Mercury and Methylmercury in the South Central Kentucky Karst: its Transportation, Accumulation, and Potential Effects on Vulnerable Biota

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Abstract

Toxicity and bioaccumulation studies of mercury (Hg), the most toxic nonessential heavy metal, on karst ecosystems are virtually nonexistent. Available data suggest organisms at higher trophic levels generally biomagnify Hg at a similar rate and, once it is stored in their tissue, excrete it very slowly. Further, biota with a slow metabolism and long life span likely bioaccumulate high levels of Hg. The data presented here are studies of other nonessential heavy metals taken from extensive literature searches. Bats are vulnerable to Hg bioaccumulation because they are mobile and generally consume 40-100% of their body mass in prey each night. Bats that feed heavily on emerging aquatic insects (e.g., Trichoptera), which spend their larval stages in contaminated sediments, are particularly susceptible to biomagnification of Hg. Bats exposed to cadmium have been found with damage to their heart, kidneys, and lungs. Another study indicated cadmium concentration was higher in a troglobitic (i.e., obligate cave-dwelling) crayfish (i.e., *Orconectes australis australis*) than in a troglophilic (i.e., facultative cave-dwelling) crayfish (i.e., *Cambarus tenebrosus*). The authors also attributed significantly higher (p.05) concentration of nonessential metals in almost all *O. a. australis* tissues, relative to *C. tenebrosus*, to its increased longevity. A vigorous research program on the toxicity and bioaccumulation of Hg would enable wildlife managers to better predict the effects of future increases in Hg deposition on vulnerable biota.

Introduction

Atmospheric deposition of mercury from power plant emissions, a major input of mercury into the environment, is coming under close scrutiny by concerned agencies. With increasing demands for power applications for many new power plants, including over twenty new applications in the Commonwealth of Kentucky, are currently being considered around the country. An understanding of the current levels of mercury is critical, particularly in a karst aquifer system (such as in South-central Kentucky) where transport of contaminants can be rapid.

The proposed Thoroughbred Generating Station is a potentially large source of mercury (Hg) deposition on South Central Kentucky Karst ecosystems. Indeed, according to Peabody's own estimates absence of baseline knowledge of environmental concentrations of Hg's most toxic molecular form (that is, methylmercury) in South Central Kentucky Karst ecosystems, the author recommends a vigorous research program be initiated.will be the fourth largest Hg emitter in the state of Kentucky (Table 1). Because prevailing winds tend to blow northeast, Thoroughbred Generating Station would likely have the *second* largest impact in the state, in terms of Hg deposition, on South Central Kentucky Karst ecosystems. Currently little data are available that would enable researchers to predict the effects of such a large increase in Hg deposition on South Central Kentucky Karst ecosystems.

While research into the toxic effects of Hg bioaccumulation on organisms has increased recently, largely on commercial species, the toxic effects on ecosystems are not well understood. Further, knowledge of the toxic effects of Hg and bioaccumulation on susceptible South Central Kentucky Karst ecosystems ranges from poor (surface ecosystems) to nonexistent (subsurface ecosystems). Due to the threat of increased Hg deposition and the absence of baseline knowledge of environmental concentrations of Hg's most toxic molecular form, methylmercury, in South Central Kentucky Karst ecosystems, the author recommends a vigorous research program be initiated.

Plant Name	City	Utility Owner	Estimated Hg Emissions (Pounds)
Paradise Fossil Plant	Muhlenberg	Tennesee Valley Authority	519
Big Sandy	Lawrence	Kentucky Power Co	485
Ghent	Carroll	Kentucky Utilities Co	480
Thoroughbred Generating Station	Muhlenberg	Peabody Energy	420

Table 1. *Rank estimated output of four top mercury emitting facilities in Kentucky. Note Paradise and Thoroughbred are in close proximity. Data for top three emitters were compiled from Environmental Protections Agency and Department of Energy data by the Environmental Working Group (Coequyt* et al*. 1999). Estimated emissions by Thoroughbred Generating Station are from Thoroughbred PSD/Title V/Phase II Application, 10/25/2001.*

