associated with the formerly identified dead and stressed areas of vegetation. Areas of contaminated soils (0 - 2 ft) where arsenic concentrations exceeded 1,000 mg/kg (maximum of approximately 11,000) were approximately 4 acres based on an isoconcentration map constructed by STS following the sampling. In the highly contaminated areas, arsenic contamination extended down to approximately 8 feet below the soil surface and at shallower depths in lower contaminated areas. Precapping arsenic concentrations maxed out at 920 mg/L in the surface waters and 800 mg/L in the "pore water" from the site. Some arsenic contamination was also found in the soils, surface water, and groundwater on the wetlands to the south side of the tracks. General summaries of the sampling for arsenic in the wetland soils and surface waters in areas outside of the cap are presented in Figures 2 and 3, respectively, in the Appendix at the end of this report.

1.2 Interim Remedial Action Implementation

Based on the results of the site investigations, the State of Wisconsin and FVWR entered into a cooperative agreement to (1) implement an Interim Action at the site, (2) for both sides to conduct monitoring and reporting, and (3) for the FVWR to conduct groundwater modeling to estimate the transport of arsenic by groundwater from the site to the Kewaunee River. The State agreed to provide partial funding for the Interim Action and provide in-kind services for toxicological and biological monitoring. The agreement was formalized in a Consent Order signed by both parties in February 1996.

WDNR conducted various monitoring at the site in 1995, 1996, and 1997 with the results summarized in periodic memos. All collected data was evaluated and used by WDNR in the baseline ecological risk assessment for the site that looked at site receptors, estimated exposure levels, toxicological endpoints, and risks to the receptors from the exposures.

Under the Consent Order, FVWR implemented the Interim Action of placement of a geotextile/wood chip cover over the area of most highly contaminated wetland soils in February of 1996. Approximately 4 acres of marsh soils were covered. Also under the agreement, FVWR constructed a chain link fence, 2,430 feet long and 6 foot high around the entire perimeter of the 15 acre arsenic contaminated marsh. Details for the construction of the cover are contained in STS's (1996) Construction Documentation Report. The cover placement essentially eliminates the areas of highest surface water and soil arsenic contamination from exposure to wildlife and waterfowl. The fence around the perimeter serves to eliminate access to the entire contaminated area by humans and larger mammals.

A more apt description of the material used in the cover would be an organic detrital mix based on the larger proportion that consisted of composted leaves and grass from yard collections in the city of Green Bay. While wood chips may be in the mix, the leaves/grass portion appeared to visibly dominate the materials when they were being unloaded at the site. The organic detrital mix was placed over an underlying geotextile fabric to a compacted depth of 2.0 to 2.5 feet which is less than the 4.0 feet of the original design plans. The underlying geotextile fabric material is permeable allowing precipitation to infiltrate through the cover, minimizing ponding and subsequent cover settling. Nitrogen fertilizer was added to the cover and then seeded with a mix of annual rye, bluegrass, timothy, and redtop. Also, under the consent agreement, two dug pond remnants to the north and southeast of the covered area were filled in with the organic materials. Relatively high levels of arsenic in the water of these two ponds had previously been measured.

Over a period of years, the organic material in the cap may be subject to decomposition, mineralization, and loss. Loss of the organic materials may mean a subsidence or loss of the thickness of the cap material. Significant loss could lead to exposing standing water associated with the underlying arsenic contaminated soils. The cap may need to be monitored for elevation loss over a number of years.

1.3 Decisions On Further Remedial Measures and Site Monitoring Considerations

Section 6 of the Consent Order states *"The parties acknowledge and agree that although approved only as an interim action, the work to be undertaken pursuant to this order may, depending on the results of long term monitoring and / or hydraulic modeling, be a reasonable and appropriate final remedy".*

Results of outputs of the modeling efforts, the baseline ecological risk assessment, human health considerations, and compliance with applicable groundwater and surface criteria and other applicable regulations will be used to assess the potential of the interim action for its short- and long-term effectiveness as determined by its ability to reduce or eliminate exposures by receptors to toxic levels of arsenic and reduce mobility of arsenic in the impacted wetland. The decision that needs to be made at the site is whether (1) the interim actions taken to date will be effective or acceptable only to some date in the near future and more permanent remedial actions must be considered at that time, (2) if the interim remedial actions taken to date can be considered a practicable and appropriate final remedy, or (3) if more site monitoring is needed before a decision can be arrived at as to the acceptability of the interim action as a final remedy. NR 722.09 (1) Wis. Admin. Code states that remedial action(s) chosen to meet the water quality criteria as cleanup objectives will be done to achieve restoration of the environment to the extent practicable.

1.4 Environmental Setting

(A large part of the following is derived from the Wetland Assessment performed as part of the NR 103 evaluation process proceeding the interim action performed at the site. (Evaluators: Trochlell and Janisch

The wetland area impacted by the arsenic spill is located in the Southwest 1/4, Section 7, Township 23 North, Range 25 East, Township of Pierce in Kewaunee County. The wetland is approximately one mile upstream from the State Highway 42 bridge in the city of Kewaunee and located along a section of track currently owned by the Fox Valley & Western Railroad known as the "ferry yard lead". The actual spill site is approximately 1,200 feet the west of the Kewaunee River. Approximately 15 acres of the wetland have been impacted by the transport of arsenic from the original 1943 spill site.

The wetland is located in the floodplain and is part of a large complex of the riverine wetlands along the Kewaunee River. Approximately 27 acres of surrounding upland drains to wetland area that contains the site. Land uses within the surrounding area (the area bordered by the Kewaunee River and the railroad) are mainly open space/wetland (70%) and agricultural cropland (30%). The wetland is at the base of steep terrain, so surface water runoff and groundwater add to the river flooding to make up the wetland's hydrology. The impacted area of the wetland is located below the ordinary high water mark of the Kewaunee River. There are two slough channels that begin on the eastern edge of the impacted wetland and connect directly to the river. Depending on water levels, either river water will flow on to the wetland from these channels or the channels will serve to drain some portion of the marsh. At some time in the past apparently as an attempt to manage for waterfowl usage of the area a series of ponds were dug in the wetland. Eight of the ponds are to the north of the security fence and two remain along the north side within the fenced area. There were at least three more at one time but they are now indistinct depressions that filled in with emergent plants over time. As part of the interim remedial actions for the site, two of the ponds were filled to eliminate exposures to the arsenic contaminated waters in the ponds.

The Natural Resources Conservation Service (NRCS) soil map classifications for the wetland are Borosaprist and Carbondale muck. These soils are nearly level, very poorly drained organic soils underlain by organic layers or by loamy or sandy sediment. Soils observed in the field were consistent with the NRCS classification description except that organic soil layers consisted of mucky peats as opposed to mucks. The underlying soils consist of coarse sands and gravely loams at 15-18 feet below the organic surface in the western portion of the impacted wetland and then changes to silty clays and clays at 26-28 feet beneath the eastern edge of the wetland.

The wetland vegetation communities in the wetland complex (includes impacted wetlands to the north and south) along the river are shallow marsh dominated by *Typha latifolia*, sedge meadow (*Carex stricta*) and Canada bluejoint grass (*Calamagrostis canadensis*), and shrub-carr dominated by *Salix exigua.* The vegetation of the impacted wetland is dominated by the first two communities with monotypic cattail stands on the southern and eastern portions of the area and the sedge meadow community on the northern and western portions of the area. The shrub-carr community is to the north of the impacted area and to the southwest, south of the railroad tracks. The sedge meadow community to the west and northwest of the site has very diverse vegetation composed almost exclusively of native wetland plants. The sedge meadow plant community has been designated as rare or uncommon in the state. Based on the integrity of the native plant community and the scarcity of the sedge meadow wetland type, a rating of exceptional is assigned to the floristic diversity functional value in assessing the wetland. The vegetation in the wetland complex was analyzed by the modified time meander method. The plant species observed are listed in Table VEG-1 in the Appendix.

Eupatorium perfoliatum and *Carex stricta*, which are vegetation found within parts of the wetland, are indicative of a groundwater hydrologic component. Iron floc and an oily sheen indicating iron bacteria also are indicative of a groundwater discharge function. One of the dug wildlife ponds has a clear, colorless water that supports a dense cover of *Chara* sp., suggesting an active groundwater component.

Use of the impacted wetland area by wildlife, birds, fish species, and other aquatic life and associated exposures to arsenic while on the area will depend very much on the yearly and seasonal periodicity of water levels on the area. The shallow marsh and meadow areas are typically subject to inundation during periods of high precipitation and snow melt and runoff in the spring. How long and at what depth surface water remains on the marsh into the summer may depend on the particular year. Water levels on the marsh may also be influenced by the long term trends of water levels in Lake Michigan. In the period from early 1996 until mid-June of 1997 water levels had increased over 2 feet in the river based on staff gauge readings at the railroad bridge. This in turn increased the depth and seasonal length of standing surface water over the marsh. Since gauge reading were not taken after this date, it is not known if water levels increased, remained the same, or decreased. Bird species observed at the reference wetland and the impacted wetland are listed in Table BIRD-1 of the Appendix.

The Kewaunee River is a large slow gradient stream with a 25-year average flow of 86.3 cubic feet per second (cfs) and low flows ($Q_{7,10}$) of 0.05 cfs (WDNR, 1995). Monthly mean flows (cfs) in the Kewaunee River for 1964-1994 are summarized in the following table.

Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
37.5	58.8	277	213	85	78.9	42.2	34.7	59.4	46.5	69.8	57.3

The wetland is extensively used by a variety of wildlife species including game species such as white-tailed deer and waterfowl; furbearers such as raccoons and muskrats; non-game species of birds (rails, marsh wrens); and reptiles and amphibians. The floodplain of the Kewaunee River forms a corridor of wetland and upland habitat which may be important to migrating species and those species that require a large home range.

The Kewaunee wetland is located in an area of special natural resource interest as defined in NR 103.04, Wis. Admin. Code. It is located directly adjacent to and within the floodplain of the Kewaunee River which is a tributary to Lake Michigan. This constitutes a direct hydrologic connection to the lake. The site is within range of a State Special Concern Bird, the Black-crowned night-heron. Black-crowned night-herons were observed flying over the impacted wetland as they flew toward their foraging sites near or in the Kewaunee Harbor. Black-crowned night-herons nest in trees, shrubs, cattails and occasionally concealed in dense undergrowth (Ehrlich et al., 1988), so they could conceivably be using this wetland for nesting. No nests were found. Roosting sites were observed in trees located along the Kewaunee River upstream of the site.

The wetland is visible from roads, public lands, and houses on the adjacent hills overlooking the area. The city of Kewaunee is located less than a mile downstream, offering the local population and visitors with an opportunity to view and use the area. Although the wetland is partially in public ownership, access is rather limited due to the lack of a direct road link to the public land. The area can be accessed by boat from the Kewaunee River or by foot along the railroad tracks. Because of its size and location in the Kewaunee River floodplain, long views are present of the site within the viewshed of adjacent upland areas and from within the wetland due to the open nature of the wetland complex.

Specific conductance of surface waters measured in June of 1997 in the area between the cap and the perimeter fence at seven locations ranged from 650 to 938 umhos/cm and averaged 807. Specific conductance measured in the Kewaunee River upstream and adjacent to the site ranged from 481 to 579 umhos/cm and averaged 530 at seven locations. These are hard carbonate waters that are generally reflected in the high conductivity levels. The ph of the river water ranged from 7.16 to 7.62.

A summary of the of the functional assessment of the wetland complex done for the NR 103 evaluation showed the following:

FUNCTION	SIGNIFICANCE			
	Low	Medium	High	Exceptional
Floral Diversity				X
Wildlife Habitat				X
Fishery Habitat		X		
Flood/Stormwater Attenuation		X		
Water Quality Protection			X	
Shoreline Protection		X		
Groundwater			X	
Aesthetics/Recreation/ Education			X	

2.0 Conceptual Framework of the Ecological Risk Assessment

2.1 Purpose of the Risk Assessment (Basically from Pascoe et al., 1994)

The purpose of the ecological risk assessment for the arsenic-impacted wetland area of the C.D. Besadny Fish and Wildlife Area was to identify (a) sensitive environments and species in the aquatic and wetland habitats; (b) species-specific exposure pathways and arsenic exposure concentrations in these habitats; (c) appropriate end points for ecological and toxicity studies; and (d) the likelihood of adverse effects of arsenic to individuals and populations. A specific goal of the risk assessment was to identify wetland soil, river sediment, and surface water concentrations that may be related to adverse biological and ecological effects at the site. Together with human health considerations, the ecological risk assessment was to provide a framework and information base to assist in evaluation the short- and long-term effectiveness of the interim remedial action carried out at the site.

2.2 Guidance On Approach and Methodology

A suite of interrelated components produced information for use in a weight-of-evidence approach in the ecological risk assessment. The components consisted of (a) chemical analysis of the environmental matrices (soil, sediment, surface water, groundwater, and biota) to establish the presence and levels of arsenic contamination within the matrices; (b) ecological surveys and observations to establish potential receptor wildlife, birds, fish, and other aquatic life that may be utilizing the site to meet their needs, identify sensitive species or habitats, and identify if any adverse ecological effects that might be distinguishable; (c) conducting toxicity tests on site waters and soils utilizing indigenous or surrogate species to attempt to establish a link between site matrices and toxicity and interpolate these results to adverse effects in the site's aquatic system and food chain; (d) relating the results of numerical criteria (e.g. promulgated water quality criteria for arsenic in NR 105), sediment quality guidelines, and literature studies of toxicity to the site measurements and toxicity testing results; (e) evaluating the environmental factors affecting the speciation, toxicity, transport, and bioavailability of arsenic; and (f) using a food chain model for various site receptors to estimate daily intake of arsenic compared to toxicological intake levels. These information components were integrated and used to evaluate potential threats to individual species and establish, if possible, a firm, casual relationship between site conditions, toxic effect levels to the site receptors in each trophic level of the wetland food web, and adverse ecological effects.

The U.S. EPA risk assessment guidance is an eight step process. The first two steps of the process consist of screening level assessments that takes initial site information and does a screening level exposure estimate and risk calculation. These steps were done early in the process resulting in a handout of compiled information that is available for review. The result of the screening level assessment was a decision for the need to proceed with Step 3 of the assessment beginning with the problem formulation stage of a baseline ecological risk assessment (BERA). The problem formulation step includes several activities:

- refining the screening-level problem formulation
- further characterizing of ecological effects of contaminants by reviewing toxicity information
- reviewing and refining information on contaminant fate and transport, complete exposure pathways, and species and habitats potentially at risk
- develop a conceptual site model with questions that the site investigation will address; and
- select assessment and measurement endpoints.

A conceptual site model of the site where ecological concerns and potential exposures to site receptors are initially identified was developed

The focus of the problem formulation stage is collecting information to design the exposure and ecological effects studies to be conducted in Step 4 of the assessment. Based on the results of the studies conducted in Step 4 and other toxicological data sources, analysis of exposures and biological/ecological effects assessment are done in Step 6. The exposure assessment quantifies the magnitude and type of actual or potential exposure of ecological receptors to site contaminants whereas the effects assessment attempts to establish if these exposure levels lead to impairments or toxic effects to the site receptors. Determining the ecological relevance of the measured biological responses and the estimated responses based on criteria, guidelines, and literature of arsenic to the site receptors is important in characterizing risks to the wetland ecosystem.

In Step 7 of the BERA involving risk characterization, data on exposure and effects are integrated into a statement about the risk to assessment endpoints established during the problem formulation stage. A weight-of-evidence approach is used to interpret the implications of different studies or tests for the assessment endpoints. The risk characterization section of the BERA should include a qualitative and quantitative presentation of the actual or potential adverse effects to receptors and ecological components (which may include organisms [i.e. individual receptors], populations, communities or the wetland ecosystem and and associated uncertainties in the risk estimates.

Step 8 of the BERA involves risk management. In risk management decisions, risk reductions at sites from implemented remedies either interim or intended permanent remedies must be balanced against a number of factors. To make these decisions, inputs from risk assessors, responsible parties, staffs of applicable WDNR programs, and local stakeholders should be considered.

2.3 Conceptual Site Model

Food chains in wetland systems are complex involving a wide array of consumers including bacteria, fungi, invertebrates, amphibians, reptiles, fish, birds, and mammals. To put some perspective on the development of the food web/food chain as part of the conceptual site model, the following points from Murkin (1989) are relevant. Murkin discusses the complicated characteristics of various food chain interactions of wetlands and the lack of or limiting information on the basic life history and ecology of some of the consumers present in wetlands. All of the above limits in cases a full understanding of wetland food chain interactions and evaluating secondary production.

- The temporal use patterns or the period of time consumers use the wetland varies. Some consumers depend on the wetland for all their annual requirements and other species only use it for some of their requirements. Some complete their entire life cycle in the wetland and others use it only for a short periods in their life cycles.

- There is a lack of information on the basic life history and ecology of some of the consumers present in wetlands. Some information is available on waterfowl and furbearer use of wetlands. Data on other groups is essentially nonexistent. Data on bacteria, fungi, and other microconsumers is lacking. Information and data about insect families or the algal community inhabiting freshwater systems is limited in some cases. Fully evaluating secondary production in freshwater wetlands is not possible because of the lack of basic life history information on most of the dominant invertebrate groups within these wetlands. For many of the invertebrates, the trophic status of the species of interest is not available and there is little known about habitat selection, indicator species for habitat conditions, and community structure. Some consumer groups for which we do have information change their trophic status over the course of their life cycles.

A conceptual site model showing potential site receptors and exposure pathways is shown in Figure 1 (in Appendix). The exposure pathways to larger mammalian receptors such as whitetail deer and fox to the arsenic contaminated wetland is judged to not be complete because the security fence around the entire perimeter effectively excludes them from the impacted area. Raccoon and opossum may also be limited from but not totally excluded from the impacted area by the fence. Additionally, because of the security fence around the area, domestic animals cannot access the area. The fence keeps them off of the site. Based on this, these mammals will not be considered further in the BERA process. These receptors may be subject to exposure to low levels of arsenic if they access the perimeter outside of the fence or if their prey leave the impacted area and go beyond the fence. Based on the probable size of the use area of these species (limited time spent in the area immediately around the perimeter fence seeking food and water) and the probable low levels of arsenic they would be exposed to beyond the perimeter fence, no or minimal risks from arsenic exposures to these species is predicted.

2.4 Assessment and Measurement Endpoints

Assessment endpoints are defined as the ecological component(s) that are considered to be of value to the particular ecosystem. Individual assessment endpoints usually encompass a group of species or populations with some common characteristics, such as a specific exposure route or contaminant sensitivity.

The assessment endpoints initially identified for the impacted portion of the wetland associated with

Kewaunee Marsh involved a variety of species from different trophic levels and a number of exposure routes. A variety of species in the wetland food chain were looked at ensure all habitat niches remain occupied, trophic level species groupings remain intact, and the trophic level functioning and energy transfers are maintained within the food web complex of the wetland system. Any actual or potential adverse effects to the growth, reproduction, or survival of the biota from various trophic levels would in turn effect the system functions listed above. The species involved are in Figure 1 of the Appendix, the conceptual site model. The measurement endpoints for the BERA become effects measurements to the above receptors as determined in toxicity testing studies or estimations of daily intake rates of arsenic from site exposures compared to toxic effect intake levels, and existing criteria and/or guidelines for arsenic in the environmental media. Measurement endpoints are measurable environmental characteristics that are related to the valued characteristics chosen as the assessment endpoints.

2.5 Exposure and Biological Effects Assessment

Steps 4 - 6 of the BERA involving the exposure and biological effects assessment are essentially contained in the following Sections 5.0 to 13.0 of this report. The discussion under each section generally includes the study design for each component, the measurement endpoints, and integrates the results of the field studies and laboratory toxicity testing with literature studies to arrive at a preliminary characterization of risks. The essence of the risk characterization outcomes in Sections to will be incorporated into Section 14.0 below related to a summary of risk characterization for the site.

3.0 Arsenic Toxicity

The toxicity of arsenic related to each one of the study components is discussed in the Sections that follow so this information generally will not be repeated here. What will be discussed here is some additional toxicity information that may pertinency in the risk characterizations for the site. Some of these factors include those as noted by Spehar et al. (1980), Eisler (1988), and Pascoe et al. (1996). These points are:

- Little work has been done on the long-term effects of arsenic on organisms at chronic concentrations (blocking or depressing enzyme systems, pathological changes in tissue, limiting development of growth, reproduction, metabolism, and other physiological processes).

- Additional long term studies and studies involving sensitive life stages such as embryos, larvae, or early juveniles are needed to more accurately assess the toxicity of arsenic forms to fish and other aquatic organisms.

- While there is not enough data available to allow derivation of numerical criteria for aquatic organisms for pentavalent arsenic (As^{+5}) or any organic arsenic compound, indications are that some organisms are more or at least as sensitive to As^{+5} and organic arsenic as they are to exposure to As^{+3} for which water quality criteria has been developed.

- Exposure to low levels of arsenic to organisms at certain trophic levels may have significant ecosystem implications. For example, Eisler (1988) indicates that chronic studies with mass cultures of natural phytoplankton communities exposed to low levels of arsenate (As+5) of 1.0 to 15 ug/L showed that certain species of algae were differentially inhibited, causing a marked change in species composition, succession, and predator-prey relations. The significance of these changes on carbon transfer between trophic levels is unknown.

➢ Generally studies on arsenic exposures from which dose-response information is derived are based on the inorganic form of arsenic, usually sodium arsenate. Speciation data on arsenic in consumable substances from wetlands are not available. It is assumed for the food chain model that all exposures to arsenic are to the inorganic form. However, the actual exposures may be a mix of inorganic forms in the water and soil and largely organic forms in food items. Organic forms of arsenic generally pass through the body unutilized and are less toxic than inorganic forms partly because of rapid excretion. Also ingested inorganic arsenic is detoxified by methylation to various organic forms. Because of detoxification and rapid excretion of arsenic, chronic poisoning is infrequently seen in wildlife. From these considerations, the assumption in the food chain model (Section 13.0) that all exposures at the wetland are to inorganic arsenic may overestimates exposure to this more toxic form.

4.0 Environmental Characteristics and Fate and Transport of Arsenic In Wetland Habitats

A number of reviews have been done on this topic in the literature. Some of the more important points are reviewed below. A number of the initial points were contained in the July 16, 1998 comment memo that reviewed the GeoTrans groundwater modeling effort for the site. Because the important roles that of redox, pH, and other components in the soil matrix play in arsenic speciation and availability, these points are reiterated here. The references from which these points are derived as listed in the July 16 memo are included in the reference listing for this report with an asterisk proceeding the authors name.

➢ Alterations in the oxidation state of arsenic, as influenced by redox potential and pH, greatly affect its solubility in water. At higher redox levels (200 to 500 mV), arsenic solubility was low and the major part was present as As(+5). An alkaline pH, or the reduction of As(+5) to As(+3), released substantial proportions of arsenic into solution. Under moderately reduced soil conditions (0 to 100 mV), arsenic solubility was controlled by dissolution of iron oxyhydroxides. Arsenic was coprecipitated as As(+5) with iron hydroxides and released upon their solubilization. Upon reduction to -200 mV, the soluble arsenic content increased 13-fold as compared to 500 mV.

➢ Numerous studies have dealt with As sorption on specific minerals and soils. Redox potential along with the clay fraction and sesquioxides play a governing role in the speciation and solubility of arsenic in contaminated soils. The transformation of arsenic in the sediment-water system is a function of redox potential and the composition of the sediments, which include mineral colloids, compounds of iron and aluminum, and the organic matter contents of sediments.

➢ Under reducing conditions, arsenite minerals are too soluble to persist in soils but arsenic sulfides were predicted to be stable.

➢ Anaerobic incubation of flooded soils and sediments will increase As concentrations in the pore waters of these materials. A portion of this increased As concentration is As(+3) since anaerobic conditions that generally exist in aquatic sediments are conductive to reduction of As(+5). The reduced state of As (As+3) has been reported to be 4 - 10 times more soluble in soils than the oxidized state.

- The increase in soluble As as the system traversed from an oxidized to a somewhat reduced environment (+100 mV) is attributed to ferric arsenate and other forms of ferric iron which are combined with arsenate, being reduced to the relatively more soluble ferrous form.

- Under oxidized conditions As solubility was low and 87% of the As in solution was present as As(+5). Upon reduction, As(III) became the major As species in solution, and As solubility increased. Total As in solution increased approximately 25 times upon reduction of sediment suspensions from 500 to -200 mV.

- More alkaline conditions (pH 7.5) led to greater dissolved As concentrations as compared to more acidic conditions. At a pH of 7.5, As solubility increased significantly under both oxidized and moderately reduced conditions (500, 200, and 0 mV) as compared to more acidic conditions. The large increase of As observed upon reduction was linked to the reductive dissolution of iron oxyhydroxides.

- Examination of sediments in a reservoir show that diagenetic sulfides are important sinks for arsenic in reduced, sulfidic sediments and they control its distribution. During reduction, oxyhydroxides of iron and manganese dissolve and arsenic either precipitates as arsenic sulfides or the arsenic is released to the groundwater system dominantly as As(+3). Observed increase in dissolved As upon reduction indicates that As solubility was not limited by the formation of insoluble As sulfide minerals.

- Under reduced conditions (0 to -200 mV), As(+3) became the major dissolved species. Up to 40% of the total arsenic present in soil became soluble.

- Iron and manganese hydroxides readily absorb As(+5) into their matrices. The larger As(+3) is probably not as readily absorbed in these structures. This suggests that under reducing pore-water conditions, redox reactions may result in increases in aqueous phase total arsenic concentrations.

- Arsenic complexation by dissolved organic matter prevents adsorptive interactions between the arsenic and solid-phase organic and inorganic materials.

- Methylation of arsenic is conducted by certain methanogenic bacteria under anoxic conditions which plays a significant role in the release of volatile arsenic from the soil to the atmosphere.

- Complex anions AsO_2^-, AsO_4^{-3}, $HAsO_4^{-2}$, and $H_2AsO_3^-$ are the most common mobile forms of arsenic.

- Arsenate ions are readily fixed by clays, humus and calcium. Most active in arsenic retention are hydrated Fe and Al oxides.

- The concentrations of aluminum, calcium, iron, and sulfur in the organic soils of the impacted wetland area of Kewaunee Marsh are shown in the following table. Generally, the levels of aluminum are comparable to the levels that may be found in and upland mineral soil, while the levels of iron are less, and the levels of calcium and sulfur are greater than what might be expected in upland mineral soils (Shacklette et al., 1984). How these levels translate into adsorption or binding ability to the arsenic would depend on the chemical and physical factors present in the wetland soils such as redox potential and pH.

Sample Site	Concentration In Kewaunee Marsh Soils - mg/kg			
	Aluminum	Calcium	Iron	Sulfur
SO-05 NE of Cap	3,300	22,000	5,400	5581
SO-06 East of Cap	3,100	21,000	5,100	5,539
SO-07 SE of Cap	4,300	23,000	6,400	5,713
SO-10 SW of Cap	2,100	40,000	4,900	4,118

5.0 Toxicity Testing Results For Soil and Water Samples Collected From Kewaunee Marsh

5.1 Methods

Toxicity bioassays were performed on wetland soils and surface water collected from the Kewaunee site in 1995 and 1996 and on surface water collected in 1997. A summary of the test organisms, measurement endpoints, and number of sites tested for each of the three years is summarized in table TOX-1 on page 30. The bioassays were conducted by the State Laboratory of Hygiene Aquatic Life Toxicity Testing Laboratory using established testing protocols (WDNR, 1996; U.S. EPA,1994). Water samples were collected from standing surface water on the marsh or in the previously dug ponds on the area and in the river. The only exception to taking surface water samples was collecting the water from below the ground surface that came into a dug pit (site KM-2W taken in 1995). For the toxicity testing, one cubitainer (7.5 gallons) of water was collected at each sample site. Concurrently, a water sample was taken at the collection site in a 250 ml polyethylene bottle for arsenic analysis. The water samples were placed on ice in large coolers for transport to the Testing Laboratory.

For the wetland soil toxicity testing, a cleaned 5 gallon polyethylene bucket was filled using a spade to obtain the soil at each sample station. The spade was used to cut a 7 in. square of soil material to a depth of 6 inches. Enough of these spade cuts were taken in a 10 foot square area to fill the 5 gallon bucket. Subterranean rhizomes and larger vegetative material in each spade cut were removed from the soil sample prior to placement in the bucket. The 5 gallon buckets of soil samples were placed in large coolers on ice, covered with an insulating blanket, and transported to the Testing Laboratory. At the Testing Laboratory, each 5 gallon bucket of soil was placed in a Hobart mixer for homogenizing. A subsample of the mixed soil was taken and placed in a 250 ml polyethylene for arsenic analysis.

The results of the 1995 and 1996 soil and water toxicity testing results were written up in March 26, 1996 and May 2, 1997 memos (Janisch to Reyburn. The summary of results of the three years of toxicity testing is shown in Tables TOX-2 (1995 soils), TOX-3 (1995 water), TOX-4 (1996 soils), TOX-5 (1996 water), and TOX-6 (1997 water). These tables are on pages 31-35. In each table, the sites are arranged in order of increasing arsenic concentrations in the wetland soils and surface waters tested, with the references site concentrations being the lowest.

5.2 Wetland Soil Toxicity Testing

1995 Acute Testing

Hyallela azteca

Survival of *Hyallela azteca* in the control exposure (66%) did not meet the test acceptability requirement of 80% or greater for the 10 day acute test. Survival in the sites tested including the reference site was similar to the control. Failure of the test organisms to survive in the control sediments may mean the cultured organisms were not healthy at the beginning of the testing.

Daphnia magna

In the 48 hr acute test with *Daphnia magna*, no significant mortality was observed in the water column over soils that had up to 440 mg/kg of arsenic. In sediment exposures, the arsenic concentration in water that may result from the release of arsenic in the sediments is not measured.

Ceriodaphnia dubia

In the 48 hr acute test with *Ceriodaphnia dubia*, the control exposure survival (mean of 80% in the control replicates) did not meet the test acceptability requirement of 90%. There was no statistically significant difference in survival between the study soils and the control or the reference site soil using statistical tests because of the variability of recovery of test organisms within the replicates of each site tested. While apparently not statistically significant, survival was reduced at sites KM-4 (60% survival), KM-5 (65% survival), and KM-6 (50% survival) compared to the 80% survival at the control and 85% survival at the reference site (KM-1). These reductions in survival begin in soils with arsenic concentrations as low as 35 mg/kg (KM-4). KM-2 at 91 mg/kg did not have reductions in survival (90%) while KM-3 at 120 mg/kg experienced some reduction (75%) but not as great as KM-4, KM-5, and KM-6 (35 mg/kg, 230 mg/kg, and 440 mg/kg, respectively.

In an alternative method of analysis for the *C. dubia* data , the 25% Inhibition Concentration level currently applied to wastewater discharge data can be used. Any response greater than 25% inhibition (25% or greater reduction in survival) compared to the lab control is considered toxic. Under these criterion, the exposures in water over the sediments from sites KM-4 (25% reduction) and KM-6 (38% reduction) would be interpreted as toxic.

1995 Chronic Testing

Chironomus tentans

Ten-day chronic toxicity exposures were conducted with *Chironomus tentans* (survival and weight endpoints). Exposure to site sediments were not toxic to the survivability of C. *tentans* over the 10 day period. However, C. *tentans* growth was significantly inhibited (P=0.05) at all of the sample sites with the exception of the reference site. However, the ammonia levels in the water increased substantially over the duration of the C. *tentans* test at the sites where growth was inhibited. These ammonia levels may confound the results in attributing all of the toxicity reflected in growth inhibition to arsenic alone. If the toxicity is related to arsenic alone, the lowest arsenic concentration in the soils that the growth inhibition effects are associated with is 35 mg/kg (KM-4).

Daphnia magna

In the 10 day chronic test using *Daphnia magna*, significant mortality (P=0.05) occurred (average of 63% survival in site replicates) in the organisms in the water over the sediment at KM-2 (91 mg/kg arsenic). Survival at all other exposures, including those with greater arsenic concentrations in the soil was greater than 80%. *D. magna* reproduction was significantly impaired in the water over the reference site sediments (KM-1 @ 4 mg/kg) and at KM-2 (91 mg/kg) and KM-3 (129 mg/kg). Reproduction was reduced at KM-4 and KM-5 but not to statistically significant levels. Apparently, in treatment KM-2, significant changes in pH, alkalinity, and hardness occurred over the test to the extent that the changes may have influenced the mortality and lack of reproduction that occurred.

A large confounding factor is that reproduction was impaired in water over sediments collected at the upriver reference site (KM-1). The conclusion from this is that either arsenic at background concentrations in the sediments is being released to the overlying water to affect the *D. magna* reproduction or some other sediment component is responsible. The assumption is that arsenic at the reference site is not the cause of the toxicity and some other sediment component is. If this is the case, it is not possible to distinguish if this component or the elevated levels of arsenic in the soils at the downstream sample sites are responsible for the reduced reproduction of *D. magna.*

1996 Acute Toxicity Testing

Hyallela azteca

Survival of *H. azteca* in the control exposure (66%) again did not meet the test acceptability requirement of 80% or greater for the 10 day acute test. Survival at all the study sites was greater than 90% which means all treatments performed better than the lab control. Why the test organisms performed better at the study sites compared to the control sediments is not known. In 1995, the *H. azteca* performed poorly both in the control sediments and the study site sediments. The range of arsenic concentrations in the study sediments were comparable between the two years. The reasons why there was so disparate response in the study sites between the two years is unknown. In 1995, the health of the *H.azteca* before the tests seemed to be questionable based on similar low survival in the lab control and study site sediments. In 1996, survival was again low in the lab control but at greater than 90% at the study sites.

Daphnia magna

Survival was significantly reduced (77% survival) at ST-05 (67 mg/kg) compared to the lab control (93% survival) and the reference site, ST-01 (97% survival). Survival was also reduced at ST-02 (77%) @ 150 mg/kg and ST-04 (57%) @ 220 mg/kg but not statistically significant because results within the replicates from these two sites are so variable and a statistical significance wasn't determined.

Ceriodaphnia dubia

Survival was reduced at two sites, ST-02 (85%) @ 150 mg/kg arsenic in soils and ST-04 (80%) @ 220 mg/kg. Neither reduction in survival was statistically significant in their differences from the lab control or the reference site.

1996 Chronic Testing

Chironomus tentans

Exposure to site sediments were not toxic to the survivability of *C. tentans* over the 10 day test period from any of the test sites.

Growth of *C. tentans* was significantly reduced at all of the study sites including the field reference site compared to the lab control. When the growth results are compared to the field reference site results (ST-01) , significant reductions in growth were present at sites ST-02, ST-04, and ST-06. This would appear to mean that some factor(s) in the system sediments are reducing growth at all of the sites compared to the lab control and the growth reduction is greater and significantly different at the sites where arsenic is elevated in the soil sites compared to the reference site. Assuming arsenic at background levels is not causing growth reductions at the reference site but some other factor is, it is not possible to distinguish if this factor or elevated arsenic levels at the study sites is responsible for the growth reduction. This situation is comparable to the difficulty in interpreting the 1995 chronic toxicity testing results for *Daphnia magna*.

Daphnia magna

Survival in the 10 day chronic test was significantly different than the control in treatments ST-02, ST-02, ST-04, ST-05, and ST-06. Survival at the reference site was only 53% but it was not significantly different from the lab control due to the large variation in the replicates survival within the field reference treatment. Reduced survival would reduce the production of young at all of the sites relative to the control. It would appear matrix factors are influencing survival and growth at all the study sites including the field reference site. Again, assuming arsenic is not causing the toxicity at the reference field site but other factors are, there is no way to distinguish whether elevated arsenic levels in the study site soils are responsible for the toxicity observed at these sample sites or other factors are involved.

5.3 Discussion of Results For the Toxicity testing of Wetland Soils

In order to attempt to determine what tests yielded valid and useful results to draw some conclusions with some degree of certainty as to the relationships between the levels of arsenic contamination in the wetland soils and toxic effects to the benthic and water column organisms, the summary table that follows was put together. The table attempts to identify what tests, organisms and endpoints gave the clearest results without associated confounding factors that make the results and association solely with potential arsenic toxicity uncertain or less certain.

From the table it would appear that the 10-day chronic toxicity test measuring survival in *Chironomus tentans* and the 48-day acute toxicity test measuring survival in *Daphnia magna* provide the most valid and therefore certain results based on test outcomes largely free of confounding influences. The growth endpoint of the 10-day chronic toxicity test for *Chironomus tentans* was subject to confounding factors in both years of testing and therefore the results have some uncertainty associated with them. Results from other tests that have less certain results that should be weighed in considerations of soil concentrations and effects are also included in the table.

Review of the Useability of Toxicity Testing Results Performed On the Wetland Soils from Kewaunee Marsh

Useability or Validity of Data	**Soil Exposures to Benthic Test Organisms**				**Lab Water Over Soil Exposures to Water Column Test Organisms**					
	Acute Toxicity Test		**Chronic Toxicity Testing**		**Acute Toxicity Tests**				**Chronic Toxicity Test**	
	H. azteca		***C. tentans***		***D. magna***		***C. dubia***		***D. magna***	
	1995	**1996**	**1995**	**1996**	**1995**	**1996**	**1995**	**1996**	**1995**	**1996**
Valid test Results			X survival	X survival	X survival	X survival				
Somewhat Valid Results		X								
Growth or Survival Reduced but Not Stat. Significant						X	X	X		
Uncertain or Questionable Results, Confounding Factors			X growth						X	
Test Acceptability for Survival of Control Not Met.	X	X					X			X
Reference Site Effects	X			X growth					X reprod.	X

The associated sediment concentrations associated with the above tests and endpoints are as follows:

Year of Test Result	Test and Endpoint	Associated Arsenic Concentration in Soil
Results With Some Degree of Certainty		
1995	*Chironomus tentans*, chronic-survival	No effects at 5 study sites; As in soils from 35 - 440 mg/kg
1995	Daphnia magna, acute-survival	
1996	*Chironomus tentans*, chronic-survival	No effects at 5 study sites; As in soils from 2.2 - 220 mg/kg
1996	*Daphnia magna* acute-survival	Survival was significantly reduced at ST-05 at soil As of 67 mg/kg
Results With Less Degree of Certainty		
1995	*Ceriodaphnia dubia* acute-survival	Sites KM-4, KM-5, and KM-6 had reduced survival that was not statistically significant from control. Soil concentrations were 35, 230, and 440 mg/kg
1996	*Daphnia magna* acute-survival	Survival reduced at sites ST-02 and ST-04 at soil As concentrations of 150 mg/kg 220 mg/kg but not statistically significant

From the test results, it appears that for the survival endpoint, *Chironomus tentans* is tolerant to range of arsenic concentrations that it was exposed to from the site sediments which ranged from 2.2 to 440 mg/kg of arsenic. In the 1995 tests, *Daphnia magna* in water over the sediments was also tolerant of the range of arsenic in the soils. Apparently, no arsenic was released from the sediments to the water column or was otherwise unavailable to the *D. magna.* Daphnids are generally considered water column organisms, although *D. magna* does graze on the sediment surface. In the 1996 soil testing, *D. magna* survival was affected only when exposed to the study site that had 67 mg/kg of arsenic. Exposure to other sites with greater arsenic concentrations in the soils did reduce survival but the reduction was not significantly different or distinguishable from the control survival. Survival was reduced in some of the other tests over a range of arsenic soil concentrations but not at statistically significant levels distinguishable from the control survival.

It would appear from the results of the *C. tentans* and *D. magna* tests that both are relatively insensitive in the whole sediment toxicity tests performed. *Hyallela azteca* are typically more sensitive to contaminated sediments than are *Chironomus* sp. In whole sediment toxicity tests, Kemble et al.(1994) found that in terms of relative sensitivity in exposures to metal contaminated sediment, the amphipod *Hyalella azteca* was more sensitive than *Chironomus riparius* with *Daphnia magna* being the least sensitive of the test species. Spehar et al. (1980) indicated that amphipods were the most sensitive species to arsenic exposures. Meehan (1931) as cited in Spehar et al. similarly found amphipods (*Hyalella knickerbocker*) to be among the most sensitive organisms when exposed to arsenic (+3), whereas damselflies, dragonflies, and isopods were much more tolerant, surviving concentrations of 10,500 to 21,000 ug/L. However, none of the toxicity testing for the Kewaunee Marsh soils using *H. azteca* yielded useable results because the test acceptability requirement for 80% or greater survival in the control exposure was not met in either year of testing. However, it is noted that in the 1996 testing that even though the test acceptability of the control was not met, survival of *H. azteca* in the five study site soils ranged from 93 to 98% when exposed to a range of soil arsenic concentrations from 2.2 to 220 mg/kg.

The sediment test results do not yield information that clearly reflects an increasing degree of effects related to increasing arsenic concentrations in the sediments. *Chironomus tentans* was tolerant of all the concentrations it was exposed to and survival of *D. magna* was reduced to a statistically significant level in only one site. Generally, concentrations of contaminants in bulk sediments are not always a good indicator of toxicity because of the multiple factors that affect the release bioavailability of the contaminant in the sediment pore-water compartments. .

In the Kewaunee Marsh shallow wetland habitat, it is expected that midges of the family Chironomidae would dominate the numbers (70%) and mass (70%) of emerging adult insects based on McLaughlin et al. (1990). Tilton et al. (1980) reviewed studies where Chironomidae dominate the invertebrate communities of emergent wetlands. Many Chironomidae are tolerant of degraded conditions and sediments with elevated metal concentrations (Canfield et al. 1994; Winner et al. 1980)). Given the relative insensitivity of *Chironomus tentans* based on the test exposures and the dominance of the Chironomidae in the marsh insect populations, it appears this species component and the food base it provides is minimally affected by exposures to elevated levels of arsenic in the wetland soils, at least up to the known levels of test exposure of 440 mg/kg.

The table below shows a summation of the arsenic concentrations at the lower and upper levels related to effects to benthic macroinvertebrates from existing studies and sediment quality guidelines. Generally, the lower number is a concentration below which toxicity is rarely observed and the majority of benthic macroinvertebrates can tolerate. The upper number is a concentration above which toxicity is frequently observed and only a small number of macroinvertebrates can tolerate. The upper level concentrations from the guidelines were generally exceeded in 8 out of the 12 wetland soil samples from Kewaunee Marsh that were used in the *Chironomus tentans* toxicity testing in 1995 and 1996 without any apparent effects.

Arsenic Concentrations (mg/kg) In Sediments Related To Effect Levels to Benthic Macroinvertebrates

Ontario MOE		**Ingersoll et al.**		**Smith et al.**		**MacDonald**		**Long & Morgan**		**MENVIQ/EC**	
LEL	SEL	ER-L	ER-M	TEL	PEL	ER-L	ER-M	ER-L	ER-M	MEL	TOEL
6	33	13	50	5.9	17	8.2	70	33	85	7	17

LEL = Lowest Effect Level ER-L = Effects Range- Low TEL = Threshold Effect Level MEL = Minimum Effect Level
SEL = Severe Effect Level ER-M = Effects Range-Median PEL = Probable Effect Level TOEL = Toxic Effect Level

Factors that could account for this tolerance to the metals exposure could include 1) *C.tentans* as a species may have developed mechanisms that facilitate survival at threshold toxicity levels that involves detoxification of arsenic after entrance into tissues or mechanisms that prevent uptake in the first place, 2) laboratory toxicity testing performed under oxygenated conditions may alter the arsenic species and therefore bioavailabilty that may be different under natural field conditions, and 3) the chemical and physical characteristics associated with wetland soils (potentially high total and dissolved organic carbon, total volatile sulfides, complexing components) may affect the bioavailability of arsenic differently than the characteristics of the river and harbor sediments generally associated with development of the existing guideline effect values. The latter two situations would be responsible for making the arsenic in the soils less bioavailable and thereby reduce exposures and uptake by benthic organisms. The tolerance for arsenic exposures by members of the family Chironomidae can be seen relative to the species mean acute water value (U.S. EPA, 1995) for a midge species in this family, *Tanytarsus dissimilis.* This value is 97,000 ug/L.

Other macroinvertebrate species that make up the benthic and water column community of Kewaunee Marsh may not be as tolerant to the soil arsenic levels as *C. tentans.* The above sediment quality guideline values may be applicable to these other taxa that make up the benthic marsh community.

Another invertebrate taxa that can make up a large proportion of the benthic community of wetlands are annelid worms, the most common of which are the oligochaetas or aquatic worm species that inhabit the bottom muds. Oligochaetes are also very tolerant of high metals concentrations in the sediments and surface waters of systems ((Winner et al 1980; Canfield et al.1994). Whether Oligochaeta are as tolerant to as high a level of arsenic in soil as the species *C. tentans* appears to be is not known. High populations of Oligochaeta and Chironomidae can be representative of natural wetland habitats. High populations of these two with low proportions of other taxa are also representative of metal contaminated systems (Canfield et al. 1994). Since no samples or studies were done of the benthic community structure in the Kewaunee Marsh, it is not known how other taxa besides Oligochaeta and Chironomidae may have been affected by the elevated levels of arsenic in the soils. Due to the possible elimination of some species that are sensitive to exposures to the levels of arsenic in the wetland soils in the Kewaunee marsh, the benthic community may have a lower diversity or taxa richness.

The role of arsenic in the porewaters of the wetland soils related to toxicity of benthic organisms would depend on the chemical conditions that determine the form of arsenic which in turn will determine if some portion is in solution and potentially more bioavailable or is adsorbed and unavailable. While no pore water samples were collected from the Kewaunee marsh soils, water that came into excavated pits (approx. 1 ft. x 1 ft. x 1 ft.) from five locations in the wetland were analyzed for arsenic. The results are below.

Sample Site	Arsenic in Pit Soil -mg/kg	Arsenic in Pit Water- ug/L	Comment
PW04-01 (1996)	4.29	3.7 (Unfil.)	Reference Site
PW01-01 (1996)	5.4	7.6 (Unfil.)	South of RR Tracks
PW03-03 (1996)	63	3,000 (Unfil.)	Sampled 24 hrs. after pit dug
KM-2 (1995)	90	500 (unfil.)	South of RR Tracks
PW02-02 (1996)	427	9,900 (unfil.)	Sampled 48 hrs. after pit dug

At the time of sampling, the surface of the groundwater table was just below the soil surface. The concentrations of arsenic in the soil water would be in the bioavailable zone for benthic macroinvertebrates (0 - 6 inches of soil). When the marsh soils are sampled for toxicity testing, the above in situ bulk soil and soil water arsenic relationships are likely lost (i.e. in preparation of the soils, the soil water concentration likely decreases from the *in situ* situation). If this is the case, laboratory exposures of the benthic test organisms to site soils may underestimate the exposure risks. Because some of the highest arsenic concentrations in the soil water were measured after a period of settling, it is assumed a large portion is dissolved in solution or associated with suspended microparticulates. It is also assumed that at least some portion of this would be bioavailable to benthic organism that are in the soils. However, loss of overlying surface waters during a dry portion of the hydrologic cycle may reduce or eliminate the benthic macroinvertebrate community from the soils.

The benthic community in the Kewaunee River which has slow flows and a soft, silty bottom likely also has a large portion made up of Oligochaeta and Chironomidae along with other taxa. The concentrations of arsenic in the surface sediments of the river off of and downriver from the contaminated marsh area ranges from 6 to 17 mg/kg (see the July 16, 1998 memo associated with the STS groundwater and surface water model comments for a discussion of these values). While these concentrations are above the background concentration of 4 mg/kg, they generally are at or below the lower effect concentrations in the sets of sediment guideline values in the above table. For these reasons, it is believed the river benthic community downriver from the impacted marsh is not or only minimally impacted from the presence of the low level of elevation of arsenic in the river sediments.

5.4 Surface Water Toxicity Testing

1995 Acute Testing

Daphnia magna

In the 48-hr acute toxicity test, *D. Magna* survival was 100% in the surface waters from the 5 study sites. Arsenic concentrations in the water ranged from 62 to 3,700 ug/L.

Ceriodaphnia dubia

In the 48-hr acute toxicity test, *C. dubia* survival was 100% at 4 study sites and 90% at one (KM-5W at 3,400 ug/L).

Fathead Minnow

In the 4 day acute test, minnow survival was 95 - 100% at 4 of the study sites and 75% at one (KM-5W at 3,400 ug/L). The latter is not statistically significant from the control. However based on the criterion applied to wastewater discharge data (the Inhibition Criterion at which there is a greater than 25% inhibition relative to the control) this latter response is considered toxic.

1995 Chronic Testing

Ceriodaphnia dubia

C. dubia reproduction was significantly impaired (P=0.05) in the water from study sites KM-2W (500 ug/l) and KM-5W (3,400 ug/L).