Transportation of Mercury and Methylmercury to Ecosystems

The abundance and distribution of pollutants in the environment, their bioavailability, and their toxicity to aquatic and terrestrial organisms are best understood in terms of molecular form (Witters 1998). Methylation is the important step that influences the ecological fate and effects of Hg. This is because all forms of Hg (for example, Hg(II), Hg^0, $(CH_3)_2Hg$) can be converted to methylmercury (MeHg) by natural processes in the environment [(Figure 1) (Keating *et al.* **1997)]. MeHg is the most toxic form of mercury, has a remarkable ability to pass through biological membranes, high chemical stability, and is excreted from most organisms very slowly (Micallef 1984, Eisler 1987, Keating** *et al.* **1997, Downs** *et al.* **1998, French 1999, Boening 2000, Mason** *et al.* 2000).

Methylation of Hg is strongly influenced by biological and chemical processes that occur in soil and water (Figure 1). An extensive literature review of the factors that affect methylation indicates Mammoth Cave National Park's riparian habitats and rivers possess most of the attributes that enhance methylation. Soil is a major reservoir for anthropogenic mercury emissions and ambient conditions determine the rate of MeHg produced in soils. For example, soil fertilization increases the availability of Hg for methylation and so waterways with high levels of anthropogenic nitrogen deposition also show increased production of MeHg (Keating *et al.* **1997, Guimarães** *et al.* **2000, Cooper and Gillespie 2001, Matilainen** *et al.* 2001). These conditions already exist in the South Central Kentucky Karst due to its receipt of nitrogen loads through long-range transport and subsequent deposition (Division for Air Quality 2001). In aquatic ecosystems, anaerobic sulfur-reducing bacteria in sediments are a major source of MeHg (Zillioux *et al.* **1993). Indeed, sediments contaminated with Hg can also serve as important reservoirs, with sediment-bound Hg recycling back into the aquatic ecosystem for decades or longer (Keating** *et al.* **1997, French** *et al.* **1999, Mason** *et al.* **2000). Mammoth Cave National Park's Green and Nolin Rivers sediment deposits increase in the impoundment zones created by Lock and Dam #6 increased, relative to non-impounded zones, due to reduced flow (Olson and Leibfreid 1999). If these deposits are already contaminated with Hg, they are likely a significant source of MeHg production in the Green and Nolin Rivers.**

Hg and MeHg input from groundwater can be relatively constant temporally and spatially (Zelewski 1999), but increased Hg concentrations and production of MeHg in streams and rivers is highly seasonal. Indeed, most Hg and MeHg input to waterways is associated with snowmelt, storm-generated runoff (bound to suspended soil/humus or dissolved organic carbon), and throughfall or rainwater that passes through a vegetation canopy (Keating *et*