Fathead Minnow

Over the 7 day test period, significant mortality occurred at study site KM-5W (3,400 ug/L). At the other 4 study sites (including site KM-3W at 3,700 ug/L), minnow survival was 93% or greater. Growth was significantly impaired at sites KM-2W (500 ug/L), KM-5W (3,400 ug/L), and KM-6W (3,700 ug/L).

1996 Acute Testing

Daphnia magna

There was 100% survival of *D. magna* at all 5 of the study sites that ranged in arsenic concentrations in the water from 37 to 8,300 ug/L.

Ceriodaphnia dubia

While not statistically different from the control survival, water from two study sites reduced survival of *C. dubia* somewhat (WT-04, 85% survival @ 2,400 ug/L and WT-02, 80% survival @ 8,300 ug/L).

Fathead Minnow

The 4 day survival was reduced for minnows at sites WT-02 (40% @ 8,300 ug/L), WT-03 (55% @ 1,400 ug/L), and WT-04 (70% @ 2,400 ug/L). The reductions in survival at sites WT-02 and WT-03 were not statistically significant compared to the control survival. The reduction in survival at WT-04 is statistically significant. A confounding factor may be that dissolved oxygen levels dropped in the test chambers during the tests requiring aeration to maintain the oxygen levels. The low oxygen levels before aeration and the starting up aeration may have stressed the minnows. How much this may have contributed to the test outcome is unknown.

Microtox

The Microtox test is a sensitive, reproducible, rapid screen for acute toxicity. The test measures the amount of reduction of emission from the luminescent bacterium, *Photobacterium phosphoreum*, when exposed to various toxicants. A significant result is interpreted where there has been a 25% reduction in light emission in a study site water sample compared to the laboratory control.

No Microtox results for any of the test site water exposures resulted in a 25% reduction in light emission. The results for only one site, WT-02 (6% reduction @ 8,300 ug/L) showed lower light emission when compared to the control. At the other 4 study sites, light emission was greater than the control which may indicate *P. phosphoreum* may have been stimulated by the presence of nutrients or other factors in the waters tested.

1996 Chronic Testing

Ceriodaphnia dubia

Survival of *C. dubia* over the seven day test period was 10% with no young produced in water exposures from site WT-02 (8,300 ug/L) and 0% survival of organisms at site WT-04 (2,400 ug/L). Survival at the other 3 study sites was 100% (37 - 1,400 ug/L).

The statistical comparison of number of young produced in treatment WT-03 (1,400 ug/L) and the lab control shows a statistically significant reduction.

Fathead Minnow

Survival of minnows was significantly reduced at sites WT-02 (43% @ 8,300 ug/L) and WT-03 (33% @ 1,400 ug/L). Survival at the other study sites was 90 - 100%. Growth of the surviving minnows at WT-02 and WT-03 was significantly reduced.

Selenastrum capricornutum

The assay using the green algae, *S. capricornutum*, over a 96 hour exposure period is a standard test used for determining the toxicity of waters and effluents (U.S. EPA, 1994). The endpoint is measured in terms of changes in the number of cells.

Algal growth was significantly inhibited in water from sites WT-02 (8,300 ug/l) and WT-03 (1,400 ug/l). Exposures of the algae to the waters of the other sites (includes WT-04 @ 2,400 ug/L) resulted in an increase in the number of cell compared to the control cell numbers. Factors in these waters appear to be stimulating algal growth compared to the control.

1997 Acute Testing

Ceriodaphnia dubia

No toxicity impacts. Arsenic concentrations at all study sites were low (2 - 24 ug/L).

Fathead Minnow

No toxicity impacts.

1997 Chronic Testing

Ceriodaphnia dubia

No toxicity impacts

Fathead Minnow

With the exception of some toxicity at WT-03 (7 ug/L), no chronic toxicity was exhibited at any of the other study sites. At WT-03, minnow survival was reduced 23.2% compared to the laboratory control survival. Given the low levels of arsenic present it is believed any toxicity is due some other factor present in the water other than arsenic or it is an artifact of the testing.

Selenastrum capricornutum

All of the study sites including the reference site exhibited toxicity in the chronic *S. capricornutum* test as compared to the control. A toxicity determination is based on a 20% reduction in cell growth compared to the laboratory control. Since the upstream reference site with < 1.0 mg/kg of arsenic in the water is also exhibiting chronic toxicity, it is assumed some factor in the water other than arsenic is responsible for the toxicity. If this is the case, this same factor may be present in the water to cause chronic toxicity in the algal test at the study sites where arsenic concentrations in the water are relatively low (1.0 - 24 ug/L).

5.5 Discussion of the Results For the Toxicity Testing of Surface Waters

Compared to the whole sediment toxicity testing results, the results of the surface water testing generally yields more valid and useable data in order to draw inferences about arsenic water concentration and toxicity effects. The concentrations of arsenic associated with the study sites and resulting test effects exhibited are summarized in the following table.

Year of Test Result	Testand Endpoint	Associated Arsenic Concentration in Water
D. magna - survival	1995	No effects at 5 study sites - As in water 62 - 3,700 ug/L
	1996	No effects at 5 study sites - As in water 37 - 8,300 ug/L
Ceriodaphnia dubia - survival	1995	No effects at 5 study sites - As in water 62 - 3,700 ug/L
	1996	No effects at 3 sites (As @ 37 - 1,400 ug/L); survival reduced at 2 sites (As @ 2,400 and 8,300 ug/L)
Fathead Minnow - survival	1995	No effects at 3 sites (As @ 62 - 860 ug/L and 3,700 ug/L; survival reduced at one site (As @ 3,400 ug/L)
	1996	No effects at 2 sites (As @ 37 and 430 ug/L and survival reduced at 3 sites (As @ 1,400 - 8,300 ug/l)
1996	Microtox - light emission reduction	No effects (As @ 37 - 8,300 ug/L)
Ceriodaphnia dubia - reproduction	1995	No effects at 3 sites (As @ 62, 860 and 3,700 ug/L); reproduction significantly reduced at 2 sites(As @ 500 and 3,400 ug/L)
	1996	1 site low survival and no young produced @ 8,300 ug/L: another site no survival @ 2,400 ug/L; another site no. of young reduced when compared with control (As @ 1,400 ug/L)
Fathead Minnow - growth	1995	1 site, significant mortality (As @ 3,400 ug/L); growth significantly impaired at 3 sites (As @ 500, 3,400, and 3,700 ug/L) ; 2 sites, no effects (As@ 62 and 860 ug/L)
	1996	2 sites, significant mortality (As @ 1,400 and 8,300 ug/L); growth significantly impaired at 2 sites (As @ 1,400 and 8,300 ug/L); no effects at 3 sites (As @37, 430 and 2,400 ug/L)
1996	*Selenastrum capricornutum* - growth	2 sites, growth significantly inhibited at 2 sites (As @ 1,400 and 8,300 ug/L); no effects at 3 sites (As @ 37, 430, and 2,400 ug/L
1997	All tests	No effects; As water levels all low, < 24 ug/L

The table below summarizes the significant effect-related concentrations from the above table with the arsenic concentrations ordered from lowest to highest based on the 1995 and 1996 results. If an effect concentration was lower than a non-effect concentration, only the effect concentration is included in the table.

Arsenic Water Concentration - ug/L in 1995 and 1996 Samples	Test Result Effect Related Concentration
37	
62	
430	
500	← Reproduction of *C. dubia* reduced (1995) ← Growth of fathead minnow significantly impaired (1995)
860	
1,400	← No effect on *C. dubia* survival (1996) ← Survival of fathead minnow reduced (1996) ← Significant mortality to fathead minnow (1996) ← Significant growth impairment to fathead minnow (1996) ← Growth of *Selenastrum* significantly inhibited (1996) ← Number of young of C dubia reduced (1996)
2,400	← Survival of *C. dubia* reduced (1995) ← No survival of *C. dubia* (1996)
3,400	← Survival if fathead minnow reduced (1995) ← Significant mortality to fathead minnow (1995)
3,700	← No effect on *C. dubia* survival (1995)
8,300	← No effect on *D. magna* survival (1995) ← Survival of *C. dubia* reduced (1996) ← Low survival and no young for *C. dubia* (1995) ← Limited effects on Microtox results

Some points from the above table related to no observed adverse effect level concentrations (NOAEL) and lowest observed adverse effect level (LOAEL) concentrations are:

1. The resulting test data shows that *D. magna* survived at all arsenic concentrations it was exposed to in water from the study sites with the highest concentration being 8,300 ug/L.

2. Reductions in survival of *C. dubia* began to be exhibited at an arsenic concentration of 2,400 ug/L.

3. Chronic effects in reproduction to *C. dubia* began to be exhibited at an arsenic concentration of 500 ug/L.

4. Chronic effects in growth to fathead minnows began to be exhibited at an arsenic concentration of 500 ug/L.

5. Reductions in the survival of fathead minnows began to be exhibited at arsenic concentrations of 1,400 mg/kg.

6. Inhibition of growth of *Selenastrum capricornutum* began to be exhibited at an arsenic concentration of 1,400 ug/L.

7. No effects in the Microtox test were exhibited with the highest concentration of exposure being up to 8,300 ug/L.

In terms of relative sensitivity to exposures to arsenic based on the acute test results, the general ordering from most resistant to most sensitive appears to be:

D. magna = *P. phosphoreum* < *C. dubia* < *S. capricornutum* = *P. promelas*
(Microtox) (Algae) (Fathead Minnow)

In comparing the above results with criteria development documents (U.S. EPA, 1995), two things are noted:

1. The ranking of the species in the criteria document from the most resistant to the most sensitive based on the Genus Mean Acute Value (GMAV) in exposures to sodium arsenite has the fathead minnow (14,065 ug/L GMAV) ranked as being more resistant than *Daphnia magna* (4,449 ug/L GMAV). This is opposite to what was found in the surface water testing for Kewaunee Marsh based on the above relative relationships.

2) The concentration of arsenic in the water at which acute toxicity was observed in the Kewaunee Marsh samples appears to be lower than the GMAVs of the criteria documents for fathead minnows and higher for *D. magna.* For example:

Test Organism	**Mean Acute Value (As^{+3}) From Criteria Documents - ug/L (Exposure to Na Arsenite)**	**Total As Concentration (ug/L) When Significant Mortality noted to Test Organism (Exposure to mix of arsenic forms)**
Pimephales promelas	14,065	1,400 (55% survival)
Daphnia magna	4,449	No mortality at 8,300

The testing done to arrive at the GMAV values involves exposing the test organisms to solutions of the toxic arsenite form dissolved in laboratory water. Field collected water samples would have a mix of arsenic forms and natural components such as particulate and organic matter that would control the bioavailabilty of the arsenic. It is assumed that only some portion of the total arsenic in a field collected sample would be present in solution as the toxic, dissolved arsenite form. Yet it appears that concentrations of total arsenic in the field samples that would only contain some portion of the toxic arsenite dissolved in solution elicit a toxic response at a lower concentration than the criteria based-arsenite numbers for fathead minnow.. The reasons for this are not known other than the presence of some toxic cofactor, possibly natural, in the wetland soils. The same may be true for the chronic effects. The toxicity tests showed growth effects to fathead minnow at total arsenic concentrations of 500 ug/L. Literature values associated with measurable effects during chronic exposures were at concentrations of 4,800 ug/L as As+3 (Lima et al. 1984) and 1,500 ug/L as As+5 (EPA, 1985).

The ambient water quality criteria for arsenic in NR 105, Wis. Admin. Code for the protection of aquatic life are that applies to the use classification of the Kewaunee River are:

Acute Toxicity Criteria	**Chronic Toxicity Criteria**
As Total Recoverable Arsenic +3 - ug/L	
339.8	152.2

The water quality criteria are designed to protect most of the aquatic species inhabiting the classified surface waters and are based on toxicity data from a variety of taxa with a range of sensitivities to the criteria contaminant.

Wetlands can have natural components and cofactor components present that affect the bioavailability and toxicity of a contaminant far outside the ranges used in standard toxicity testing for criteria establishment (e.g. total organic carbon, particulate matter, pH, and dissolved oxygen). Some of the wetland cofactors themselves can be directly toxic to organisms (e.g. un-ionized ammonia, high or low pH, hydrogen sulfide, low dissolved oxygen, and production of quantities of water soluble organic acids from anaerobic decomposition of organic matter, some of which can be toxic to higher plants McKee [1993]). The criteria for some of these water quality characteristics can be naturally exceeded in wetlands (Hagley et al. 1991). All of these factors are affected by the hydrologic regime of the wetland. In shallow water marshes and the sedge meadow area of Kewaunee Marsh, the surface water levels will fluctuate seasonally and yearly. At times water levels on the area will drop below the ground surface and standing surface water will only be present in limited depressional areas in the marsh topography and in the four or five wildlife ponds dug on the area in the past.

Surface water samples were collected from Kewaunee Marsh at various locations in 1995, 1996, and 1997. The data has been summarized in past memos. Some samples were collected in association with toxicity testing discussed above and others were collected for general characterization purposes. Yearly and seasonal differences in hydrological and water chemistry conditions make identification of any trends in differences of arsenic water concentrations over the area difficult from year to year. Placement of the cap over the highly contaminated area of soils in early 1996 eliminated the surface water areas that had very high arsenic concentrations. Sampling in the spring of 1996 generally showed relatively high concentrations of arsenic in the surface waters in the marsh area to the south (1,400 and 8,300 ug/L) and southeast (2,400 ug/L) of the cap. Sampling of the surface waters in these same areas one year later after cap placement showed much lower arsenic concentrations (260 and 86 ug/L, respectively). Whether this decrease is due to cap placement or seasonal hydrological and chemistry conditions is not known. More long term monitoring is needed to establish the seasonal and yearly surface water concentration of arsenic for the site (see Appendix Figure 3 for general locations of surface water sampling sites)..

The table below summarizes the most current sampling data for arsenic concentrations in the surface waters of the site (collected in 1997) compared to the ambient water quality criteria for arsenic and the effect-concentrations associated with toxicity testing performed on surface waters collected from the site discussed above. What is designated as the "Slough-River Interface" samples in the table below were water samples taken on the marsh end and mouth of two slough channels that drain the impacted area of the marsh to the river. Water samples taken at the marsh end of the two sloughs had elevated arsenic concentrations (76 and 430 ug/L) in 1996. The samples taken at the marsh end of the sloughs in 1997 had lower concentrations (9 and 24 ug/L). Concentrations at the mouth of the sloughs at the river in 1997 were lower (2 and 7 ug/L). Reasons for differences in arsenic concentration at the marsh end of the sloughs between the two years is unknown other than hydrological and water chemistry differences between the two years. The role the cap may have played is unknown. Sampling at the marsh end of the sloughs in 1996 was farther up the slough compared to 1997 where the sample was taken at the chain link fence. Some mixing with the river may have occurred at this point.

From the table below, the arsenic concentration in some portion of the wetland area has the potential to reduce the reproduction of *Ceriodaphnia dubia* and the growth of fathead minnow based on the toxicity testing results using site waters. These are more chronic toxic effects. None of the site samples exceed the 1,400 ug/L toxicity testing concentration associated with more acute effects related to reduction in survival of fathead minnows.

The conservative assumption that all arsenic concentrations measured in the site waters are in the trivalent or more toxic form would mean that the ambient acute and chronic water quality criteria are exceeded in surface waters over some portion of the site. In reality, components in the natural waters will control the form and bioavailability of the arsenic present and assuming all site measured arsenic is in the trivalent form may overestimate the toxic effects of the site waters.

A rough estimate of what proportion of the total arsenic measured in the site water is in the more toxic trivalent form can be gained by dividing the ambient criteria by the toxic effects total arsenic related concentrations from testing of the site waters as follows:

$$\text{Acute} = \frac{\text{340 ug/l Acute Toxicity Criteria}}{\text{1,400 ug/L Toxic Effects Concentration}} = \text{25\% of total arsenic as trivalent}$$

$$\text{Chronic} = \frac{\text{152 ug/L Chronic Toxicity Criteria}}{\text{500 ug/L Toxic Effects Concentration}} = \text{30\% of total arsenic as trivalent}$$

To determine the approximate toxic proportion of any site measured total arsenic concentrations, the above factors could be applied.

Sample Site	Total Arsenic (ug/L) in Water Sample	Ambient Water Quality Criteria Arsenic $^{+3}$		Tox. Testing Effects Concentrations - Total As	
		Acute	**Chronic**	**Mortality to minnows: Algal Inhibition**	**Reproduction & Growth**
		339.8	**152.2**	**1,400**	**500**
Slough-River Interface	2 - 24	<	<	<	<
SW-15	26	<	<	<	<
SW-10	86	<	<	<	<
SW-11	120	<	<	<	<
SW-9	260	<	Exceeded	<	<
SW-12	320	<	Exceeded	<	<
SW-13	530	Exceeded	Exceeded	<	Exceeded
SW-14	810	Exceeded	Exceeded	<	Exceeded

The results of the toxicity tests ran on both the water and sediment samples from Kewaunee Marsh and the literature indicate that *Daphnia magna* is tolerant of exposures to relatively high levels of arsenic. If *D. magna* is indigenous to the surface waters of the Kewaunee Marsh wetland means that it will remain as part of the established food chain of the marsh. *Daphnia* has the ability to bioconcentrate high levels of arsenic from the water into their tissues. This in turn may serve as a source of arsenic to organisms that feed on this zooplankton. Spehar et a. (1980) exposed *D. magna* to concentrations of 100 and 1,000 ug/L of both As+3 and As +5. The arsenic accumulation in *D. magna* increased with increased exposure concentration, and residues were highest in daphnids exposed to As+3. Bioconcentration factors calculated for *Daphnia* exposed to As+3 (979 and 96 ug/L) were 50 and 219, respectively after 21 days. However, while arsenic will bioconcentrate in organisms it does not biomagnify up the food chain. As+3 concentrations in the *D. magna* at the two levels of exposure were 20 and 50 mg/kg. As+5 concentrations in the two levels of exposure were approximately 5 and 20 mg/kg.

Generally, lower level trophic organisms such as algae and daphnids have higher bioconcentration factors for arsenic than higher trophic level organisms such as fish. Arsenic does not biomagnify up the food chain. The reason for the low toxicity and low accumulation of arsenic in the higher trophic level organisms such as fish may be their ability to metabolize the arsenic and remove it from the body in a short period of time (Spehar et al., 1980). Fish can apparently biosynthesize organoarsenic compounds within the gastrointestinal tract. The main source of arsenic for fish is primarily organoarsenic compounds that are synthesized at lower stages in the food chains (May et al. 1981). There is some suggestion that organisms at each trophic level convert inorganic arsenic to a detoxified organic form and then organisms at the next higher trophic level rapidly excrete the ingested organic arsenic, precluding food chain bioaccumulation (Penrose et al. 1977). Maeda et al.(1990) found in a freshwater food chain consisting of an algae (*Chlorella* sp.), grazer (zooplankton), and predator (goldfish), that the total arsenic accumulated decreased one order of magnitude and the relative concentration of methylated, organic arsenic increased successively up the food chain.

Table TOX-1. Toxicity Tests Conducted On the Wetland Soil and Water Samples Collected From Kewaunee Marsh.

Media Tested	End Points	Test Organisms	No. of Sites	Period Collected
Soils	Acute	*Hyallela azteca*	Reference site plus 5 study sites and lab control	June 1995
	Chronic	*Chironomus tentans*		
Lab Water Over Soils	Acute	*Daphnia magna*		
		Ceriodaphnia dubia		
	Chronic	*Daphnia magna*		
Site Surface Water	Acute	*Daphnia magna*	Reference site plus 5 study sites and lab control	
		Ceriodaphnia magna		
		Fathead minnow		
	Chronic	*Ceriodaphnia dubia*		
		Fathead minnow		
Soils	Acute	*Hyallela azteca*	Reference site plus 5 study sites and lab control	May 1996
	Chronic	*Chironomus tentans*		
Lab Water Over Soils	Acute	*Daphnia magna*		
		Ceriodaphnia magna		
	Chronic	*Daphnia magna*		
Site Surface Water	Acute	*Daphnia magna*	Reference site plus 5 study sites and lab control	
		Ceriodaphnia dubia		
		Fathead minnow		
		Microtox		
	Chronic	*Ceriodaphnia dubia*		
		Fathead minnow		
		Algal Assay		
Site Surface Water	Acute	*Ceriodaphnia dubia*	Reference site plus 5 study sites and lab control	June 1997
		Fathead minnow		
		Algal assay		
	Chronic	*Ceriodaphnia dubia*		
		Fathead minnow		
		Algal assay		

Table TOX-2 . Results of 1995 Toxicity Testing on Kewaunee Marsh Soils and Lab Water Overlying Soils.

Sample Site	Soil As mg/kg	Soil Exposures to Benthic Test Organisms		Lab Water Over Soils Exposures to Water Column Test Organisms		
		Acute Toxicity Test	Chronic Toxicity Test	Acute Toxicity Tests		Chronic Toxicity Test
		Hyallela azteca	*Chironomus tentans*	*Daphnia magna*	*Ceriodaphnia dubia*	*Daphnia magna*
KM-1 (Reference Site)	4	NT[1]	NT	NT	NT	Reproduction significantly impaired
KM-4 (NE of dead area)	35	NT	Growth impaired but qualified	NT	Toxic but qualified	Reproduction reduced but not significant
KM-2 (S. of RR tracks)	91	NT	Growth impaired but qualified	NT	NT	Reproduction significantly impaired. Significant mortality
KM-3 (E. of dead area)	120	NT	Growth impaired but qualified	NT	NT	Reproduction significantly impaired
KM-6 (E. of dead area)	230	NT	Growth impaired but qualified	NT	Toxic but qualified	NT
KM-5 (N. of hot area)	440	NT	Growth impaired but qualified	NT	NT	Reproduction reduced but not significant

NT = No Toxicity. No statistically significant differences between control and site results.

Table TOX-3 . Results of 1995 Toxicity Testing On Kewaunee Marsh Surface Waters

Sample Site	Water As ug/L	Surface Water Exposures to Water Column Test Organisms				
		Acute Toxicity Tests			Chronic Toxicity Tests	
		Daphia magna	*Ceriodaphnia dubia*	Fathead Minnow	*Ceriodaphnia dubia*	Fathead Minnow
KM-1W (Upstream Kewaunee R.)	3	100% survival NT	100% survival NT	NT	NT	NT
KM-4W (Pond #6)	62	100% survival NT	100% survival NT	NT	NT	NT
KM-2W (Pit water S of RR tracks)	500	100% survival NT	100% survival NT	NT	Reproduction significantly impaired	Growth significantly impaired
KM-6W (Dug Pond)	860	100% survival NT	100% survival NT	NT	NT	Growth significantly impaired
KM-5W (Pond #12)	3400	100% survival NT	90% survival NT	Toxic but qualified	Reproduction significantly impaired	Significant mortality. Growth significantly impaired
KM-3W (Dug pond)	3700	100% survival NT	100% survival NT	NT	NT	NT

Table TOX-4. Results of 1996 Toxicity Testing on Kewaunee Marsh Soils and Lab Water Overlying Soils.

Sample Site	Soil Arsenic mg/kg	Soil Exposures to Benthic Test Organisms		Lab Water Over Soils Exposures to Water Column Test Organisms		
		Acute Toxicity Test	Chronic Toxicity Test	Acute Toxicity Tests		Chronic Toxicity Tests
		Hyallela azteca	*Chironomus tentans*	*Daphnia magna*	*Ceriodaphnia dubia*	*Daphnia magna*
ST-01 (Reference Site)	2.6	NT[1]	NT	NT	NT	NT
ST-06 (S. of RR tracks)	2.2	NT	Toxicity[4]	NT	NT	Toxicity
ST-05 (SE of cap)	67	NT	NT	Toxicity[3]	NT	Toxicity
ST-02 (N. of cap)	150	NT	Toxicity[4]	Tox NS[2]	NT	Toxicity
ST-03 (NE of cap)	220	NT	NT	NT	NT	Toxicity
ST-04 (SE of cap)	220	NT	Toxicity[4]	Tox NS	NT	Toxicity

1. NT = No toxicity
2. Tox NS = Toxicity was noted in the replicates from the site but compared to the control, the differences were not statistically significant.
3. Toxicity = Impairments were present related to either survival or reproduction of young and the differences compared to the control results were statistically significant.
4. *Chironomus tentans* survival in the lab control was >70%, the test acceptability requirement. However, growth of *C. tentans* was significantly reduced in the field reference and at all the study sites compared to the lab control. In comparing the growth results of the study sites to the field reference, sites ST-02, ST-04, and ST-06 resulted in significantly reduced growth relative to the field reference.