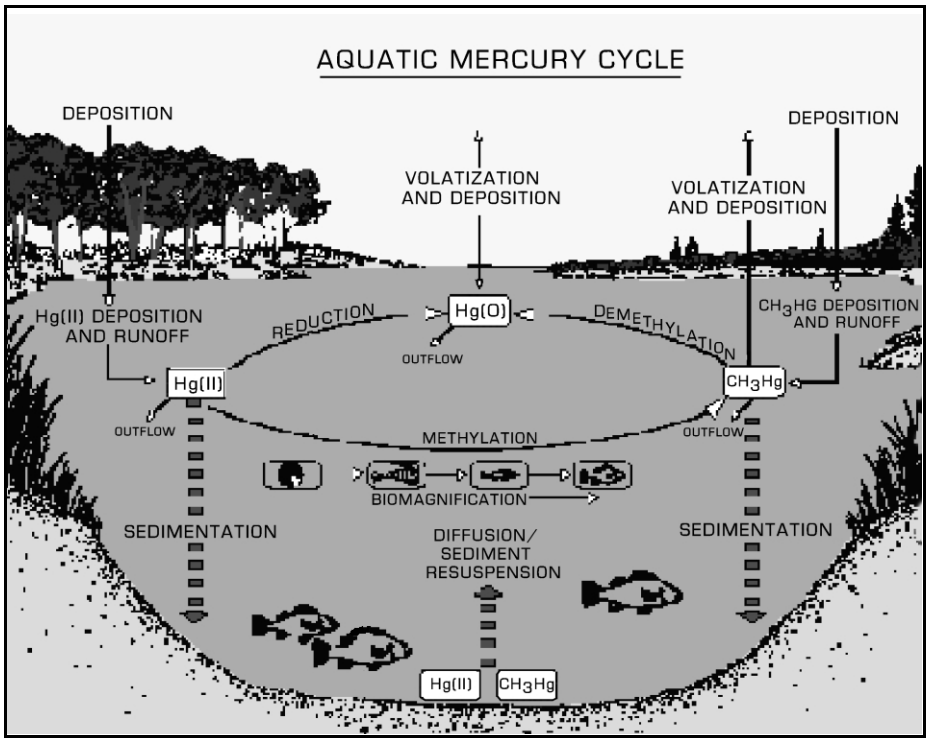

Figure 1. *The cycling of various molecular forms of Hg through an aquatic ecosystem. Deposition occurs in several ways: rainwater that passes through a vegetation canopy (throughfall, left), direct deposition (wet, right), and dry deposition. Hg also readily adsorbs onto surfaces and so aquatic organisms may also take in MeHg adsorbed to the surface of contaminated prey.*

al. **1997, Allan and Heyes 1998, Balogh and Johnson 1998, Mikac *et al.* 1999, Mason *et al.* 2000). In summer, high levels of MeHg in aquatic sediments are a result of elevated temperatures and increased activity of methylating microbes (Weber 1993, Hintelmann and Wilken 1995, Watras *et al*.** 1998, Cooper and Gillespie 2001) and so production of MeHg coincides with the most productive periods in aquatic ecosystems. Mammoth Cave National Park is heavily forested and possesses extensive riparian habitat and so likely MeHg production in aquatic habitats is also highly seasonal.

Bioaccumulation of Hg and MeHg

Hg and MeHg are bioaccumulated rapidly because organisms are exposed through multiple pathways. Bioaccumulation refers to an organism's net uptake through all possible pathways including bioconcentration and biomagnification. Bioconcentration refers to the accumulation of Hg and MeHg that occurs when an organism is in direct contact with its surrounding medium (for example, uptake from water through a fish's gills) and only accounts for a small percentage of an organism's total accumulated Hg and MeHg. However, Hg and MeHg are highly toxic and so even exposure to low levels can lead to toxic effects and death. Biomagnification is the largest contributor to the accumulation of MeHg in living tissue (Eisler 1987, Keating *et al.* **1997, Watras *et al.* 1998, Boening 2000, Mason *et al.* 2000). Biomagnification refers to increased concentration in organisms at successively higher trophic levels through ingestion of contaminated organisms at lower trophic levels.**

Exposure Pathways of Hg and MeHg in Mammoth Cave National Park's Ecosystems

A. Surface aquatic ecosystems

In aquatic ecosystems MeHg concentration generally increases with trophic level (Figure 2). Primary producers accumulate MeHg within their cytoplasm at levels several orders of magnitude higher than water (Bloom 1992, Keating *et al.* **1997, Boening 2000, Mason *et al.* 2000, Simon *et al.* 2000). Phytoplankton are ingested by zooplankton which biomagnify MeHg approximately 3-10 times that amount (Downs *et al.* 2000, Mason *et al.* 2000). Organ-**