Table TOX-5. Results of 1996 Toxicity Testing On Kewaunee Marsh Surface Waters

Sample Site	Water As ug/L	Surface Water Exposures to Water Column Test Organisms						
		Acute Toxicity Tests				Chronic Toxicity Tests		
		C. dubia	*Daphnia magna*	Fathead Minnow	Microtox	*C. dubia*	Fathead Minnow	Algal Assay[5] 96 hr.
WT-01 (Ref. Site)	1.0	100%[1] survival	100% survival	NT[2]	NT	NT	NT	NT
WT-06 (S. of RR)	37	100% survival	100% survival	NT	NT	NT	NT	NT
WT-05 (SE of cap)	430	100% survival	100 survival	NT	NT	NT	NT	NT
WT-03 (S. of cap)	1,400	100% survival	100% survival	Tox NS[3]	NT	Toxicity[4]	Toxicity	Toxicity
WT-04 (SE of cap)	2,400	Tox NS	100% survival	Toxicity	NT	Toxicity	NT	NT
WT-02 (SW of cap)	8,300	Tox NS	100% survival	Tox NS	Tox NS	Toxicity	Toxicity	Toxicity

1. 100% survival = The same number of viable test organisms were present at the end of the test period as were present at the start.
2. NT = No toxicity.
3. Tox NS = Toxicity was noted in the replicates from the site but compared to the control, the differences were not statistically significant.
4. Toxicity = Impairments were present related to either survival or reproduction of young and the differences compared to the control results were statistically significant.
5. Algal Assay = Uses *Selenastrum capricornutum*.

Table TOX-6. Results of the 1997 Toxicity Testing On Kewaunee Marsh Surface Waters

Sample Site	Water As ug/L	Surface Water Exposures to Water Column Test Organisms					
		Acute Toxicity Tests			Chronic Toxicity Tests		
		C. dubia	Fathead Minnow	Algal Assay	C. dubia	Fathead Minnow	Algal Assay
WT-01 (Reference Site)	< 1.0	NT	NT	NT	NT	NT	Toxicity[2] (64.5%)
WT-02 (Upper end-N. slough)	24	NT	NT	Toxicity[1] (30.5%)	NT	NT	Toxicity (78.4%)
WT-03 (N. slough, juncture @ river)	7	NT	NT	Toxicity (30.5%)	NT	Tox NS[3]	Toxicity (69.7%)
WT-04 (Upper end-S. slough)	9	NT	NT	NT	NT	NT	Toxicity (41.5%)
WT-05 (S. slough, juncture @ river)	2	NT	NT	NT	NT	NT	Toxicity (68%)
WT-06 (Downriver S. of RR bridge)	1.0	NT	NT	Toxicity (40.0%)	NT	NT	Toxicity (74.4%)

1. In the acute *Selenastrum* algal test as compared with the control, the following sites exhibited acute toxicity: WT-02, WT-03, and WT-06. The number in parentheses is the amount of reduction in algal cells compared with the laboratory control. A toxicity determination is based on a greater than 20% reduction from the laboratory control.
2. In the chronic *Selenastrum* algal test as compared with the control, the following sites exhibited chronic toxicity: WT-01, WT-02, WT-03, WT-04, WT-05, and WT-06. The number of cells in parentheses is the amount of reduction in algal cells compared with the laboratory control. A chronic toxicity determination is based on a greater than 20 % reduction from the laboratory control.
3. There was no toxicity exhibited in the chronic fathead minnow test as compared with the laboratory control survival although site WT-03 exhibited 23.2% reduction in test organism survival. A chronic toxicity determination is made based on a reduction of greater than 25% from the laboratory control.

6.0 Significance of the Arsenic Concentrations Measured In the Kewaunee Marsh Surface Waters to the Fisheries of the Marsh and Kewaunee River

The Kewaunee River is a large, low gradient stream that supports a warmwater sport fishery and has seasonal runs of coho salmon and steelhead (rainbow) trout from Lake Michigan. WDNR operates a salmon egg taking facility on the river.The lower river has extensive wetlands which serve as nursery grounds for the 28 species of fish supported by the river (WDNR, 1995). The most common species of fish found in the lower Kewaunee River are: smallmouth bass, northern pike, channel catfish, white sucker, yellow bullhead, redhorse (undefined species), pumpkinseed, bluegill, carp, gizzard shad, and alewife. Of these species, only the northern pike and carp use wetland areas as their primary spawning habitat. Generally, most of the species make major upstream spawning runs in the spring, likely further upstream than the arsenic impacted area. However, young-of-the-year fish may use wetland areas including the impacted wetland for foraging and shelter (Amrhein personnel communication with Steve Hoegler, WDNR fisheries manager in Manitowoc, August 1998).

STS's monitoring in 1996 included a river sample site next to the impacted marsh. Concentrations of arsenic in the unfiltered samples from this site over the year were 118, 108, 50, 3.2, and 3.7 ug/L with the highest levels associated with the April and May 1996 monitoring events. These samples are below the acute and chronic criteria for the protection of aquatic organisms. At different times and under different hydrological conditions, higher concentrations of arsenic in surface waters on the marsh may be transported or flushed to the river. Any elevated levels would be transitory and soon mixed in with the larger volume river flows. A concentration of 430 ug/L of arsenic was measured in the marsh end of one of the two sloughs that drain the marsh to the river in early 1996. Assuming that only a portion of the 430 ug/L was toxic, the toxic level would probably be below the acute and chronic toxicity criteria. Based on the data we have to date, fish utilizing or passing through that portion of the river next to the impacted marsh or moving up the slough channels connected to the river are at probably at low risk from exposure to any elevated levels of arsenic in the water.

Collections of various fish species in the Kewaunee River in 1994 showed detectable levels in game fish ranging from 0.1 to 0.5 mg/kg (wet weight) in skin-on fillets from three different fish species (channel catfish, northern pike, and smallmouth bass). Whole fish analysis of carp showed levels that were at less than detection. Assuming the whole fish water content is 50 to 80% of the fish weight, the dry weight concentration of arsenic in the fish tissue would range from 0.2 - 0.5 mg/kg at the low end and 1.0 to 2.5 at the upper end based on the above range of wet weight values. The upper end values dry weight values of 1.0 - 2.5 mg/kg are generally higher than the dry weight concentrations of arsenic found in fish (primarily white suckers) in other Lake Michigan tributaries by U.S.G.S. (1997). Generally, the U.S.G.S. study found arsenic tissue concentrations of 0.4 mg/kg or less (dry wt.) in whole fish.

Generally, whole fish concentrations might be expected to be greater than fillet concentrations. If this is the case, the whole fish arsenic tissue concentrations for the three Kewaunee River fish may be even greater than the fillet concentrations that range from 0.1 to 0.5 mg/kg (wet weight) as discussed above. Gilderhus (1966) found in his study where he exposed bluegills in ponds to various water concentrations of arsenic that the residues of arsenic in fillets of adult fish averaged 60% as high as the concentrations in whole fish. In doing two conversions, one involving going from wet weight to dry weight (assumes fish water content is 50% water by weight) and the other conversion going from fillet to whole fish concentrations (1.67 factor based on 60%), the possible concentration of arsenic in the whole fish based on the wet weight fillet concentration range above would be 0.33 - 1.67 mg/kg (dry wt.). While arsenic concentrations in fillets are important in human health considerations, arsenic concentrations in whole fish are important in doing ecological considerations because predator and upper trophic level species will be consuming the whole fish.

The upper end values of 0.4 and 0.5 mg/kg (wet wt.) which were found in smallmouth bass fillets in the Kewaunee River fish are generally above the arsenic concentrations taken from fish from other rivers of the state where arsenic was detected in fish tissue. The arsenic concentrations in these fish which was generally was 0.2 mg/kg (wet wt) or less on a whole fish basis. Based on the statewide fish data base, all the arsenic concentrations in other smallmouth bass collected from around the state are reported as < 0.5 mg/kg. This does not allow comparison with the results from the Kewaunee River or to make associations with the possibly naturally elevated arsenic levels in the crayfish and intake by the larger smallmouth bass (see discussion below). The exception to this is other species of fish collected from the Menominee River in Marinette that has arsenic contaminated sediments and surface water from a former arsenical herbicide manufacturing facility. Arsenic levels from the fillets of two fish from the Menominee River were 0.5 and 0.6 mg/kg (wet wt.), a value that matches the upper end values from smallmouth bass taken in the Kewaunee River. Another exception is fish collected from a site in the Ashland Harbor. Analysis of burbot from this site indicated arsenic levels of 0.5 mg/kg (wet wt.) in whole fish. The history of this site is unknown as far as a potential arsenic source. If the fish were taken in or near industrialized area of the harbor a source of arsenic may be leachate from coal storage piles or coal ash.

One explaination for arsenic being elevated in smallmouth bass and not other fish species from the Kewaunee River is that the smallmouth bass sampled were large specimens (greater than 15 inches) that because of their size are top level predators that feed especially on crayfish. Even at natural background levels of arsenic, crayfish accumulate arsenic into the chitin of the cuticle of the body and appendages. Smallmouth bass consuming the whole body of the crayfish ingest the elevated arsenic in the exoskelton. As an example of bioaccumulation from background sources, crayfish taken from a reference site in the Sheboygan River had whole body concentrations of arsenic of 0.4 - 0.6 mg/kg (wet wt.), the bulk of which is probably stored in the exoskelton. Since this food link is missing in the other fish species collected in the Kewaunee River such as the white sucker and carp who are omnivores, it may be a reason why their whole body arsenic concentrations were low. This could also mean crayfish from the impacted wetland are accumulating above normal concentrations of arsenic into cuticle chitin. If larger smallmouth bass from the river have access to these crayfish, it could result in elevated body burdens in the fish. Site sampling for crayfish would be needed to determine if arsenic levels are elevated above normal background levels in crayfish and if the crayfish are available to the fish in the river from the site.

On a comparative basis, Gilderhus (1966) in a study of farm ponds treated with arsenic found that at tissue levels of 1.3 mg/kg were associated with reduced growth rate and increased mortality in immature bluegills. Among adult bluegills, tissue residues of about 5.0 mg/kg of arsenic were associated with severe loss of weight and high mortality. Growth in adults was slowed to some degree when the residues in tissue were between 1 and 3 mg/kg. The Gilderhus study does not identify if the above 1.3 mg/kg value is expressed on a wet weight or dry weight basis. It is assumed to be expressed as a whole fish concentration on a wet weight basis. Adjusting the upper end smallmouth bass fillet concentration of 0.5 mg/kg (wet wt.) taken from the Kewaunee River to a whole fish concentration (times 1.67) yields a whole fish concentration of 0.84 mg/kg (wet wt.). This value is below the above effect value of 1.3 mg/kg from the Gilderhus study.

The U.S. Fish and Wildlife Service National Contaminant Biomonitoring Program (NCBP) samples and analyzes fish tissue from 117 surface water bodies nationwide (Schmitt and Brumbaugh, 1990; Lowe et al., 1985). Arsenic in NCBP samples have historically been the highest in bloaters (*Coregonus hoyi)*, a species of ciscoe, from a collection station along the western shore of Lake Michigan off of Sheboygan.. These values were the highest found in any of the 117 collection stations nationwide. Tissue concentrations of arsenic in lake trout were 1/3 the levels in bloaters at this station. In 1984 at the western Lake Michigan collection site, arsenic tissue concentrations in bloaters and lake trout were 11x and 3.6x the national mean. Actual 1984 tissue concentrations were 1.45 for bloaters and 0.50 mg/kg (wet wt.) for lake trout. Bloaters have a planktivorous diet and lake trout are primarily piscivorous. Chub are not preyed upon by lake trout to any great extent. Lake trout concentrations of arsenic may not be indicative of food chain transport through chub consumption and other uptake routes must account for the source. More recent monitoring by WDNR showed whole fish arsenic levels of 1.56 mg/kg and 1.69 mg/kg (wet wts.) in two composite samples of bloater chubs collected off of Door County and concentrations of 1.5 mg/kg in a composited sampled of cisco/lake herring collected off of Milwaukee.

At the present time based on the data available, there is no reason to believe that the Kewaunee site is responsible for any significant flux of arsenic to Lake Michigan to contribute to the arsenic bioaccumualtion in the species of Lake Michigan fish collected along the western shore. The upper end levels of arsenic in smallmouth bass collected in the Kewaunee River may need further investigation.

During June of 1997, carp were observed on the impacted area of the marsh around the capped area. Their roiling and splashing activities could be heard and seen. During the course of their activities in foraging and stirring up the bottom, they may incidentally be ingesting arsenic contaminated soils and detrtus. If the carp analyzed in 1994 engaged in similar activities at some earlier time, they did not appear to be absorbing and bioaccumulating any of the ingested arsenic into their tissues. The carp may not have accessed the area, or the marsh may not have been flooded to allow access prior to the collection. During observations of the unvegetated mudflats of the impacted area of the marsh in early 1995, carcasses of large carp were noted among the waterfowl carcasses. The carp either became trapped in the area as seasonal water levels dropped or they succumbed after ingesting acutely toxic concentrations of arsenic from the water, soils, or food associated with the devegetated area of the marsh. The access route that larger fish like adult carp have to the impacted marsh that is surrounded by the chain link fence is up two slough channels that begin on the eastern edge of the marsh and connect to the river. The carp swim underneath the bottom of the fence in the channels.

Fish moving off the river and onto the impacted area of the marsh will be exposed to elevated levels of arsenic, the levels of which vary in different areas of the marsh based on the previous sampling. Looking at the ranked species mean acute values (SMAV) for As^{+3} (U.S. EPA, 1995), the three fish species (fathead minnow, brook trout, and rainbow trout) for which toxicity data is available are ranked in order based on having similar acute toxicity values. The species specific acute values and related chronic value for the fathead minnow are as follows:

Species	Species Mean Acute Value (ug/L As+3)	Species Mean Acute-Chronic Ratio	Species Mean Chronic Value (ug/L As+3)
Brook Trout (*Salvelinus Fontinalis*)	14,065	-----	-----
Fathead Minnow (*Pimephales promelas*)	14,065	4.199	3,350
Rainbow Trout (*Oncorhynchus mykiss*)	13,340	-----	-----

Assuming all the other fish species that may access the impacted marsh area are at least as sensitive as the fathead minnow, including the hatchlings and young-of-the- year, to exposure to arsenic, toxicity would likely not be exhibited based on a comparison of the above criteria with the 1997 surface water concentrations discussed above. Based on site-specific toxicity testing, some chronic toxicity may be present to fish accessing some portions of the impacted marsh.

Emergent marshes are usually dominated by small minnows such as fathead minnow, mudminnow (*Umbra* sp.), and sticklebacks (Weller, 1980). Wetlands can have naturally low dissolved oxygen levels and this will determine the fish species that can survive in the habitat. Fathead minnows have a tolerance for dissolved oxygen levels down to 0.5 mg/L and in cases can survive periods when no measured dissolved oxygen was present (Peterka, 1989). Other fish species are not as tolerant to low dissolved oxygen levels. Dissolved oxygen levels would tend to be the highest in marsh waters in the spring when the young fish would be accessing the waters for feeding and protection. As the summer proceeds, dissolved oxygen levels would tend to decrease and may reach levels that stress fish species other than fathead minnow. In these cases, these fish species may be subject to mortality or they would leave the marshes for the river. Spring use of the impacted wetland would expose the young fish to arsenic for only part of their life cycles.

Besides exposure to arsenic in the water by ingestion and uptake of the dissolved forms across the gills, fish can also be exposed to arsenic in the food they ingest. Generally, biomagnification of arsenic does not occur through the food chain and bioaccumulation is small. But the amount of metal transferred by food can be high enough to attain biologically harmful concentrations in fish (Woodward et al., 1995). Younger fish may be more susceptible than older fish because their diets consist totally of drifting benthic invertebrates and zooplankton.

Woodward et al. (1995) found that benthic organisms in the mine-contaminated river system they were studying were implicated as a dietary source of metals that may have been a chronic problem for young-of-the-year rainbow trout(*Oncorhynchus mykiss*). Arsenic, as well as cadmium, copper and lead contamination was involved at this site. Rainbow trout fed a diet of invertebrates collected from the contaminated river that had average arsenic tissue levels of 6.5 and 19 mg/kg yielded fish tissue levels of approximately 0.2 and 0.6 mg/kg, respectively. Because there was multiple metal uptake, the chronic health effects to the fish cannot be attributed to arsenic alone.

7.0 Effect of Arsenic Exposure On Amphibians and Reptiles

Amphibians fall into two taxonomic groups: anurans (frogs and toads) and urodeles, or tailed amphibians (salamanders). Wetlands are required for breeding purposes. Some may be somewhat terrestrial outside the breeding season but most species depend on water or moist ground for egg laying and maturation. Reptiles use the wetlands for food and cover but move to the wetland edge or to drier land to deposit their eggs.

Little data exists to establish the roles of amphibians and reptiles in the structure and function of shallow marsh and sedge meadow wetland ecosystems. Little information exist as to the effects of arsenic on the larval, juvenile, or adult life stages of amphibians and reptiles. While not a highly visible part of the wetland system, they are important food organisms for a large variety of fish, birds, and mammals and can be of major ecological significance (Nebecker et al., 1995).

Amphibians are sensitive to anthropogenic and natural stresses and because of this they are often considered valuable bioindicators of water quality and environmental perturbations. Under certain conditions, naturally occurring metals along with site pH and hardness can be toxic to certain amphibians at breeding sites (Horne et al., 1995). Amphibians have permeable eggs and skin which readily absorb materials from the water. Over the course of their life cycles, they undergo trophic level shifts that subject them to a variety of conditions and food sources (DuBois December 21, 1995 memo to Water Resources Managers). Generally, amphibian embryos are the most sensitive life stage, followed by newly hatched larvae and older larvae (Freda et al., 1990).

One available laboratory study (Birge, 1978) using narrow-mouthed toad tadpoles (*Gastrophryne carolinensis*) measured an LC_{50} of 40 ug/L for waterborne arsenic as arsenite at pH 7.4 and 22^0C with a 7 day exposure interval (from fertilization to 4 days post-hatch). Based on this one study, the U.S. Fish & Wildlife Service suggested that the current national freshwater aquatic life criterion for arsenic (and therefore the NR 105 chronic aquatic life criterion at 153 ug/L) may be underprotective. No other data from toxicity testing is available. What the intra- and inter-species sensitivity is to a similar life stages of amphibians and reptiles indigenous to the Kewaunee Marsh wetlands is unknown.

Clark et al. (1998) found that Ranid tadpoles collected from a lake and pond in Texas contaminated by releases from a manufacturing plant producing calcium arsenate had arsenic concentrations in two species of tadpoles that ranged from 1.64 - 9.52 mg/kg (wet wt.). Arsenic concentrations in tadpoles collected from the reference site ranged from 0.56 - 1.76 mg/kg. The dry weight concentration of arsenic in the tadpoles from the contaminated pond was 51.3 mg/kg compared to the sediment concentration of 420 mg/kg. In the contaminated lake, the dry weight concentration of arsenic in the tadpoles was 23.6 mg/kg compared to 81 mg/kg in the sediment. The authors indicate that even at these high arsenic body burdens, tadpoles were still living in the contaminated lake and pond. While tadpoles were observed, snakes were not. The authors thought this may indicate that snakes are more sensitive to arsenic exposures or their exposure through the food chain is greater than that of frogs. The high body burdens of arsenic of tadpoles in the study means prey organisms consuming the tadpoles like waterfowl could have relatively high intake rates of arsenic from this food source. The sediment concentrations in the above study are in a range of the concentrations in the impacted wetland soils at Kewaunee marsh.

No specific studies or surveys were carried out on Kewaunee Marsh for amphibians or reptiles. The amphibian and reptile component of the site may need a study component in the future to use these groups as bioindicators of the site conditions and potential for exposing prey species to elevated arsenic levels in their food.

8.0 Small Mammal Trapping Study

8.1 Habitats and Ecological Relationships of Small Mammals Utilizing Wetlands

A general observation can be made that while there has been a large body of research conducted on small mammals, their utilization of various types of wetland ecosystems has not received a large amount of study. The role of small mammals in the energy and nutrient dynamics of wetland ecosystems is poorly understood. Their functional roles in wetlands must be inferred from studies in other environments.

The small mammals trapped in Kewaunee Marsh and others that were not trapped but could potentially utilize the habitat are considered habitat generalists i.e. they have no special needs or unique adaptations to wetland habitats. They generally can be found in a variety of habitats. Their distribution in wetlands may be influenced by soil moisture and vegetation types along the wetland - upland continuum. Some small mammals such as meadow voles may be associated with sites having higher soil moisture. Deer mice may be found at drier sites along the edges of wetlands. Masked shrews may be found in transitional habitats intermediate in moisture (Fritzell, 1989). Because the small mammals have such small home ranges (typically 1/4 to 1 to 2 acres), estimation of exposure in areas of arsenic contamination by resident species can generally be assigned an area use factor of 100% without having to make relative assumptions of a part time area use factor as needs to be done for more mobile, larger site receptors in exposure calculations. Accounting for exposures to variable concentrations of a contaminant at a waste site by organisms with a smaller home range generally is done by averaging the concentrations over the area of exposure.

The deer mouse (seeds and fruits) and meadow vole (green vegetation but adds seeds, nuts, fungi and a few insects) are herbivores while the major food items for the masked shrew are insects, vertebrates, and centipedes. All of the species burrow in the soils which can lead to increased exposure to the contaminants from the consumption of contaminated food items and water, and the incidental ingestion of contaminated soil associated with the food and water and the burrowing activities.

As to the role of small mammals in the energy and nutrient dynamics of wetland systems, Fritzell (1989) has the following to say:

"The role of small mammals in the energy and nutrient dynamics of wetland ecosystems is poorly understood. Their relative contribution to ecosystem energetics and nutrient processing has not been measured in North American wetlands, but is probably not of great importance."

While probably not important in wetland energy and nutrient budgets (e.g. based on very small consumption of above ground and below ground plant parts), the production of small mammals can be important to the presence and abundance of other conspicuous and important secondary and tertiary consumers that forage in wetland areas or at the wetland edge such as foxes, hawks, owls, weasel, and mink. Small mammals carrying body burdens of arsenic can serve as an exposure route to these higher trophic level organisms through ingestion of the small mammals.

8.2 Methods

Small mammal populations (mice, shrews, voles) were sampled in the wetland vegetation communities within the arsenic-impacted areas of Kewaunee Marsh and comparable unimpacted wetland habitats in 1995 and 1996. The locations of the traplines for both years in the impacted and reference wetland areas are described in WDNR memos summarizing the results of the sampling. Reconnaissance was done to identify the dominant wetland vegetation types in the impacted areas of Kewaunee Marsh and to find comparable unimpacted reference site vegetational communities.

1995 Trapping, May 15-17, 1995

Reference Wetland Site

In 1995, 30 small Victor snap traps were set 30 feet apart in the selected reference area along two sections of trapline. The first section of trapline consisting of 15 traps was in a meadow community between the Kewaunee River and the entrance road/parking area to the WDNR hatchery rearing area north of CTH E. The second section of reference site trapline consisting of 15 traps was placed in the edge of a cattail marsh along the base of the bank slope CTH E.

Impacted Wetland Area

At the impact area, 20 snap traps were placed along the base of the north slope of the railroad grade consisting of a mixture of wetland vegetation types (cattail, sedge, marsh grasses, and shrub). To the north and adjacent to this trap line was the unvegetated "dead zone" or area of high arsenic concentration in the soils. Ten snap traps were placed in a second section of trapline in an impacted sedge area to the northwest of the "dead zone".

The traps in all the areas were secured with nylon twine to flagged dowels. The traps were placed in what was judged to be suitable microhabitat with overhead cover that would be used by the small mammals of interest. The traps were baited with a mixture of peanut butter, oatmeal, and ground raisins. Traps were baited early in the day and traps checked early the next morning for two days. Small mammals were removed from the traps, identified to species, placed in a labeled Zip-Loc bag, and put on ice in a cooler. The carcasses were placed in a freezer when later delivered to the Biomonitoring Laboratory. When the carcasses were being prepared for analysis, they were removed from the freezer and allowed to thaw. Upon thawing, the carcasses with the skin on were placed in a small blender and homogenized. The tissue material was transferred to a clean glass jar and placed in a refrigerator until delivery to the SLOH for arsenic analysis.

The results for the two days of trapping are shown in the table below. Over the two trapping days, a total of 10 small mammals were trapped in the reference wetland habitats and only one in the impacted wetlands.

!996 Trapping, September 9-11, 1996

Trapping in 1996 used Sherman box traps. Thirty box traps were placed in the reference wetland habitats and thirty were placed in the impacted wetlands. The areas for the placement of the reference and the impacted area traplines differed form those in 1995. The reference area traplines were placed in two different sections in cattail and sedge meadow areas south of the railroad tracks. The impacted wetland traplines were placed in two sections, one section east of the capped area in cattail and one section in the impacted sedge area northwest of the cap. Traps were again placed in what was judged to be suitable microhabitat being use by the small mammals. The same type of bait was used in the traps as 1995. Trapping was again done over two days and two nights. The trapped small mammals from the impacted wetland were handled and prepared as were the 1995 samples. Small mammals collected along the reference site trapline were released. The results of the 1996 trapping are also shown in the table below.

Reference Area	Impact Area
1995 Trapline Results	
May 16, 1995	
2 Deer Mice (*Peromyscus leucopus*)	Redback Vole (*Clethrionomys gapperi*)
2 Masked Shrews (Sorex cinereus)	
May 17. 1995	
1 Deer Mice (*Peromyscus leucopus*)	None
5 Masked Shrews (*Sorex cinereus*)	
Total Trapped - 10	1
1996 Trapline Results	
September 10, 1996	
see below	1 Masked Shrew (*Sorex cinereus*)
September 11, 1996	
see below	1 Jumping Mouse (*Zapus hudsonicus*)
Total Trapped - Counts not made, trap and release. Numbers low.	2

8.3 Results

Number of Small Mammals Collected On Traplines

For the 60 trap nights in the reference wetlands (30 traps left in place over two nights) in 1995, the trapping success was 16.6% (10 trapped mammals for 60 attempts). The success in the impacted wetlands for the same period was only 1.7% (1 trapped mammal in 60 attempts).

The number of traps used and the duration of deployment was not adequate to make any attempts at population or density estimates (number of small mammals per acre). The results from the different traplines do provide for some general comparisons based on relative numbers of mammals trapped. However, there may be explanations other than the presence of arsenic in the soils, water, and vegetation to account for these differences. It does not automatically follow that any discernible differences in relative number of mammals trapped between the reference and impact area traplines are due to the exposure of the small mammals to arsenic in the impacted area. Other explanations include:

1) The available upland and more mesic habitats adjacent to the marsh may be adequate to provide food and shelter for the existing small mammal populations, negating their need to move in any great numbers to the wetlands.

2) Given a choice, the small mammals may prefer habitats at the dryer edges of the marsh and the adjacent vegetated uplands rather than along the wetter continuum into the marsh. In 1996, both the reference areas and the impacted areas had standing water at the base of the emergent vegetation which seemed to be associated with a trend of increasing water levels in the system from year to year. Standing water may have deterred migration of small mammals into any marsh areas or made the habitat less suitable for occupancy. The trapping success along the reference area traplines in 1995 may have been due to their location in dryer areas at the upland - wetland transition.

Arsenic Body Burdens In Small Mammals

The results of the arsenic analysis in the bodies of the collected small mammals is shown in the table below. Eight of the small mammals collected along the reference traplines were combined for a single arsenic analysis. Each of the three small mammals collected along the impact area traplines in 1995 and 1996 were analyzed separately.

Whole Body Skin-On Arsenic Concentration (mg/kg-wet weight)			
Reference Wetland Traplines	**Impacted Wetland Traplines**		
Composite of 8 Small Mammals	Redback Vole 1995	Masked Shrew 1996	Jumping Mouse 1996
0.1 (Detected between 0.1 [LOD] and 0.3 [LOQ] mg/kg)	2.0	0.5	0.2 (Detected between 0.1 [LOD] and 0.3 [LOQ] mg/kg)
mg/kg-dry weight (assumes small mammal 50% water by weight)			
0.2	4.0	1.0	0.4

It appears arsenic levels are elevated in the redback vole collected in 1995 and the masked shrew collected in 1996 along the impacted area traplines possibly from exposure and ingestion of contaminated food, water, and incidental ingestion of soil. The vole is primarily herbivorous and the shrew is insectivororous. This may indicate that both the vegetation and the insects associated with the impacted area contain tissue burdens of arsenic that are being passed to the voles and shrews from intake of contaminated food items.

The redback vole collected in 1995 was taken along a section of the trap line near the base of the railroad grade and near the "dead zone" or unvegetated area of the impacted marsh. The home range of this vole may have taken it into or near this highly contaminated area which may be related to the estimated 4.0 mg/kg (dry weight) arsenic body burden in the vole. The capping of this area in early 1996 eliminated access to the soils, water, and vegetation of this zone by any wildlife. It may be that the level of 1.0 mg/kg (dry weight) of arsenic found in the masked shrew in 1996 may be more typical of the arsenic body burdens that will be found in small mammals that reside on the site around the capped area.

Since the fur of the trapped small mammals did not undergo any washing or cleaning prior to analysis, some of the measured arsenic concentration associated with the carcasses may be from arsenic associated externally with the fur. While the external arsenic is not harmful to the small mammals themselves, it may be consumed by predators of the small mammals. Also, the measured body burden in the trapped small mammals may be associated with food items or incidentally digested soil in the gut and not incorporated into any body organ. In the gut, the arsenic may not be adsorbed but eliminated from the body. Again, the arsenic may not be harmful to the small mammal if in the gut and will eventually be eliminated, but it may be ingested by upper trophic level predators who ingest the whole carcass of the small mammal.

The estimated body concentrations of arsenic from 1.0 to 4.0 mg/kg (dry weight) in the trapped mammals from the impacted area are not immediately translatable into potential health impairments. Toxic effects are normally expressed as a daily ingested dose in mg As / kg of body weight. The intake doses expressed this way are relatable to no observed adverse effect level (NOAEL) concentrations or lowest observed adverse effect level (LOAEL) based on feeding studies. The effects could be related to reduced reproduction, reduced growth, or changes in behavior that may effect survival. Toxicity values (Pascoe, et al., 1996) derived from toxicology databases expressed in mg As / kg of body wt.-day related to NOAELs and LOAELs for the site small mammals or similar species are shown in the following table. The table also contains some calculated daily doses (mg/kg-day) of arsenic the small mammals would be estimated to be ingesting from contaminated food, water, and incidental soils from a site (Pascoe, et al., 1996). The site from which the estimated levels ingested arsenic are being made is a riverine wetland contaminated by metals and arsenic from mining wastes. The level of arsenic contamination in the soils of the wetland associated with the mining wastes is less than the levels in the wetland soils in the impacted area of Kewaunee Marsh. Based on this, it can be assumed that the calculated daily dose of arsenic that the small mammals would be ingesting from the Kewaunee site would be greater than the estimated dose values in the table. However, it would appear that the estimated daily ingested dose of arsenic would need to increase by one to two orders of magnitude before toxicity effects would be evident in the small mammals. Whether these arsenic ingestion levels are reached by small mammals at the Kewaunee site is unknown. It is suspected these levels are not reached.

Receptor Species	Toxicity Values mg As / kg body wt.-day	Estimated Daily Dose From Ingested Food and Water mg As / kg body wt.-day
Vole	0.5	0.092
Mouse	2.3	0.041
Shrew	5	0.191

All values from Pascoe, G.A., R.J. Blanchet, and G. Linder. 1996. Food Chain Analysis of Exposures and Risks to Wildlife at a Metals-Contaminated Wetland. Arch. Environ. Contam. Toxicol. 30:306-318.

It appears there may be no direct toxic effect to small mammals from the site based on ingestion of arsenic. The study by Pascoe et al. (1994) conducted on a wetland impacted by arsenic-containing mining wastes concluded that the bioavailable fraction of arsenic (and the other metals of concern) was limited and was lower than originally anticipated. The findings of such low bioavailability in small mammals suggested that arsenic intake for other higher trophic organisms at the site may be similarly limited.

The consumption of the small mammals with their arsenic body burdens by predator organisms in the food chain such as mink red-tailed hawk is looked in Section 13.0 below based on the food chain modeling.

9.0 Plant Tissue Analysis For Arsenic

Herbivores or consumers of the living plant tissue in wetlands ranges from microcrustaceans (zooplankon) grazing and filtering algae in the water column, muskrats feeding on cattail rhizomes, voles and shrews consuming seeds and other plant parts, geese grazing on above ground plant parts during spring and summer, and waterfowl feeding on submerged pondweed. Also, many larvae of the species of the Chironomidae family (order Diptera) are filter feeders that build tubes on plant material or bottom sediments. Planktonic algae and detritus are their main food sources. Algae make up the majority of their diet during spring and summer when algal productivity is high (Lambert et al., 1984 as cited in Murkin, 1984). Consumption of algal containing body burdens of arsenic by the Dipteran larvae leads to bioaccumulation that in turn is passed on to higher food chain organisms. The dominant herbivore in freshwater wetlands is the muskrat.

The impacted wetland area is part of the C.D. Besadny Wildlife Area which in turn is part of a large complex of wetlands along the Kewaunee River. The impacted area's vegetation communities are shallow marsh dominated by cattail (*Typha latifolia*) and sedge meadow dominated by *Carex stricta*. The cattail community dominates in the southern and eastern portions of the site next to the river and the sedge community dominates along the northern and western portions of the site. The cattail portions of the site are fairly monotypic as are the areas of sedge meadow community. To help to determine if the dominant wetland plants are serving as possible exposure routes to herbivores consuming parts of the plants, cattail and sedge plants were sampled and analyzed for arsenic content. The above ground leaves of both plant species were sampled.

Herbivore consumers of the above ground leaves may include muskrats and voles. Whitetail deer also potentially consume these plants. However because of the chain link fence surrounding the impacted wetland, deer are effectively eliminated from accessing the area for foraging. They may access some of the low contaminated areas just beyond the perimeter of the fence. For a number of reasons including small area of low contamination involved outside the fence, large home range of the deer, and probably only limited use of cattail or sedge for forage, deer would appear to be at minimal risk from exposure from arsenic in the food base or water associated from the site. Based on this, the exposure route to deer will be considered incomplete and they will be given no further consideration in the risk assessment. Other plant species and other plant parts from the site are also utilized by other receptor species such as deer mice consuming seeds and fruits and waterfowl consuming submergent plant leaves, stems, and seeds.

9.1 Methods

The above ground portions of cattail and sedge plants consisting of leaves were sampled on September 10 and 11, 1996. The early September time period is generally the time when wetland plants have achieved their maximum above ground biomass and accrued the greatest levels of nutrients and minerals in the leaves (Bayly et al. 1972; Lindsley et al. 1977; Linde et al. 1976; and Gustafson, 1976). Assuming the arsenic is bioavailable in the wetland soils, it was believed this would also be the period of maximum accumulation of arsenic in the leaves. After the September period with the onset of leaf senescence, nutrient, mineral, and arsenic (assumed) concentrations in the leaf would generally decrease due to translocation and leaching.

The locations for sampling the leaves of cattail and sedge at the Kewaunee site were determined by the arsenic levels in soils of the impacted area based on previous sampling. Based on the results of the soil samples, cattail and sedge plant leaves were sampled from four areas that represented a range of arsenic concentrations in soil from background, to low, medium, and high levels. The arsenic concentrations in the leaves of cattail and sedge from these four areas and the arsenic concentrations in the soils of these areas are shown in the table below.

At sample sites where cattail was collected, 5 plants were collected from a 5 ft x 5 ft area. At sites where sedge was collected, 10 to 15 plants were collected within a 5 ft x 5 ft area. The sedge plants were generally growing on hummocks and the plants were taken from the crown and sides of the hummock. The plants were cut off at ground level and any outermost dead leaves were discarded. The leaves from the plants were cut up into small pieces with a scissors and composited into one sample, usually of 25 to 30 grams. The sample was placed in a glass jar and put on ice in a cooler in the field for later delivery to the SLOH for arsenic analysis.

For the wetland soil samples taken at the plant sampling locations, 3 shovel cores were taken within 5 feet of a stake marking the site, to a depth of 8 to 10 inches. Each core was sectioned vertically into four quarters and one of the sections from each of the three cores was randomly selected, placed into one mixing pan and homogenized. The three quartered sections from each core that was not utilized were placed back into the sampling hole. Fibrous roots and coarser plant materials such as detritus and rhizomes were separated out and not included in the sample. Adhering soil was removed and placed into the mixing pan prior to discarding the plant parts.

9.2 Results

The results of the leaf analysis for arsenic on the two plant species from the four areas of arsenic concentration in the soil are shown in the table below. The results show uptake of arsenic into the leaves of both species with differences in uptake between cattail and sedge and differences in uptake between the areas of varying arsenic concentration in the soils. Dividing the arsenic concentration in the leaf (dry weight) by the concentration in the soil yields a bioavailability factor range from 0.003 to 0.089 for the medium and high soil concentration sites. Sedge had the greatest uptake. Whether this is reflective of factors in the soil that limit availability of arsenic to the plants or is related to the physiology of the species and their uptake mechanisms is not known.

Most studies of metal and arsenic toxicity to plants have been carried out on agricultural or commercial crops. Very few studies have been done on uptake and toxicity studies involving wetland and aquatic plants. Arsenic is present in most plants but little is known about its biochemical role. There is no evidence that arsenic is essential for plant growth. Concentrations of arsenic in plants grown on uncontaminated soils vary from 0.009 to 1.5 mg/kg dry wt with excessive or toxic levels in leaves being in the 5 to 20 mg/kg dry wt.range (Kabata-Pendias et al.1984). In a study (Lee et al. 1982) using the marsh plant *Cyperus esculentus* or umbrella sedge, phytotoxic symptoms were observed when the arsenic leaf concentrations were 10 mg/kg. The arsenic concentration in the soil that the plants in the study were growing in was 131 mg/kg. The bioavailability factor using the calculation above is 0.08 which is similar to the high end uptake in sedge plants from the Kewaunee site. If the leaf concentration of 10 mg/kg of arsenic from the above study is applicable to the sedge species on Kewaunee marsh, the sedge plants with 8 mg/kg in the leaves could be just below the threshold of toxicity. In a review of studies by Catallo (1993), *Typha* sp. was rated in a range of "medium" to "high" with 10 other emergent plant species for their ability to uptake or remove trace metals (didn't include arsenic) from contaminated sediments and water.

Also in the Lee et al. (1982) study, the plant leaf arsenic content for the same plant species grown in the same sediment under upland conditions was 1.45 mg/kg. The lower uptake was attributable to the oxygenated, upland conditions that caused the arsenic to become precipitated and adsorbed to soil particles, making it less available. Given the standing water and moisture conditions during the plant sampling on Kewaunee Marsh, these latter conditions generally did not exist to make the arsenic unavailable for uptake. Other factors were involved in the Kewaunee wetland to make the arsenic unavailable for uptake by plants.

The symptoms of arsenic toxicity are leaf wilting, violet coloration (increased anthocyanin), growth reduction, small leaves, necrotic, chlorotic or otherwise discolored leaves, early leaf fall, stunted root growth, either browning or death of the root meristem and suppressed development of lateral roots (Lejune et al. 1996).

Crops have differing degrees of tolerance to soil arsenic levels. Generally, the literature indicates that the arsenic soil concentrations above which plant toxicity is likely is from 20 to 50 mg/kg (Alloway, 1990; Kabata-Pendias et al. 1984). For most plants, a significant reduction in crop yields was evident at soil arsenic concentrations ranging from 25 to 85 mg/kg (Eisler, 1988). This toxicity data is generally related to agricultural crops. Plants vary considerably in their tolerance to high levels of soil arsenic. Also, total arsenic in soil is not a good predictor of water soluble arsenic or arsenic phytotoxicity when these relationships are compared among soils with widely differing characteristics. The textural class of soils has been related to plant toxicity with lighter soils such as sands and loams being more toxic than clay loams and clays at a given arsenic concentration (with toxicity being reflected in reduction in weight of the plants) (Crafts et al. 1939). Relationships have been shown between the extractable forms of arsenic and plant growth (Walsh et al. 1975).

Based on the isoconcentration map for arsenic in the top 2 ft of soils in the impacted wetland area made by STS for the Kewaunee site, the original areas of dead vegetation or devoid of vegetation were generally associated with soil arsenic concentrations of 1,000 mg/kg or greater. It is possible that under present site conditions there are areas of cattail and sedge around the perimeter of the capped area that are growing in soils that have arsenic concentrations of up to or greater than 1,000 mg/kg (see Figure 2 in Appendix). Based on the plant sampling and associated soil sampling done at the Kewaunee site reported in the following table, cattail and sedge are growing in soils with an arsenic concentration that approaches 700 mg/kg. This is over 10 times greater than the soil levels associated with plant toxicity in the literature. Based on general observations, there did not appear to be any significant differences in the general health or stem densities of the cattail and sedge growing in the high arsenic concentration soils compared to the plants growing in the lower concentration areas or the reference area. If anything, the sedge plant height and plant size may have been somewhat reduced in the high arsenic soil areas. In areas around the cap that were disturbed by cap construction in early 1996 and where arsenic soil levels are probably in the 500 to 1,000 mg/kg range, cattail began regrowing into these areas during the 1996 growing season and consisted of dense stands up to the snow fence perimeter around the cap by the 1997 growing season. In the area between the cap and the railroad which served as the initial storage area for the cap material after being dumped from the rail cars, it appeared the cattails started from seedlings that germinated in this area.

Relative As Soil Level	Plant Site Sample No.	As in Plant Tissue mg/kg-wet wt (Dry weight in Parenthesis[1])	Soil Site Sample No.	As In Soils mg/kg-dry weight
Sedge (*Carex stricta*)				
Reference Site	SE01	< 0.1 (< 0.5)	SO15	4.29
Low	SE02	0.7 (3.5)	S002	39.2
Medium	SE03	1.2 (6.0)	S001	219.1
High	SE04	1.6 (8.0)	S013	692
Cattail (*Typha latifolia*)				
Reference Site	CTO1	< 0.1 (< 0.5)	SO15	4.29
Low	CT02	< 0.1 (< 0.5)	S003	42
Medium	CT03	0.4 (2.0)	S001	219.1
High	CTO4	0.4 (2.0)	S014	685
1. Wet weight to dry weight conversion based on assuming the plant is 80% water by weight.				

It appears that the cattail and sedge species in the impacted area wetlands are tolerant genotypes that have adaptations that allow them to survive under the high arsenic concentrations in the soils. Cattail species can tolerate a wide range of ecological conditions. They have a demonstrated wide amplitude of adaptability in their physiological systems to survive in the conditions they are growing in. *Typha latifolia*, the species of cattail to be growing in the Kewaunee wetland, has been identified as the most adaptable, therefore most cosmopolitan distributed member of the genus (McNaughton, 1966; Weller, 1975; and Huenecke, 1950). The observations from the impacted area of Kewaunee Marsh would appear to indicate a very tolerant genotype of *Typha latifolia* is growing on the area. Nothing is known about the ecological adaptability of the sedge species growing in the impacted area of Kewaunee Marsh other than its ability to grow in prolonged flooded conditions. It, like the cattail species, must have some physiological adaptations that allows it to tolerate and grow in elevated arsenic soil conditions.

Species tolerant of growing in waterlogged soils with an hypoxic root environment are generally capable of maintaining uptake and translocation of N, P, K, and Ca to the shoot while limiting uptake and movement of potentially toxic nutrients such as Fe and Mn (McKee et al., 1993). This physiological feature also may allow cattails and sedges to control the uptake af arsenic from the roots to the above ground portions of the plant, Cattails have a demonstrated ability to grow and survive in areas with high metal concentrations in the soils. Since no plant community studies were done to determine species makeup and densities of other wetland plants, it is not known if other plants are able to tolerate the high arsenic soil concentrations like the cattail and sedge species, or if they are reduced or eliminated from the cattail and sedge communities growing in areas of elevated arsenic concentrations in the soil. Since the vegetational communities within the fenced area of Kewaunee Marsh are generally dominated by areas of either monotypic cattail or sedge plants, contributions of other plant species to the overall stem densities are likely small.

The number of species of emergent aquatic plants in Wisconsin marshes is typically low compared to most terrestrial communities (Curtis, 1959). This paucity of flora in individual stands is evidenced by an average species density of 11 and a species density of only three or four over large areas that would be typical of a monotypic cattail areas in southern Wisconsin marshes like areas of the impacted wetland at the Kewaunee Marsh. In the wetland continuum, sedge meadows occupy a position between the grass-forb areas on the edge of uplands and the emergent marsh community with the plant communities grading into the other where they meet. In sedge meadows, the ground surface is flooded in the spring and after heavy precipitation events but typically lies just above the water table. Periods of standing surface water over sedge meadow areas may stress many plant species in the sedge community because of disturbed oxygen conditions. This will in turn limit the number of plant species present. The sedge *Carex stricta* that is present in the impacted wetland area of Kewaunee Marsh has a growth form that results in plant groupings growing on individual hummocks or tussocks. Well developed aerenchyma cells in the roots and rhizomes allow the plant to survive periods of flooded conditions by transmitting atmosphere air to submerged plant parts.

It would appear that based on the tolerant species of cattail and sedge able to grow in the wetland areas with elevated arsenic levels and limited number of other plant species in the community types, there does not appear to be any noticeable effects at the plant community level from the arsenic. It appears the cattail and sedge species have been able to survive the high arsenic soil areas by having a mechanism that limits the uptake of arsenic into the plant tissues. The limiting of the uptake of arsenic into the plant leaves means that herbivores who use the plant leaves from the site as a

food source may not be exposed to any significant levels of arsenic through the ingestion route. The exception may be muskrats who consume the rhizomes or the underground parts of the cattail plant. Pascoe et al. (1996) found that the underground rhizomes of emergent wetland plants growing in arsenic contaminated soils accumulated much greater levels of arsenic in the rhizome tissues compared to the above ground leaves (3.6 mg/kg of arsenic in leaves versus 52 mg/kg of arsenic in the rhizomes). Since cattail rhizomes were not sampled and analyzed for arsenic from the Kewaunee site, it is not known whether this variable uptake of arsenic between above and below ground plant parts was present. Muskrats can be a major consumer of cattail at times to the point of causing complete eatouts or removal of all cattail plants over wide areas of wetland.
Given that rhizomes of cattail can dominant the food base of muskrats especially during the winter and the muskrat home range is relatively small, arsenic levels in this food source from the impacted area of Kewaunee Marsh may be important. The Pascoe et al. (1996) study concluded that even though the estimated intake rate of arsenic was high for muskrat based on their high consumption of cattail rhizomes, evidence of a viable muskrat population was also observed at the contaminated wetland under study and therefore the exposure risk was estimated to be minimal.

Evidence of old muskrat houses consisting of cattail plant parts which were used for either rearing their young or winter dens were seen in the impacted area of Kewaunee Marsh during the periods of site investigations. However, given the shallow water conditions present in Kewaunee Marsh, the habitat may not be suitable for sustaining overwintering populations of muskrats. When water depths are shallow, ice depths may become so great that all of the food source in the form of rhizomes is sealed in frost. When muskrats cannot access rhizomes underwater from their winter lodges, they chew out of their houses and leave in search of food. They generally fail to find food above the ice and succumb to the cold and predators (Mathiak, 1966). Generally, one to two pairs of muskrats could be expected to inhabit the approximately 15 acres of wetland within the fenced-in area of Kewaunee Marsh. They would move into the area in the late spring when much of the natural restocking of muskrats takes place.

In terms of exposure and ingestion of the leaves of cattail and sedge by herbivores and exposure to the levels of arsenic that were measured in the leaves, consideration needs to be given to the growth stage of the leaves. It is assumed that the early summer more tender growth of the shoots would be more digestible and more used by herbivores than later, less succulent older growth. Since the early shoot growth may not have the arsenic levels that are present in later growth from translocation from the roots and rhizomes, there may be an even lower risk in consuming the early growth.

The food chain model in Section 13.0 below looks at the estimated intake of arsenic in plants from the site by consumers.

10.0 Impacts of Arsenic on the Algal Community

Very little research has been done on the ecological role, physiology, and the taxonomy of algae in freshwater wetlands (Crumpton, 1989). Some of what is known comes from the limited knowledge of algae in the littoral zone of lakes. Limited knowledge of algae in these habitats is in contrast to the large numbers of studies conducted on planktonic algae in lakes. Algae along with vascular wetland plants are the primary producers are the link between the consumers and resources of the system. The primary producers fix carbon through photosynthesis and incorporate inorganic nutrients into organic forms. The net primary production of a system may ultimately limit secondary production.

While algae may not have a large standing biomass at any point in time, turnover rates are high resulting in significant annual production. Algal production may be important because of the potentially high nutritional value of algae.

There are basically three groups of algae in wetlands based on the habitats they occupy:

1) Phytoplankton - planktonic algae that remain suspended in open water.
2) Metaphyton - assemblages of unattached algae that are found loosely associated with substrata. Floating mats of filamentous algae are in the metaphyton group.
3) Periphytic or periphyton are attached algae that includes epiphytic algae attached to plants, and epipelic algae growing in or on sediments.