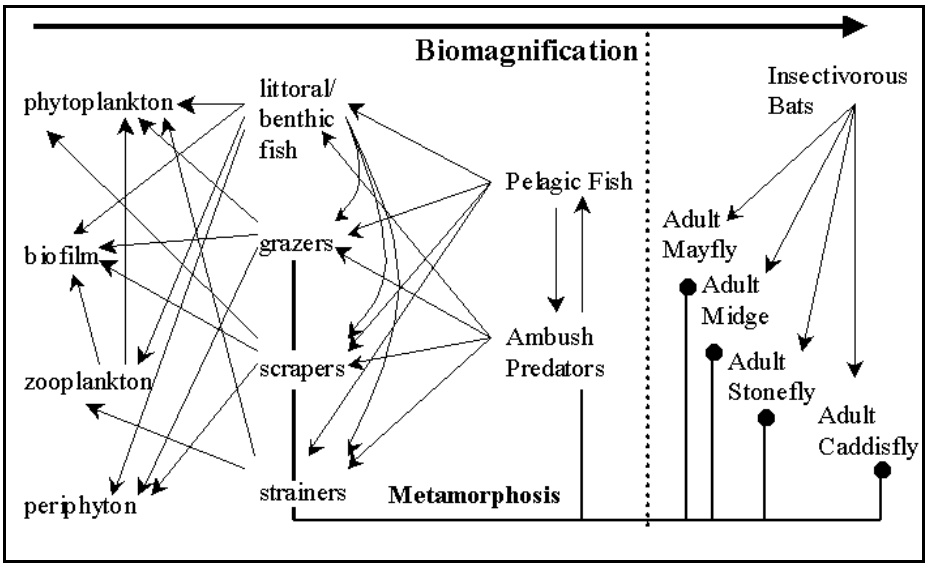

Figure 2. *Hypothesized food web for biomagnification of Hg and MeHg within aquatic community and between aquatic and terrestrial communities. Note insectivorous bats accumulate Hg and MeHg from terrestrial (i.e., adult) forms of contaminated aquatic insects.*

ismsathighertrophiclevelsgenerallybiomagnifyMeHgatasimilarrateand,onceitisstored in their muscle tissue, excrete it very slowly (Downs*et al.* 2000). Hgalsohasahighaffinity of surface adsorption and so organisms that feed on seston (that is, suspended particulate matter such as plankton) or detritus (that is., dead organic matter) can also ingest it in this manner(Keating*et al.* 1997, Ledin*et al.* 1997). Uptake of Hg can also occur through skin or gillsandisheavilyinfluencedbyaconsumer's size (that is, surface area/volume ratio) and functionalgroup(Boening2000, Downs*et al.* 2000, Canivet*et al.* 2001).

B. Subsurface aquatic ecosystems

Mammoth Cave's subsurface aquatic ecosystems are subsidized by the storm-generated influx of runoff and detritus from the surface, so transport of contaminants is most likely episodic (Meiman and Hall 1995). Storm-generated subsidies that enter through sinkholes or vertical shafts mostly acquire Hg from throughfall and detritus (Watras *et al.* **1998, Mason** *et al.* **2000). These periodic subsidies likelyformthebasisformethylatingconditions in accumulated sediments between storm events(Barr1985). Further, during upstream floods on the Green River, backflooding throughspringorificesbringsthedirectinflux of river water into the cave (Hess** *et al.* **1989) which also contributes MeHg contaminated waterandsediment.**

Subsurface aquatic organisms may accumulate MeHg in much the same manner as their epigean congeners. However, it is not known which exposure pathway determines the toxic effects of contaminants. Laboratory experiments indicated long-term exposure (10 days) to low concentrations of toxic metals (.5 milligrams/Liter) were lethal to subsurface amphipods (*Niphargus rhenorhodanensis*) and that surface and subsurface amphipods (*Gammarus fossarum* and *N. rhenorhodanensis*, respectively) bioaccumulated pollutants at the same rate (Canivet 2001). However, cave biota undoubtedly biomagnify Hg and MeHg through absorption and feeding on detritus and/or prey (Figure 3). In addition, the life history of subsurface organisms may make them particularly vulnerable to Hg and MeHg contaminated water and detritus. Indeed, due to their slow metabolism and long life span subsurface invertebrates likely bioaccumulate high levels of Hg and MeHg. Finally, invertebrate larval stages are particularly sensitive to Hg and MeHg (Boening 2000). Some subsurface invertebrates (for example, Cave crayfish, *Orconectes pellucidus*) may take years to reach maturity which increases the length of time they are most vulnerable to contaminants.