Sediments of wetlands can support an abundant epipelic community consisting of chlorophytes. cyanobacteria, and diatoms that are more diverse and distinct from the other groups.

As result of the toxicity testing on the Kewaunee Marsh waters using *Selenastrum capricornutum*, inhibition of growth was being exhibited at an arsenic concentration of 1,400 ug/L. Eisler (1988) summarized the results of toxicity studies using algae, and reported the following effect-related concentrations:

Algae Species	Arsenic Concentration	Effect
Various Species	1,700 ug/L as As+3	Toxic
	4,000 ug/L as As+3	Decomposition
	2,300 ug/L as As+3	95% to 100% kill in 2 to 4 weeks of 4 species
	75 ug/L as As +5	Decreased Growth
Scenedesmus obliquus	48 ug/L as As+5	EC-50 (14 days)
Selenastrum capricornutum	690 ug/L as As +5	EC-50 (4 days)

Moore et al. (1984) in summarizing the literature indicated that the "no effect" concentrations for As+3 and As+5 and total arsenic ranged from 0.16 to 1,000 mg/L in several algal species. The authors indicated that the inconsistency on the results reflected the differences in test conditions which emphasizes the need for the quantification of the form of arsenic in the test waters.

Anderson et al. (1980) conducted studies designed to evaluate algal arsenic uptake and algal growth in the presence of arsenic. Algal species used in the test were all common to Lake Michigan and Green Bay. Significant differences were found to exist between the algal species in arsenic accumulation. Arsenic and phosphorus share a common mode of uptake because of their chemical similarity. Under phosphorus limiting conditions, it is possible arsenic causes decreased cellular activity and growth by disturbances of basic metabolic processes through substitution of the inoperative arsenic for phosphorus. Anderson et al. (1980) discussed that alteration of phosphorus requirements caused by stress at sublethal arsenic concentrations may favor and give dominance to more resistant algal species at the expense of others since phosphorus is the element most likely to control aquatic productivity. Differential species sensitivity to stress might alter established patterns of phytoplankton dominance and succession in nature. Species diversity changes in phytoplankton could also be reflected by diversity changes in the entire food web.

How representative the results of the toxicity testing done on the green algae *Selanastrum capricornutum* when exposed to the Kewaunee Marsh surface waters are to the assemblage of algae in the groups above that might be present in Kewaunee Marsh is unknown. A number of different algae species may be present in wetlands from the families Chlorophyceae (green algae), Cyanophyceae (blue-green algae), and Bacillariophyceae (diatoms). Interspecies sensitivity to arsenic exposure is unknown.

11.0 Effect of Arsenic On Plant Decomposition and Nutrient Cycling

There are generally two basic avenues through which nutrient and energy resources of primary production are made available to heterotrophic secondary consumers (Murkin, 1989). One is by consumption of living plants by herbivores. The second is utilization of plant detritus by assorted detrivores and microbial decomposers or the dertital food chain. A lot of the trophic transfer of many trace metals in emergent wetland plant communities is mediated primarily by detrital systems rather than direct consumption of aerial plant material by insects, mammals, birds, or fish (Catallo, 1993). Plant material enters the detrital compartment throughout the growing season and with senescence at the end of the growing season. Litter decomposition involves the leaching of soluble substances like nutrients and organics from the dead material and biological decay from the oxidation of detritus by bacteria, fungi, and other consumers. Various invertebrates feed on the detritus, breaking it into small particles. The resulting feces of the invertebrates are utilized by microbial populations whose decomposition activities additionally recycle nutrient and energy to maintain system functioning. The anaerobic decomposers gasify and recycle to the atmosphere carbon, nitrogen, and sulfur. Phosphate is converted from insoluble sulfide forms to soluble forms that are available to plants and other organisms.

The dynamics of all the interactions taking place during the decomposition and mineralization process are poorly understood. The microbial decomposers are heavily preyed upon by microscopic meiobenthic organisms (i.e. sediment invertebrates passing through a 500 um sieve but retained on a 63 um sieve), chiefly nematodes, that are in turn a food source for larger macrobenthic organisms (Mitsch, 1986), fish and larval amphibians. Under conditions found in wetland soils, the total biomass of meiofauna can be extremely high and serves as a food base for higher trophic levels in the wetland system. The literature on meiofaunal system response to contaminants is not well developed for wetlands. Neither is an understanding of the interactions between and pollutional effects on microbe-meiofaunal relationships in wetlands. The microbe-meiofaunal communities are involved in a large portion of the biogeochemical and organic matter transformation related to wetland functioning and they also support higher trophic levels (Catallo, 1993). Uptake by of arsenic by meiofaunal could be an important route of arsenic transfer from the soils and contaminated detritus via microbes (bacteria, fungi) → meiofauna→macroinvertebrates→ amphibians, fish, birds, mammals.

The decomposition activities of the microbes can cause high sediment and water column oxygen demands in the wetland that leads to low oxygen levels in which only tolerant organisms can survive.

Toxicity testing using surface waters collected from impacted marsh and the luminescent bacterium *Photobacterium phosphoreum* showed only a slight but not significant effect (reduction in light transmission) only at the highest level of exposure (8,300 ug/L). At all other levels of arsenic exposure, light emission from bacteria was greater than the control indicating components in the marsh water were stimulating the bacteria.

Data on arsenic effects to upland soil biota are limited (Eisler, 1988; the papers and discussion below are largely from this source). It would follow that the known effects to wetland soil biota are similarly limited. From what is known, it would appear that soil microorganisms are capable of tolerating relatively high concentrations of arsenic. Adding arsenic to soils does not appear to influence the decomposition rate of plant tissues by soil microorganisms (Wang et al. 1984). Tolerant soil microbes can withstand concentrations up to 1,600 mg/kg. Growth and metabolism were reduced in sensitive species at 375 mg/kg and at 150 to 165 mg/kg, soils were devoid of earthworms and showed diminishing quantities of bacteria and protozoans (NRCC, 1978).

In aquatic systems, arsenic can affect the populations of nitrifying microorganisms. The nitrification process of transforming nitrogen from a reduced state, such as ammonia, to a more oxidized state, such as nitrite and nitrate, is carried out by bacteria like *Nitrosomonas*, who get energy for growth by oxidizing ammonia to nitrite. *Nitrobacter* oxidizes the nitrite to nitrate. Holm et al. (1978) found that the oxidation of nitrite to nitrate by *Nitrobacter* was affected by a concentration of 100 ug/L of arsenic as arsenate that delayed the oxidation of nitrite. A concentration of 100,000 ug/L inhibited the process. The inhibition of the *Nitrobacter* population may promote the accumulation of nitrite in the environment. Nitrites can be toxic to aquatic organisms.

12.0 Aquatic Insect Body Burdens of Arsenic

12.1 Background

Benthic invertebrates are important food sources for small mammals, fish, birds and amphibians and play an important role in trophic energy transfer and nutrient cycling. Aquatic insects who have spent the larval, pupa, nymph, and immature portions of their life cycles in or near metal and arsenic contaminated sediments and water may externally adsorb or internally assimilate the arsenic. With the exception of a small proportion of contaminants shed with larval or pupal exuviae (external skin), body burdens of contaminants are retained following emergence to the adult flying stage from the immature form (Larsson, 1984). In this fashion, the arsenic may be passed on and present in the emerged adult insect life form and as such may serve as a link for the food chain transfer of the contaminants to organisms in higher trophic levels in the aquatic and nearby terrestrial ecosystems (Steingraeber et al. 1995; Hare et al. 1991). For example, flying adults of the Dipterean (Chironomidae) family emerge from the sediments through the water column and become a significant portion of the diet of bats, swallows, redwing blackbirds, terns, and amphibians. Small mammals and some ducks and most ducklings also may ingest contaminated insects that have been associated with metal contaminated sediments. The diet of laying female dabbling ducks like mallards and blue-winged teal in the spring will consist primarily of insects and other invertebrates to satisfy protein demand for egg production. The ducklings of all species consume a diet dominated by invertebrates during early stages of development. Many species of birds time their breeding cycles to take advantage of the seasonally abundant supply of emerging insects (Fairchild et al. 1992) with their protein content. Some omnivorous bottom-feeding forage fish feeding directly on metal contaminated invertebrates and in contact and consuming contaminated sediment particles as part of sifting and feeding can have higher tissue concentrations than piscivorous fish (Hodson et al. 1984).

In 1996 and 1997 a preliminary attempt was made to collect emerging adult and immature water-associated life forms of aquatic insects by different methods from the Kewaunee site to use as biomonitors to determine what arsenic body burdens in aquatic invertebrates were potentially available to organisms higher in the food chain.

12.2 Methods

Emerging Adult Insects

Over the period from May 20-23 of 1996, floating pyramidal traps were set out on the impacted wetland area of Kewaunee Marsh and at a reference site. The traps were built on a 2 ft x 2 ft base with styrofoam strips fastened to the base to provide floatation. Wire screening over angled braces formed the above water pyramid, at the top of which was an inverted jar to capture adult insects as they emerged from the bottom and detritus and were directed to the jar by the pyramid. Use of and expected success of the floating pyramidal traps was based on Kimerle et al. (1967) and McLaughlin et al. (1990).

Over the period of deployment, no emerged adult aquatic insects were seen within the screened pyramids or capture jars of the floating traps in either the impacted area of Kewaunee Marsh or the reference site. It is believed the timing of placement of the traps may have been too early to capture any emergence. The type of habitats the traps were deployed in may also have had something to do with the lack of capture.

The types of vegetational habitats across the wetland continuum includes open water, sparse emergent, dense emergent, and wet meadow zones. The floating traps were generally deployed in the dense emergent and wet meadow habitats. The sparse emergent vegetation zone generally has been shown to be the zone that produces the most insects representing the greatest insect biomass (McLaughlin et al.1990) However, since no insects were captured in the floating traps, it is believed to be related to the timing of placement rather than the habitat they were placed in.

As an alternative to deploying the floating traps, terrestrial light traps, were deployed in September of 1996 and again in June of 1997 on Kewaunee Marsh to capture emerged flying adult aquatic insects. The terrestrial light traps had been previously used for a food chain study on the Sheboygan River to measure PCB levels in emerging insects. The traps were highly successful in capturing flying, emerged insects (Marcia Burzynski, WDNR, SER, personnel communication).

The light traps are manufactured by BioQuip Inc. (Gardena, CA). The traps consist of a 3-1/2 gallon polypropylene bucket inside of which is placed an aluminum funnel the same diameter as the bucket with the small end of the funnel near the bottom of the bucket.. A fluorescent light run off a 12 volt battery supported by a plexiglass tripod structure is mounted over the top of the bucket. The lighted traps placed in the evening attract flying insects to the traps who subsequently hit the plexiglass structure and fall into the bucket through the funnel. Aluminum foil is placed on the bottom of the bucket and is used to enclose the collected insects. To quiet the insects down prior to removing the funnel and folding up the aluminum foil with the insects in it, the funnel mouth was plugged and the traps left to sit overnight before opening.

Because of time constraints, only a limited amount of light trapping was done during the two summers on Kewaunee Marsh. Three light traps were available for use. Traps were not placed at a reference location. The three traps were set along the berm on the eastern end of the capped area in the middle of the impacted marsh area. The traps were set out for two nights in September of 1996 and one night in June of 1997. The traps were set out at about eight p.m. each evening and picked up two or three hours later. At the laboratory, the collected insects were placed in a small stainless steel blender cup, homogenized, and placed in a jar for freezing and subsequently analyzed by the SLOH.

Concurrent with the placement of the light traps for collecting emerged flying insects, submerged light traps, also manufactured by BioQuip, were placed in some of the ponds of the impacted wetland. The submerged traps were designed to capture nektonic, immature forms of insects. The submerged traps were 8 in. high and 10 in. across with 4 side ports with funnels. Chemically activated light sticks were placed in the traps to attract the insects. These traps captured only a small number of insects which did not result in a large enough tissue mass for arsenic analysis. Any future work for insect collection should attempt to additionally use a net to sweep the submerged bottom areas and underwater spaces between the emergent vegetation to collect aquatic insects.

12.3 Results

The two nights of trapping in 1996 yielded 9.5 grams wet wt. of insect tissue mass. The one night of sampling in 1997 yielded 2.5 grams of tissue mass. The tissue masses for both years were combined to yield one sample for arsenic analysis. The arsenic concentration in this composited insect sample was 1.7 mg/kg (wet wt.). Assuming the water content of the insect makes up 50-80% of the total weight of the insect, the concentration of arsenic in the insect tissue could be from 3.4 to 8.5 mg/kg on a dry weight basis.

The collected flying insects from the traps were largely made up of Dipterans (mosquitoes and midges) and Lepidopterans (moths). While the immature forms of the Dipterans collected can be assumed to be largely aquatic and therefore are potentially exposed to arsenic contaminated bottom substrates and water, the immature stages of Lepidopterans are less likely to be associated with water. Although there are more than 150 families of Lepidopterans, only two American genera of a single family of moths (Pyralididae) have immature stages known to be truly aquatic (Pennak, 1953). McLaughlin et al. (1990) collected moths from this family in their emergent traps from wetlands associated with Green Bay. It is assumed the genera may also be associated with Kewaunee Marsh. No attempts were made to identify the moths collected in the Kewaunee Marsh traps to family. If the moths collected at Kewaunee marsh did not belong to the family Pyralididae means the larvae would not have been exposed to the arsenic contaminated bottom substrates and water from the marsh and would not have elevated levels in tissue from this source.

Although the light traps were set out in the middle of the impacted marsh along the berm on the eastern end of the capped area, all the flying insects in the traps may not be associated or originate from immature forms in the contaminated wetlands around the cap and generally within the fenced area. Approximately 500 ft in a northerly and southerly direction from the eastern end of the capped area where the light traps were placed lies uncontaminated wetland areas. Flying insects originating from these areas could possibly fly the 500 ft distance and reach the light traps.

Based on some of the above factors, the arsenic levels measured in the light trapped insects may result in an underestimate of the arsenic levels in the tissues of flying insects that originate solely from the impacted marsh area. On the other hand, the arsenic levels measured may be a reflection of the actual exposure conditions some predators such as marsh wrens and redwing blackbirds would be subject to as they traverse the area and consume a mix of flying insects that originate from both the contaminated and uncontaminated portions of the marsh.

Unfortunately, there were no reference site flying insect collections done to compare the arsenic body burdens with the 1.7 mg/kg (wet wt.) level measured from the impacted marsh. For the Sheboygan River study, arsenic levels in both adult flying and larval insects collected along the river did not exceed 0.2 mg/kg (wet wt.) at either the upriver background site or the study sites. Arsenic was not a contaminant of concern in the river and levels in the sediments were generally associated with the expected urban area concentrations (Marsha Burzynski, WDNR, SER, personnel communication).

In eight western Lake Michigan drainages (4 in Wisconsin and 4 in Michigan) that contained no urban areas and largely forested and wetland cover types, USGS (1997) found that arsenic levels in caddisfly and stonefly larvae ranged from < 0.40 to 6.7 mg/kg and averaged 1.83 mg/kg (dry wt.). Arsenic concentrations in the streambed sediments from these eight drainages ranged from 3 to 29 mg/kg and averaged 15.5 mg/kg (dry wt.). The higher larvae arsenic concentrations were generally associated with the higher streambed sediment concentrations. The 15.5 mg/kg sediment value for the above sites is greater than the 4 mg/kg upstream reference site concentration in the Kewaunee River. Generally it is not expected that the arsenic levels in larvae or adult insects from the Kewaunee River reference site would be at the levels found at the USGS sites but would be comparable to the levels found at the Sheboygan River site.

Ingersoll et al. (1994) in their study of a river contaminated by arsenic and metals from mining wastes conducted laboratory bioaccumulation studies by exposing the amphipod *Hyallela azteca* for 28 days to sediment samples collected in the river. They also collected benthic invertebrates from riffle areas of the river near where the sediment samples were collected for the laboratory bioaccumulation studies. A summary of their results in shown in the following table. Sediment arsenic concentrations at the study sites are from Kemble et al. (1994).

Sample Station	**Simultaneously Extracted Arsenic Concentration In Sediment - mg/kg**	**Arsenic Concentration In *Hyallela azteca* After 28 days of Lab Exposure To Sediments - mg/kg (dry weight)**	**Arsenic Concentration In Riffle Collected Insects From River (Immature forms of Caddisflies Stoneflies, Caneflies and Horseflies) mg/kg (dry wt.)**
Reference Site	< 0.5	0.43	2.7
CF-01	202	7.4	34
CF-02	23.8	12	15
CF-03	24.8	3.8	13
CF-04	10.8	1.9	27
CF-05	2.7	1.1	3.4

Results from the Ingersoll et al. (1994) study generally shows increasing levels of arsenic in insect tissues as levels in sediment increase. Also, the arsenic levels in the site collected insects was greater than that in *Hyalella azteca* used in the laboratory bioaccumulation studies. Differences in accumulation were attributed to possible differences in spatial variation of the sediment contamination and sediment characteristics, taxonomic variation in arsenic uptake, and potential differences in routes of exposure.

Arsenic concentrations in the wetland soils at the Kewaunee site exceed the highest concentration of 200 mg/kg of arsenic in the sediments from the contaminated river sediments in the Ingersoll study. Site factors such as physical and chemical characteristics of the bottom substrates (e.g. particle size fractions, total organic carbon content, and redox potential) that determine the bioavailability of arsenic to invertebrates are likely different between the Kewaunee site and the Ingersoll study site.

13.0 Ecological Exposure Estimates And Risks To Mammal and Bird Species Utilizing the Arsenic Impacted Areas of Kewaunee Marsh

A food chain analysis estimating wetland and terrestrial species exposures to arsenic using exposure assumptions and prey, forage, and environmental media (soil and water) concentrations was performed to support the baseline ecological risk assessment at the Kewaunee Marsh site. Site collected data related to food, water, and soil arsenic concentrations were used in the food chain model to estimate exposures and uptake by receptor species. The estimated uptake of arsenic by the species utilizing the site was compared to intake levels related to toxicological effects derived from literature studies for the particular species or similar species.

13.1 Estimation of Exposures and Chemical Intakes

Estimated daily intakes were calculated by the following equation:

$$DD = \frac{(C_F \times I_F) + (C_W \times I_W) + (C_S \times I_S) \times AUF \times AB}{BW}$$

where:
DD = Estimated daily exposure dose through ingestion in mg/kg body weight per day
C_F = Arsenic concentration in mg/kg in food consumed (dry wt.)
I_F = Food ingestion rate in kg/day
C_W = Arsenic concentration in mg/l in water consumed
I_W = Water ingestion rate in L/day
C_S = Arsenic concentration in wetland soil incidentally consumed
I_S = Soil ingestion rate in kg/day
AUF = Area use factor. Expressed as a fraction and considers home range of the receptor and what portion of the range is made up by the acreage of the impacted area of Kewaunee Marsh (the marsh area within the fence.
AB = Fraction of arsenic absorbed into the body from the gut (Pascoe et al. 1996)
    Small mammals = 0.01 or 1%
    Marsh wren = 0.01 or 1% (Not from Pascoe; judged to be appropriate)
    Muskrat and mink =0.025 or 2.5%
    Deer, waterfowl, and birds = 0.10 or 10%
BW = Body weight in kg

Exposure Parameters

The food chain exposure parameters for each receptor species is shown in the table below. The main sources of information in compiling the parameter values in the following table were Pascoe et al. (1996); CH2M Hill (1998); and U.S. EPA (1993).

Food Chain Exposure Parameters

Receptor Species	Intake Rates						Body Weight (kg)	Absorption from the gut (fraction)	Area use factor
	Plants (kg/day)	Animals (kg/day)			Wetland Soils (kg/day)	Water (L/day)			
	Emergent Sedge[1]	Aquatic Invertebrates	Small Mammals	Fish					
Meadow Vole	0.012	0	0	0	0.00025	0.008	0.04	0.01	1.0
Masked Shrew[2]	0	0.009	0	0	0.000063	0.004	0.017	0.01	1.0
Muskrat	0.37	0	0	0	0.012	0.0625	1.25	0.025	1.0
Mink	0	0.03	0.025	0.09	0.0059	0.1	0.9	0.025	0.25
Mallard	0.0405	0.0065	0	0	0.00062	0.06	1.2	0.10	0.50
Canada Goose	0.0820	0.012	0	0	0.0082	0.165	3.3	0.10	0.50
Red-Tailed Hawk	0	0	0.136	0	0	0.067	1.2	0.10	0.05
Marsh Wren	0	0.011	0	0	0	0.003	0.011	0.01	1.0

1. It is assumed that all vegetation including leaves, stems, seeds, and below ground parts that will be consumed will contain arsenic at comparable levels to that measures in sedge leaves from the site or 8 mg/kg (dry wt.)
2. Some parameters based on the short-tailed shrew from U.S. EPA (1993).

The arsenic concentrations in the food that the site receptors were estimated to be consuming that were used in the exposure estimates were based on the site collections done for plants (cattail and sedge), small mammals, insects, surface waters, and soils are shown in the table below. The arsenic concentration in the water consumed by the site receptors was based on the average concentration of arsenic in seven surface waters samples collected on the site in June of 1997 around the perimeter of the capped area. The arsenic concentration in the impacted wetland soils at the site that are incidentally ingested by some of the receptors was estimated at 200 mg/kg. This is an estimated concentration in some of the soils over the area considering the concentrations in the soils next to the cap (700 to 1,000 mg/kg) and those at the perimeter fence. The receptors will be ranging over these areas and as with the water arsenic concentrations, the receptor exposures to the arsenic will average out.

Food Item	Arsenic Concentration mg/kg	Comment
Small Mammal	2.0 (wet wt.) 4.0 (dry wt.) (Assumes 50% water by wt.)	Maximum whole body concentration found in redback vole on trapline
Insects	1.7 (wet wt.) 8.5 (dry wt.) (Assumes 80% water by wt.)	Found in flying insects caught in light traps. Concentration assumed in immature and adult insects
Fish	0.5 (wet wt.) 1.67 (dry wt.) (Assumes 80% water by wt.)	Based on high end value for smallmouth bass collected in Kewaunee River. Also fillet to whole fish conversion.
Wetland Plants	1.6 (wet wt.) 8 (dry wt.) (Assumes 80% water by wt.)	Maximum concentration found in sedge leaves in soils of high arsenic concentration
Wetland Soils	200 mg/kg	Average concentration over the impacted wetland
Water	0.307 mg/L	Average concentration in 7 surface water samples collected on impacted area in 1997

The estimated daily intake of arsenic for the site receptors based on the formula and parameter values in the tables above is shown in the table below.

Receptor Species on Kewaunee Marsh	Calculated Daily Intake Dose mg As / kg body wt-day	Toxicity Values From Literature mg As / kg body wt-day (see below)
Meadow Vole	0.037 (74%)[1]	0.05
Masked Shrew	0.053 (106%)	0.05
Muskrat	0.11 (48%)	0.23
Mink	0.12 (52%)	0.23
Mallard	0.068 (5%)	1.25
Canada Goose	0.037 (3%)	1.25
Red Tailed Hawk	0.002 (0.2%)	0.91
Marsh Wren	0.086 (34%)	0.25

1. The number in parenthesis is the percentage that the estimated daily arsenic intake is of the toxicological related intake value.

13.2 Toxicological Data For Arsenic

Toxicological studies for arsenic were briefly reviewed or the studies and values cited in Pascoe et al. (1996) and CH2M Hill (1998) were used to derive values applicable to the site receptors or surrogate receptors. The toxicity values related to the species are shown in the table below. These values are also placed in the table above for comparison with the estimated daily intake of arsenic by the receptor species at the site.

Summary of Toxicity Information for Arsenic to Receptors or Surrogate Receptors

Receptor Species	**Experimental Values - mg As / kg body wt -day**			**Laboratory Species**	**Basis of Toxicity Value**	**References**
	NOAEL	**LOAEL**	**LD_{50}**			
Meadow Vole	0.05 (10x for LOAEL to NOAEL)$^{1.}$	0.5		Mouse	Reduced litter size	Schroeder & Mitchener 1971
Masked Shrew	0.05 (As above)	0.5		Mouse	As above	As above
Muskrat	2.3 (0.23)$^{2.}$			Rat	Chronic	ATSDR 1992
Mink	2.3 (0.23)$^{2.}$			Rat	Chronic	As above
Mallard	1.25			Mallard Duckling	Chronic	Whitworth et al. 1991
Canada Geese	1.25			As above	Chronic	As above
Red Tailed Hawk	9.1 (0.91)$^{2.}$			Chicken	Chronic	Eisler, 1988
Marsh Wren	2.46 (0.25)$^{2.}$			Cowbird	Chronic	Sample et al. 1996

1. An uncertainty factor of 0.1 was used to convert the LOAEL to a NOAEL value.
2. An interspecies uncertainty factor of 0.1 was used for interspecies extrapolation of the study results to the site receptor.

No interspecies uncertainty factor used in applying mallard study results to the Canada Goose. Goose assumed to be equally as sensitive as the mallard to arsenic exposure.

13.3 Results of the Food Chain Model

The food chain model results show that with the exception of the masked shrew the estimated daily intake levels of arsenic for the site receptors are below the estimated toxicological-related intake levels. It is noted that for some receptors such as the meadow vole, muskrat, and mink, the estimated daily intake is approximately 50% or more of the toxicological intake. For marsh wren it is 34% and for mallards and for Canada Geese it is less than 5%. For the insectivorous species, the masked shrew, estimated intake levels are slightly greater than the toxicological level which could possibly lead to impairments to populations of this species in the impacted marsh. Masked shrews were trapped at the reference site in 1995 but not along the traplines in the impacted marsh in either 1995 or 1996. As discussed above in Section 8.0, habitat preferences may account for these differences and not the presence of arsenic. However, the food chain model indicates that toxicity impacts may be possible.

The absorption fraction (AB) values used in the parameter table and calculations assumes only a fraction of the arsenic ingested is absorbed from the gut and incorporated into various body tissues. Pascoe et al. (1996) had calculated an absorption fraction of less than 0.1% for small mammals based on gastrointestinal absorption and elimination of arsenic in food items and ingested environmental media. Use of the AB of 1% results in a conservative estimates of absorption. The 1% AB factor was also used for the marsh wren. The AB factor was conservatively assumed to increase as body size of the receptor increased following Pascoe et al. (1996).

The area use factor is species specific and is an estimate based on the home range of the species. Smaller species like the voles and shrews could spend their entire life cycles in the area of the impacted wetland while wider ranging species like the red-tailed hawk will spend only a small portion of their time looking for food in the area of the impacted wetland. An area use factor of 0.5 or 50% use of the area was applied to waterfowl and geese. If the ducks or geese are with broods, they may spend more time on the area. In 1997 a pair of geese showed behavior indicating they might have been nesting on the capped area. However, even if the area use factor in the above calculation was changed to 1.0 for 100% usage of the area by ducks or geese, the daily arsenic intake would still be much less than the intake levels related to toxicity. However, impacts on ducklings ingesting a diet of largely invertebrates from the site may need to be looked at more closely assuming the goslings or ducklings may be more sensitive to arsenic exposures than adults of the species.

Precapping Wildlife Observation In the Impacted Wetland

Observations of approximately 35 - 40 duck and goose carcasses and bone piles on the dried out surfaces of the dead vegetation zones of the impacted marsh in April of 1995 were likely associated with the high water (highest measured level of approximately 200 mg/L) and soil (5,000 - 10,000 mg/kg) arsenic concentrations in these areas. The count of 35 - 40 carcasses was based on what was visible from the railroad tracks. More carcasses were likely present out farther. The shallow pools, mudflats, and dry areas in this dead zone were attractive to migrating waterfowl as resting and feeding areas. In these activities it is assumed the waterfowl received acute doses of arsenic from ingesting the contaminated components in these areas. With the capping of these areas of high arsenic soil concentration in the wetland in early 1996, exposure of this area to migrant and resident waterfowl, geese, and other marsh birds (rails and shorebirds) has been eliminated.

13.4 Risks Posed To Wildlife and Domestic Animals Based on Possible NR 105 Ambient Water Quality Criteria

The above food chain modeling would appear to be in concurrence that arsenic poisoning in animals is rarely seen outside of the laboratory. Although NR 105 currently does not contain a Wild and Domestic Animal Criterion (WDAC) for arsenic, the guidance in NR 105 can be used to determine a protective range of 32 - 50 ug/L. Toxicologists at both Michigan DNR and EPA - Duluth have independently calculated these same values for wildlife protection. The lower end of the range is based on protecting avian wildlife and the upper end of the range would protect mammalian wildlife (Goodman, 1995). The criteria apparently apply to total arsenic in the water. The values are based on laboratory studies where the test species are fed constant diets of set concentrations of arsenic in the food and water and uncertainty factors as high as 100 are used to account for interspecies differences that assumes other species are more sensitive to the arsenic exposure than the test species. For example, the LOAEL value determined in as a result of test using the mammal was 5 mg/l. To account for interspecies differences (10x) and for converting the LOAEL value to a NOAEL value (10x), the 5 mg/L value is divided by 100 to arrive at the 50 ug/L value. The food chain model above which uses exposures to site water concentrations greater than the range of 32 - 50 ug/L did not identify toxicity problems. Apparently the toxicity endpoints and toxic effect concentrations used in the model are different from the studies used to develop the criteria or do not have the uncertainty factors applied to them as done in criteria development. Also the food chain model takes into account an area use factor. This considers that the bird or mammal only uses the impacted area for only a portion of the time and therefore only gets some of its food and water needs from the area.

14.0 Summary of Risk Considerations and Characterizations

14.1 Human Health Risk Considerations

The focus of this report is on the risks to animals, birds, and aquatic organisms from exposures to arsenic associated with the contaminated marsh site. Human health risks are not normally dealt with in ecological risk assessments but some comments will be made at this point related to human health.

NR 105, Wis. Admin. Code, contains ambient water quality criteria based on human health concerns from ingesting arsenic contaminated water or fish taken from those waters. The ambient water quality criterion in NR 105 based on human cancer is 0.185 ug/L. This value is expected to result in the risk of no more than one additional case of cancer above background incidence rates per 100,000 people or a risk of 1 E-05. The value applies to all Great Lakes tributaries. Exposure assumptions in deriving the criterion includes consumption of drinking water, fish consumption, and recreational exposure. The human health criterion is partially based on a prediction of the bioaccumulation of arsenite from life-long ingestion of the edible tissues of fish. This is based on the conservative assumption that all the arsenic in tissues of edible fish is present as inorganic arsenite or in a form that is readily reduced to arsenite. However, the predominant form of arsenic in fish tissues is assumed to be an organoarsenical form which when consumed is excreted from the body. This has implications to both humans and wildlife and birds consuming tissues. Assuming all the arsenic in tissues is in the inorganic arsenite form may overestimate the exposure risks (Neff, 1997).

Background levels of arsenic in the Kewaunee River and Lake Michigan are in the 2 - 3 ug/L range compared to the 0.185 ug/L criterion above, which means there is a certain amount of risks involved even from exposure to background conditions.

The chain link security fence around the 15 acre perimeter of the impacted marsh effectively eliminates human access to remaining marsh areas outside of the cap that have elevated arsenic level in soils and surface waters. Access by humans to the impacted area can only be gained by scaling the fence and disregarding the posted hazardous warning signs. Chances of generation of arsine gases from the site are probably minimal because the extremely reduced conditions and low pHs needed for the gas production would not be present in the soil matrix. If the warnings signs are heeded, there are no exposure risks to humans from the wetland area within the fenced area.

There are elevated levels of arsenic in the wetland soils and surface waters just beyond the perimeter of the security fence but these are generally at low levels compared to the area within the fence. Arsenic levels in soils proximal to the outside perimeter of the fence ranged from approximately 40 to 70 mg/kg compared to 4 mg/kg at the marsh reference site. Arsenic levels in surface waters proximal to the outside of the perimeter fence ranged from approximately 25 to 180 ug/L compared to a background concentration of 3 ug/L. The wetland areas around the perimeter fence would probably only have limited usage from occasional hunters on the area. Boots and other protective gear normally worn in wet conditions would largely protect them from any dermal contacts with the low levels of arsenic in the soils and water. Surface water on the area does not serve as a drinking water source and this exposure route is not complete unless their is some incidental ingestion. It is unlikely recreational users would be accessing the wetland around the perimeter fence. Access by children to the perimeter of the fenced area is unlikely but is not out of the question.

There may be some unknown risk if the hunters harvest waterfowl that may have spent some time on the impacted area and bioaccumulated arsenic within their bodies. The same could be said for the waterfowl that leave the area and are harvested at some off-site location. No collections and analysis for arsenic were done in waterfowl utilizing the site to determine if arsenic was bioaccumulated. The same could also be said for fish that access the impacted marsh area, bioaccumulate arsenic into their tissues and then leave the site for the river and lake where they might be caught by people fishing. In the discussion in Section 6.0 above, it is noted that some smallmouth bass collected in the Kewaunee River had arsenic concentrations in their tissues that appeared to be above the range of natural variability from background exposures. There are no levels established for arsenic in either fish or waterfowl that would trigger a consumption advisory for humans. The relationship of the arsenic levels in the smallmouth bass and the impacted wetland is unknown. The World Health Organization (as cited by Neff, 1997) has established a maximum acceptable human intake of arsenic in food of 2 ug As /kg body weight/day which is equivalent to 140 ug As/day in a 70 kg person. To put this into perspective, a person who is doing subsistence fishing from the river, that is, consuming smallmouth bass for 250 days per year that have 0.5 mg As/kg in them would be ingesting 70 ug As/day or half the above cited maximum acceptable amount of 140 ug As/day (0.5 mg As/kg of fillet x 0.140 kg of fillet/day consumed by the subsistence fish consumer).

The presence of arsenic contaminated surface water on the marsh either inside or outside the perimeter of the security fence will depend on the yearly and seasonal hydrologic cycle. Surface water is not present at all times. When not present, there will be no risks from this exposure route to humans or from consumption of the fish, birds, and other aquatic life that depend on the presence of water.

Consideration of the implications of arsenic contaminated water leaving the impacted wetland and reaching the river and exceeding the NR 105 Human Cancer Criterion of 0.185 ug/L were discussed in the Janisch to Huffman memo dated July 16, 1998. In the memo, the groundwater discharge to the river is treated conceptually as a point source discharge and a process for developing effluent limitations for arsenic that would apply based on the Cancer Criterion was followed. The resulting effluent limitation that would apply to the groundwater is 7.98 mg/L (note the revision of this value from the July 16 memo). The maximum concentrations in the Kewaunee River adjacent to the site were measured by STS in early 1996 at approximately 100 ug/L. Concentrations near the site are typically low or at background. Levels in the river downstream of the site when monitored were always at background concentrations.

The GeoTrans groundwater model (STS, 1997) predicted that the groundwater concentration of arsenic at the river would not reach the 7.98 mg/L concentration until approximately 1,800 years in the future. As commented on in the July 16 memo, the reality of the predicted time frames of the model for increasing concentrations of arsenic reaching the river needs to be looked at closely. Generally, because the 7.98 mg/L arsenic concentration in groundwater will not reach the river until some future date, there are no river exposure risks to humans based on present groundwater concentrations of arsenic reaching the river. Other regulations applicable to groundwater such as NR 140 will be used for evaluating groundwater quality. There will be unacceptable risks to humans at some date in the future when groundwater concentrations at the river reach and exceed the 7.98 mg/L concentration.

Based on the slightly elevated concentrations of arsenic in the river sediments downstream from the impacted wetland (range 11-17 mg/kg versus 4 mg/kg background), arsenic has been transported off the impacted wetland into the river. It is believed that flooding events on the impacted wetland and interactions between the soils and surface waters put arsenic into solution. Subsequent flows of the flood waters back to the river transported the arsenic that was eventually deposited in the downriver sediments. The arsenic concentration in the surface water during these events is unknown. While these flooding events will still continue, the capping of the most highly contaminated arsenic soils in the wetland will likely reduce the concentrations of arsenic in any surface waters that flow back to the river. The periodic elevated levels of arsenic in the river that may occur because of these flooding events on the marsh and the resulting slight increases to arsenic in the river sediments are believed to pose minimal risks to human health.

14.2 Ecological Risk Characterizations

14.2.1 Acute and Chronic Toxicity Criteria in NR 105

The acute (339.8 ug/L) and chronic (148) ug/L) toxicity criteria in NR 105 were promulgated to protect most aquatic species in surface waters. In the July 16, 1998 memo (Janisch to Huffman), treating the contaminated groundwater flow from the site to the Kewaunee River conceptually as a point source discharge was discussed. Methods used for point source discharges were used to develop effluent limitations that would apply to the groundwater to protect aquatic life in the river. The effluent limitations developed that would apply to the groundwater where it discharges to the river based on the acute and chronic toxicity criteria are 925 ug/L and 679.6 ug/L (note the revision of the later from the July 16 memo), respectively.

The GeoTrans model (STS,1997) predicts that the concentration of 680 ug/L and 925 ug/L in the groundwater will not reach the river until 1,700 or 1,800 years in the future. However, a review of the current site situation shows that arsenic concentrations in monitoring wells approximately 900 feet to the east of the original spill site averages 650 ug/L. It has taken 56 years for these levels of arsenic to reach the well location or 16 feet/year If there are no differences in the hydraulic conductivity in the organic soils between the wells and the river as there are between the spill site and the wells, it is estimated it will take another 11 years for the 650 ug/L arsenic concentrations to reach the river. This does not address that the arsenic concentrations in the groundwater may already be elevated between the well location and the river which would shorten the time the 650 ug/L concentration in the groundwater would reach the river.

The arsenic transportation times between the above simple calculation and the GeoTrans model will need to be resolved. Assuming the above is applicable, the immediate risks that the "effluent limitations" to protect aquatic life as applied to the groundwater discharge to the river will be exceeded in the immediate future is high. It appears that within approximately a decade the risks are high that the acute toxicity criteria in the river could be exceeded. At some period after that, the chronic toxicity criteria will begin to be exceeded. If these ambient criteria in the river are exceeded would potentially have significant impacts to aquatic life in the river. The GeoTrans groundwater model needs to be reviewed for the accuracy in predicting of timing when the increasing concentrations of arsenic contaminated groundwater will reach the river.

14.2.2 Plant Community

14.2.2.1 Emergent Plant Community

Arsenic concentrations in the marsh soils greater than approximately 1,000 mg/kg (which are now under the cap) were responsible for the phytotoxicty to cattail and sedge plants and devegetation of the wetland areas originally observed. The literature indicates that for agricultural crops, arsenic levels in soils that ranges from approximately 50-80 mg/kg are responsible for phytotoxicty and significant reductions in crop yields. On the impacted areas of Kewaunee Marsh, cattail and sedge plants are growing in soils that have arsenic levels that approach 700 mg/kg and possibly higher or over 10x greater than highest documented soil-effect levels. Based on the isocontour map constructed by STS, the highest soil arsenic concentrations would be in the perimeter around the cap. The cattail and sedge plants appear to be growing and reproducing in a normal fashion in the areas around the cap and throughout the remainder of the area with elevated arsenic concentrations within the fence perimeter.

The cattail and sedge species involved appear to be very tolerant and may have physiological adaptations to grow in the extreme levels of arsenic present in the soil matrix. Analysis of leaf tissues collected from the cattail and sedge plants from areas of the impacted marsh with a range of arsenic concentrations in the soil showed some uptake into the plants, with sedge leaves bioaccumulating more than cattail leaves.. The levels were below a level of 10 mg/kg in the plant tissues that may be associated with phytotoxicity to these species.

Impacts of arsenic on other plant species of the emergent plant community is unknown as no vegetational community studies involving frequency of occurrence or stem density counts were conducted. Under natural conditions, there generally is a paucity of species in marsh vegetational communities compared to upland communities. The emergent marsh community on the impacted area is dominated by monotypic cattail and a largely monotypic sedge area. The contribution of any other species to these communities would be considered minor and any impacts or loss of these minor species is not believed to have any large population or community-level impacts to the wetland vegetation community due to the elevated level of arsenic in the soils.

For the reasons above (as discussed in more detail in Section 9.0), it would appear the emergent marsh and sedge meadow vegetational communities within the fenced area and outside the cap are at minimal risks due to the elevated levels of arsenic in the soils, surface water, and soil pore water of the site.

14.2.2.2 Algal Community

There are unknowns in relationship to the ecological role, physiology, and the taxonomy of algae in freshwater wetlands in general. These unknowns lead to some uncertainty in interpreting any data related to impairments to individual species and what these impairments mean to the overall functioning of aquatic system. Algae along with vascular plants are the primary producers in wetland systems and are the link between the consumers and resources of the system. Loss or reduction of primary producers would disrupt the nutrient and energy flows of the system. Biomass production over the growing season by algae can be high. Algae in wetlands can be associated with the water column as phytoplankton, as filamentous floating mats, and as periphyton attached to plants and the surface of the bottom soils.

Toxicity testing using surface waters collected from the impacted marsh in 1996 and the green algae *Selanastrum capricornutum* showed that the no observed adverse effect level (NOAEL) was 430 ug/L and that the lowest observed adverse effect level was 1,400 ug/l. No water concentrations of arsenic were tested between these two levels so the NOAEL may be higher and the LOAEL may be lower than the effect concentrations above. How representative the results of the toxicity testing done on *Selanastrum* sp.are to the possible assemblage of algae indigenous to the Kewaunee Marsh is unknown. Some literature values related to effects to various algal species are higher than this and some are lower (see Section 10.0 above). One study using *Selanastrum capricornutum* in exposures to As+5 had an EC-50 (effect concentration to 50% of the organisms) of 690 ug/l.

Other than the laboratory toxicity testing, no on-site studies of the algal community in or outside of the impacted marsh were conducted. Surface water sampling on the wetland around the cap in 1997 showed that arsenic concentrations in the water ranged from 86 to 810 ug/L and averaged 354 ug/L.

Site areas where the arsenic concentration in the water approaches the 810 ug/L may be at some greater degree of risk. Also site factors that at times may produce arsenic concentrations in water higher than those measured in 1997 may put the algal community at risk. Given the variable nature of arsenic concentrations in the surface water over the site, only the algae in a portion of the overall site may be impacted by the effect-related concentrations. Those that are unaffected in other portions of the site with lower arsenic water concentrations may carry on the algal trophic level functions.

The above assumes that all algae that may be potentially indigenous to the wetland are equally as sensitive to the levels of arsenic as *the Selanastrum* sp. used in the toxicity testing and those in the literature studies. If some species are more sensitive, risks to some portion of the algal community may be involved. The latter is an uncertainty and may change the low level risk characterization to some portion of the algal community to higher level of risk.

Levels of arsenic measured in the river near the site or downriver represent no to minimal risks to the phytoplankton community in the river. Loss of standing surface water over the wetland during the seasonal and yearly hydrologic cycle means loss of habitat and the subsequent demise of the algal community except in depressions and the dug ponds over the wetland from natural factors.

14.2.3 Fish Community

The Kewaunee River supports a warm water sport fishery and has seasonal runs of coho salmon and steelhead trout from Lake Michigan. A number of warmwater sport fish species make major spawning runs upstream in the spring, but likely further upstream than the arsenic impacted marsh. Only the northern pike and carp use wetland areas such as the impacted marsh for spawning. Young-of-the-year fish from a number of species may use the wetland for foraging and hiding.

Toxicity testing using fathead minnows (*Pimephales promelas*) in exposures to surface waters collected in the wetland showed significant reductions in growth to fathead minnows at 500 ug/L and reduced survival at 1,400 ug/L. Of the five species of organisms from four trophic levels that were used in the toxicity testing, fathead minnows generally showed effect levels at the lowest arsenic concentrations. The concentration at which survival is reduced (1,400 ug/L) would appear to be at a lower level than shown in toxicity data bases (14,065 ug/L) for fathead minnows based on exposure to As+3 in criteria development documents. The chronic toxicity value for fathead minnows in the criteria development documents is 3,350 ug/L (As+3). The reasons for the lower site-related effect concentrations are unknown. It is possible that natural site stressors in the marsh waters may be acting in a synergistic or other manner along with arsenic.

Based on toxicity data bases, fathead minnows are as sensitive to arsenic exposures as brook trout and rainbow trout and it is assumed to other warm water fish species in their larval, young, and adult life forms. Fathead minnows are also representative of the limited number of fish species that are found in marshes that can survive the periods of very low oxygen conditions. Other fish species who can possibly access the impacted marsh may succumb to low oxygen levels or they would may leave the area for the river where oxygen levels in the water are higher.

Again using the surface water arsenic concentrations over the impacted wetland found in 1997 that ranged from 86-810 ug/L and averaged 354 ug/L and comparing these values with the effect level values from the toxicity testing of 500 ug/L (growth reductions) and 1,400 ug/L (reductions in survival), some portion of the wetland (2 of the 6 results) exceeded the 500 ug/L effect-related concentration. Fish species in these areas would possibly experience reductions in growth. Depending on the size of the areas, fish would likely move out of the areas into adjacent areas where arsenic concentrations are below the effect levels during normal movement patterns and the effects on growth would be minimal.

Some uncertainty factors are that site factors may change the concentrations of arsenic in surface waters to levels different from those measured in 1997. It is also assumed that the greatest toxicity contribution to the measured amount of arsenic expressed on a total basis is due the As+3 form. Again depending on site factors, the portion of arsenic in the As+3 form may increase while the total concentration may not change. Under these circumstances, toxicity may not be associated with a total arsenic concentration one time and under a different set of conditions it may be, due to shifts in the proportion of As+3 in the total. An example would be testing a water sample in the laboratory where oxygenation of the sample may transform most of the arsenic to the less soluble and less toxic As+5 form. The resulting As+3 levels may be below any toxic effect concentrations. Under the natural conditions in site waters under low redox conditions, a greater portion of the same total arsenic may be in the As+3 more toxic form and be above effect levels.

In the fish collections done in the Kewaunee River in 1995, it was noted that some smallmouth bass had arsenic concentrations in the fillets above what might be the expected range of natural variability due to natural background levels. One possibility may be that the larger smallmouth bass are feeding on crayfish who can bioaccumulate elevated levels of arsenic in their exoskelton even at background levels. Another possibility is the fish being exposed to arsenic in the water and food from the impacted wetland site. It does not appear that the bioaccumulated levels in the fish potentially impair fish health. The bioaccumulated levels would be available to the consumers in the next trophic level.

Based on the arsenic concentrations in the 1997 surface water samples, fish species accessing the marsh would potentially be subject to a minimal level of risk in some portions of the impacted marsh area related to reductions in growth. The risks would not appear to have any population or community level impacts.

Loss of standing surface water over the wetland during the seasonal and yearly hydrologic cycle would mean loss of habitat for the fish except for depression area and the dug ponds. Natural factors at these sites including low dissolved oxygen levels would likely cause the death of most fish species except the fathead minnow.

The concentration of arsenic in the river water next to the site and downriver from the site would appear to represent no to minimal exposure risks to the fish.

14.2.4 Reptiles and Amphibians

Although no site specific studies or toxicity testing was done for reptiles and amphibians, the possible fate of these taxa in the wetland bears some comment. Little data exists in general to establish the roles of amphibians and reptiles in the structure functions of marsh ecosystems. Little data exists on the effects of any toxicant to the larval, juvenile, and adult life forms. They are important as a food source to a number of consumers in the wetland.

They are sensitive to anthropogenic and natural stresses and because of this, they are a good bioindicators of water quality conditions. One available study showed an LC_{50} (concentration causing mortality to 50% of the organisms) to tadpoles of a toad species of 40 ug/L of As+3. The chronic toxicity level would expected to be lower. The inter- and intra-species sensitivity of similar life stages of amphibians and reptiles indigenous to the Kewaunee Marsh to the above study results is unknown. If the concentrations of the study are applicable to all species at the site, the site concentrations of arsenic in the surface water of 86-810 ug/L and averaging 354 ug/L would mean that reptiles and amphibians over the entire impacted wetland are at considerable risk. Even if only a portion of the site concentration measured as total is in the As+3 form, the level of exposure risk would generally remain high. Loss of a number of species of amphibians and reptiles from the site would mean the loss of a food source to a number of consumers. These consumers may be able to find alternative food sources by ranging wider in their foraging activities. Ultimately the loss of a substantial portion of the amphibian and reptile populations of the marsh may lead to some trophic level impacts and overall impacts to the wetland ecosystem.

A review of field notes indicates that adult leopard frogs and other frogs were observed in ponds of the impacted marsh that contained arsenic concentrations up to 110 ug/L. The potential impact of arsenic levels in the soils and surface waters of the impacted marsh to amphibians and reptiles bears further study to confirm that effect levels in the one available study are applicable to the reptile and amphibian species and their various life stages that are indigenous to Kewaunee Marsh. Given the apparent sensitivities of amphibians and reptiles to toxicant exposures and the potential impacts given the one toxicity data point, a conservative estimate of risks to these taxa from arsenic exposure in the impacted wetland is put at moderate to high.

14.2.5 Surface Water and Benthic Macroinvertebrate Organisms

14.2.5.1 Benthic Organisms

In the toxicity testing (survival and weight endpoints) where the larvae of the midge fly *Chironomus tentans* was exposed to soils from the impacted wetland that contained a range of arsenic concentrations, survival was not affected at any of the exposure levels. The highest arsenic concentrations in the soil tested was 440 mg/kg. Results related to reductions in the weight endpoint were unclear due to confounding factors. There are some areas of the impacted wetland outside of the cap where the arsenic levels approach 1,000 to 2,000 mg/kg. It is unknown if exposure to these levels would elicit an effect to *C. tentans. C. tentans* is likely indigenous to the benthic community of Kewaunee Marsh.

The amphipod *Hyallela azteca* was also used in the toxicity testing of site soils. The control exposures generally did not meet testing criteria based on percent survival. However, in the 1996 testing survival of *H. azteca* ranged from 93 to 98% when exposed to a range of arsenic concentrations up to 220 mg/kg.

The exposure levels in the toxicity tests with no related significant effects are not consistent with a number of existing sediment quality guideline values that predict significant effects at much lower levels of exposure (17 to 70 mg/kg). For a number of reasons, including tolerance of the test species to arsenic (more applicable to *C. tentans*) and possibly factors in the soil that keep the arsenic from being bioavailable, the guideline values do not appear to apply to the particular site-specific situation.

Another species that along with the family Chironomidae make up a large proportion of the benthic community of wetlands are the Oligochaeta or aquatic worm species. Both of these species are tolerant to metal exposures and also tolerant to the range of conditions and natural environmental stressors found in wetland habitats. Other macroinvertebrates that may make up a smaller proportion of the benthic community of the impacted wetland may be more sensitive to the levels of arsenic in the soils. Due to the possible impairment or loss of some benthic macroinvertebrate species, the benthic community of the impacted marsh may have lower diversity and taxa richness. Sampling of the macroinvertebrate communities of the marsh is needed to confirm reductions or loss of taxa from the impacted wetland.

Levels of arsenic in the Kewaunee River sediments adjacent to and downstream are elevated (6-17 mg/kg versus 4 mg/kg background). These levels are generally at of slightly above the low effect levels of the existing sediment quality guidelines.

Based on the above, it is concluded that the benthic macroinvertebrate community in the impacted wetland is at a low to moderate degree of risk from exposure to the arsenic levels present in the soils and that the benthic organisms in the river sediments are subject to a low level of risk from exposure to the arsenic. It is not believed that risks to the wetland macroinvertebrates would significantly affect the structure and functioning of the system if adequate populations of the tolerant taxa are present. The tolerant macroinvertebrates may bioaccumulate arsenic which could be passed along to consumers.

Loss of standing surface water over the impacted wetland during periods of the hydrologic cycle will eliminate the benthic community except from depressional areas and the dug ponds.

14.2.5.2 Surface Water Organisms

Impacts to the fish community from exposures to arsenic are discussed above. In the 1995 whole sediment toxicity testing, no significant mortality to *Daphnia magna* was observed in the water column over soils that had up to 440 mg/kg of arsenic in the 48 hour acute test. In an acute test using *Ceriodaphnia dubia* and in a chronic test with *D. magna* exposures over soils, confounding factors did not yield useable results from the tests.

In toxicity testing using surface waters collected from the impacted wetland, *Daphnia magna* was not effected by any of the arsenic concentrations it was exposed to. The highest concentration was 8,300 ug/L. For *Ceriodaphnia dubia*, reproduction was reduced when it was exposed to 500 ug/L and survival was reduced when exposed to 2,400 ug/L. A comparison of the above effect-related concentrations with the measured concentration of arsenic in samples of surface water collected in 1997 (range of 86-810 and average of 354 ug/L) would indicate that *C. dubia* reproduction (if indigenous to the site and if not, some comparably sensitive indigenous zooplankton species) would be effected only in some portions of the marsh (the concentration of arsenic in the water at 2 of the 6 sampling site exceeds the 500 ug/L effect level).

A comparison of the surface water concentrations at the site and the ambient acute (339.8 ug/L) and chronic (152.2) water quality criteria in NR 105 indicates the acute criteria is exceeded at 2 of the 6 sites and the chronic criteria is exceeded at 4 of the 6 sample sites. The criteria apply to the As+3 form and assumes that all the measured arsenic concentrations in the site surface waters are also in the As+3 form. In the surface waters of the marsh the As+3 and As+5 form likely co-occur with the forms interconverting to the other depending on the environmental factors. Based on the toxicity testing results and the criteria, it is estimated that approximately 25% of the total measured arsenic concentration is in the As+3 or more toxic form. Based on this, a rough estimate of the total arsenic concentration at which acute and chronic toxicity would be demonstrated are 1,400 and 600 ug/L, respectively. Compared to the measured concentrations in site waters, only 1 of the 6 sample sites exceeds the above adjusted chronic value and no site concentrations exceed the acute value. If the As+3 proportion of the total arsenic concentration was 50%, the acute and toxic criteria would for total arsenic would be 680 and 300 ug/L, respectively. At these levels, 1 of 6 site concentrations exceeds the acute criteria and 3 of the 6 site concentrations exceeds the chronic criteria.

Given the above considerations it is concluded that the risks to water column organisms from exposures to arsenic concentrations are at a low risk level. When criteria are exceeded it is only on some portion of the impacted wetland leaving the water column organisms in other areas of the to meet trophic level structure and functioning needs in the immediate area.

14.2.6 Mammals and Birds

14.2.6.1 Large mammals

The chain link security fence around the approximately 15 acres of impacted wetland effectively excludes larger mammals from accessing the contaminated soils, water, and any possible food sources on the area. This includes whitetail deer, fox, raccoons, opossums. Domestic animals such as dogs and horses that are pastured just to the west of the site will also be excluded from the area by the fence. There may be wetland areas outside but close to the perimeter of the fence that contain elevated areas of arsenic in the soil and water. Arsenic levels in some soils immediately outside the fence had arsenic levels ranging from 40 to 70 mg/kg (compared to 4 mg/kg background) and arsenic in surface waters outside the fence of 25 to 110 mg/kg (3 ug/L background). Some food sources may leave the fenced in area of the wetland and be consumed by predators outside the fence such as fox consuming voles or muskrats. It is believed that any of these exposure routes to the excluded wildlife on the outside represents only a low level of risk.

14.2.6.2 Small Mammals and Birds

A food chain model was used to estimate the daily intake of arsenic by meadow voles, masked shrews, muskrat, mink, mallard ducks, Canada geese, red tailed hawks, and marsh wrens that obtain some portion of their food and water from the site. The portion of food and water obtained from the site varies between species based on the estimated size of their foraging areas and the time they spend on the impacted wetland area. The arsenic concentrations in the food and water they consume from the site was based on consideration of site specific data were possible. For example, arsenic concentrations in the site water, soils, plants, insects, and small mammals where used for the applicable consumer species to estimate the daily arsenic intake in their diets. Toxicity values from the literature were used to compare with the estimated intake values from the food chain model to see if their might be any potential health impairments to any of the bird or small mammal species.

The food chain model showed that for the site receptors, only the estimated daily intake dose of arsenic by the masked shrew slightly exceeded the intake dose related to toxicity effects. For species like the meadow vole, muskrat, and mink, the estimated intake was approximately 50% of the toxicity level. For the marsh wren, it was 34%. For the wide ranging red-tailed hawk, the intake was less than 0.2% of the toxicity value.

NR 105 currently does not contain ambient water quality criteria for arsenic to protect wildlife and domestic animal life (WDAC). Using the procedures in NR 105 the possible criteria would be 32 - 50 ug/L of arsenic. Comparison of these concentrations with the concentrations in surface waters of the site (86 - 810 ug/L and 354 ug/L average) would indicate considerable risk to mammals and birds consuming site water.

The results of the food chain model and the possible NR 105 criteria arrive at somewhat different risk levels for wildlife and birds. The criteria have large uncertainty factors (100x) built into the development of them. It would seem the criteria values need to be validated through further testing using appropriate mammal or bird species. The food chain model takes into account an area use factor for each species which assumes the water and food is not solely obtained from the site depending on the home range of the species. The criteria would assume all the food and water is obtained from the site. This may be lead to an overestimate of the exposure risks. The latter may not fully account for the differences in risks to mammals and birds when the food chain model results and the possible criteria are compared.

Until the possible criteria can be validated in some fashion, a moderate risk level to the health of the mammal and birds consuming food and water from the site will be used. This is a risk level between the low risk from the results of the food chain modeling and high risk from the possible criteria values. Some species that obtain all or most of their food and water needs from the site could be at a greater risk than those who obtain only some portion of their needs from the site.

14.2.7 Microbial Community Responsible For Organic Matter Decomposition and Nutrient Cycling

The bacteria species used in toxicity testing showed only slight effects at the highest levels of exposure to arsenic in the surface water (2,400 ug/L). Available literature values related to the arsenic levels in soils that affect microbial life compared to the arsenic levels in the soils of the impacted wetland would appear to indicate that the risk to microbial decomposers and detrivores appears to be low to moderate. There are uncertainties asssociated with this characterization given the paucity of the effects related information. So conservatively, the risk characterization for decomposers/detrivores from exposure to arsenic in the impacted wetland is put at low to moderate.

15.0 Recommendations For the Site

1. The seasonal and yearly hydrologic regimes for the wetland should be established to determine for what portion of the year standing surface water is present over the impacted wetland, what portions of the wetland are covered, and at what depth. Relate the ground surface elevations at a number of locations around the wetland to the river staff gauge elevation mark on the railroad bridge piling in the river. Staff gauge reading from the railroad bridge should be related to the gauge and discharge volume readings from the U.S.G.S. monitoring station that is farther upstream.

2. Based on flow information and staff gauge readings for the Kewaunee River from U.S.G.S., determine the number and duration of flooding events that occur on the impacted wetland.

3. Sample arsenic concentrations at a number of locations over time as long as standing surface water is on the impacted wetland and sample the water on the flooded wetland as it is draining to the river to determine how much arsenic loading is occurring from the wetland to the river.

4. Design a study that includes sampling and analysis of surface water, sediments and environmental factors to determine what conditions are related to the presence of the As+3 and As+5 forms.

5. More closely look at the potential toxicity of arsenic to various life stages of amphibians and reptiles using appropriate toxicity tests with site water and soils either in the laboratory or in situ. Design and conduct site surveys to establish the makeup of the amphibian and reptile communities present in reference wetlands and impacted wetlands.

6. Establish the makeup of the seasonal benthic macroinvertebrate community in the wetlands at reference sites and on the impacted wetland by sampling to determine the community makeup.

Sampling could include net sweeps, aerial light funnel traps to capture emerged insects , and submerged light traps. The arsenic tissue concentrations should be measured in the collected insects.

7. Design and implement studies that look at the makeup of algal communities in the wetland and potential toxicity of arsenic to the algal species present.

8. Evaluate the feasibility of doing any additional trapping of small mammals in order to determine if exposure to arsenic levels from the site are impacting some small mammals, especially the insectivores, or if the habitats present too much of a confounding factor to clearly establish any arsenic impacts to the small mammal populations.

9. Establish the density and frequency of all plants in the emergent plant communities at a reference wetlands and in the impacted wetlands to determine if arsenic levels are impacting minor or secondary plant species in the dominant cattail and sedge communities. Possibly use root growth and germination testing to determine arsenic phytotoxicity.

10. Conduct caged fish monitoring in the river off of the impacted wetland to determine if arsenic released from the site is bioaccumulated in the caged fish. Collect crayfish for arsenic analysis in or near the wetland and at reference sites.

11. Arsenic levels in the groundwater between the STS wells monitored in 1996 (MP-1 and MP-2) and the river should be established. If arsenic concentrations are elevated in this near-river area, it may mean it may take a shorter period of time for critical concentrations related to NR 105 to reach the river than predicted. More groundwater monitoring should be conducted over a longer term period to establish the status of the movement of the arsenic contaminated plume toward the river.

12. The subsidence or decrease in height of the cap on the area due to decomposition and loss of organic material in the cap should be monitored over time. Loss of the cap could mean exposing of underlying surface waters with high levels of arsenic.

13. Design and conduct studies to resolve the differences in risk characterization between the food chain model and the possible NR 105 criteria as it relates to some of the wildlife and bird receptors.

13. Conduct further studies to characterize the extent of arsenic contamination in the Kewaunee River sediments.

14. Investigate the extent of arsenic contamination in the soils and surface waters to the southwest of the capped area on the south side of the railroad tracks.

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APPENDIX

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Figure 1. Conceptual Site Model For Exposure Routes and Trophic Level Groups and Receptors in the Kewaunee Marsh Wetland and Kewaunee River.

Primary Arsenic Source	Primary Release Mechanisms	Secondary Sources	Primary Producers	Primary Consumers	Secondary Consumers
Railroad Car Derailment	Solubilization	Water Column	Shallow Marsh and Sedge Meadow Vegetation Communities *Typha latifolia* *Carex stricta*	Herbivores Muskrat Meadow Vole Planktivores Zooplankton *Daphnia magna* *Ceriodaphnia dubia*	Insectivores Marsh Wren Masked Shrew Waterfowl - Omnivores Mallard - Adult and Ducklings Canada Geese - Adult and Goslings
	Erosion	Soil and Sediment Porewater		Fish Fathead Minnow in early life stage	Carnivores Mink Red-tailed Hawk Game Fish Smallmouth Bass
	Wetland Soil and Sediment Resuspension	Wetland Soils and River Sediments		Macroinvertebrates Omnivores, herbivores, and detrivores *Chironomus tentans* Oligochaetes or aquatic worms	Forage Fish Fathead Minnow Amphibians and Reptiles Consume Insects, worms, other animal matter, can be omnivorous Leopard Frog Eastern Painted Turtle Snapping Turtle
	Infiltration / Percolation		Algal Community of the Wetland Phytoplankton Metaphyton Periphyton	Microbial Decomposers	Carp Omnivorous

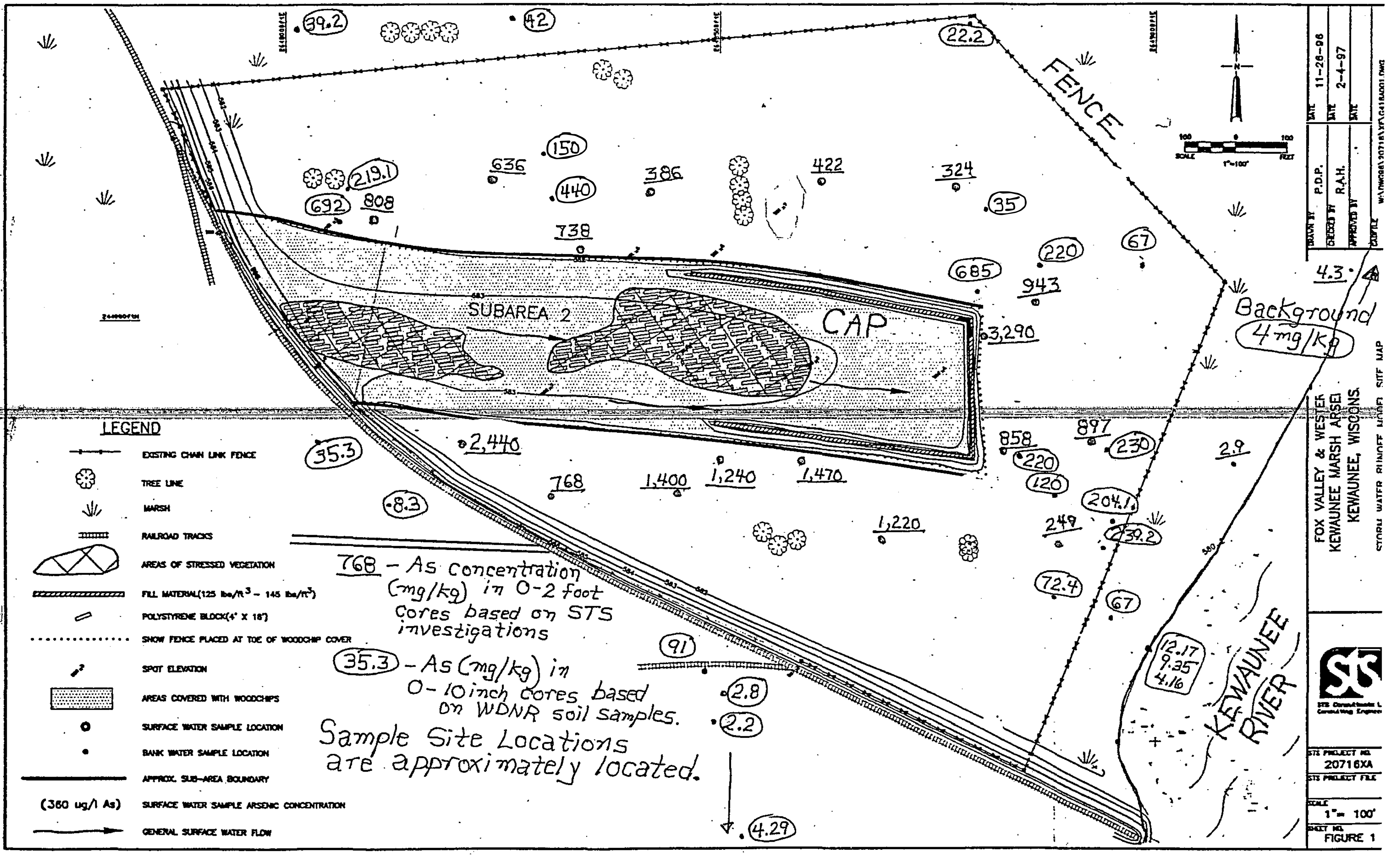

Figure 2. Arsenic Concentrations (mg/kg) in Site Soils Outside of Cap Found During Investigations By STS (0 - 2 feet) and WDNR (0 - 10 inches). Sample locations are approximate. Map Figure Adopted From STS (1997).

Figure 3. Arsenic Concentrations (ug/L) In Surface Waters Of The Impacted Wetland and Kewaunee River In Precapping and Postcapping Sampling. Sample Locations are Approximate. Map Figure Adopted From STS (1997).

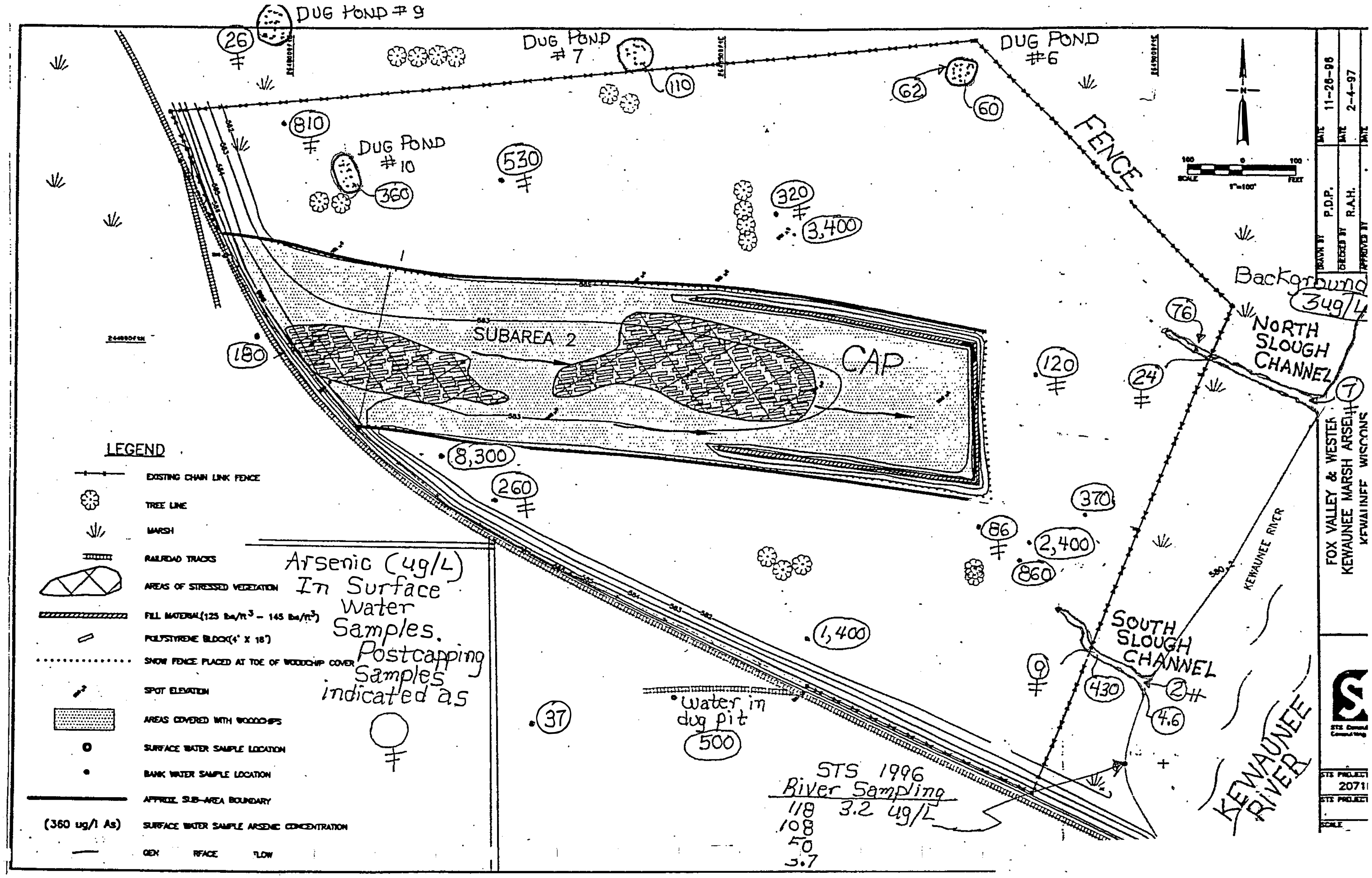

Table VEG-1. Listing of Emergent and Submergent Vegetation Observed While Transversing the Wetland Complex To the West of the Kewaunee River. Includes Wetlands to the North of the Impacted Wetland (Partial Listing).

Scientific Name	Common Name
Chara sp.	Muskgrass
Ceratophyllum demersum	Coontail
Juncus brevicaudatus	Rush
Carex stricta	Hummock Sedge
Carex lacustris	Lake Sedge
Calamagrostis canadensis	Canada Bluejoint Grass
Phalaris arundinacea	Reed Canary Grass
Phragmites australis	Giant Reed Grass
Typha latifoilia	Broad-Leaved Cattail
Iris versicolor	Blue Flag Iris
Asclepias incarnata	Marsh Milkweed
Rumex sp.	Dock
Viola sp.	Violet
Impatiens capensis	Jewelweed
Urtica dioica	Stinging Netle
Betula papyrifera	Paper Birch
Populus balsamifera	Balsam Poplar
Salix exigua	Sandbar Willow
Cornus stolonifera	Red-Osier Dogwood
Eleocharis sp.	Spike Rush
Acorus calamus	Sweet Flag
Eupatorium perfoliatum	Boneset
Aster sp.	Aster
Eupatorium maculatum	Joe-Pye Weed
Spirea alba	Meadowsweet
Solidago sp.	Goldenrod
Scirpus validus	Roundstem Bullrush
Sparganium eurycarpum	Burreed

Table Bird-1. Listing of Bird Species Observed At Reference Site Wetland and Impacted Wetland

Reference Wetland	Impacted Wetland
Canada Geese	Canada Geese
Gulls	Common Snipe
Mallard	Herring Gull
Caspian Tern	American Crow
Belted Kingfisher	Marsh Wren
Ring-Necked Pheasant	Sedge Wren
Barn Swallow	Chimney Swift
Cliff Swallow	Eastern Kingbird
Sedge Wren	Least Flycatcher
American Goldfinch	Wilson's Warbler
Yellow Warbler	Common Yellowthroat
Common Yelowthroat	American Goldfinch
Common Grackle	Bobolink
Red-Winged Blackbird	Northern Oriole
Northern Oriole	Red-Winged Blackbird
Eastern Meadowlark	Brown-Headed Cowbird
Clay-Colored Sparrow	Eastern Meadowlark
Osprey	Swamp Sparrow
Green Heron	Song Sparrow
Great Blue Heron	Mallards
Eastern Kingbird	King fisher
Sandhill Cranes	Blue Winged Teal