C. Surface terrestrial ecosystems

Several possible exposure pathways to Hg and MeHg exist for terrestrial organisms: ingestion of contaminated food or water, direct contact with soil, and inhalation (Keating *et al.* **1997). Themostimportantexposurepathway forterrestrialorganismsisbiomagnificationbecauseHgandMeHgcanaccumulateatincreas-**

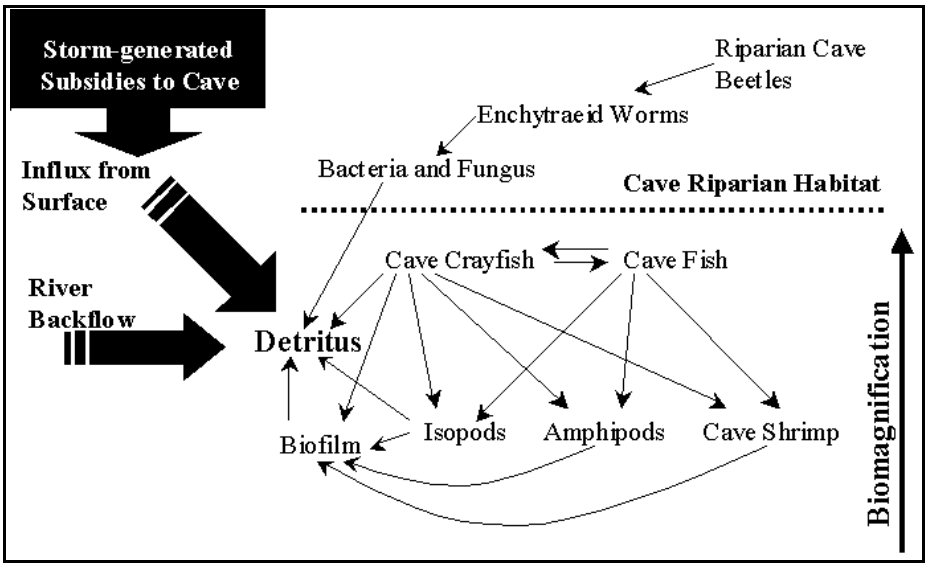

Figure 3. *Hypothesized exposure pathways for biomagnification of Hg and MeHg within cave aquatic community and between cave aquatic and cave terrestrial communities.*

ing concentrations with successively higher trophic levels. Terrestrial carnivores that consume prey from aquatic sources (for example, insectivorous bats) are particularly vulnerable to biomagnification of Hg and MeHg [(Figure 2) (Hickey *et al.* 2001)].

Potential Effects of Hg and MeHg on Biota of Special Concern in Mammoth Cave National Park

I. Surface aquatic biota

A. Mussels

Freshwater mussels readily bioaccumulate Hg and MeHg because they ingest contaminated organisms, sediment, and have direct contact with contaminated water and sediment (McMahon 1991, Hickey *et al.* **1995, Keller 1989). Transplant experiments using** *Elliptio complanata* indicated mussel growth was negatively correlated with tissue concentrations of total Hg (Beckvar *et al.* **2000). Further, exposure to Hg has been shown to disrupt gill function and may interfere with the respiratory system at every level of organization (Spicer and Weber 1991). Finally, experimental evidence suggests heavy metal contaminated water may affect Unionid mussel populations by reducing the ability of obligate parasitic larvae (glochidia) to close their valves and therefore attach themselves to host fish (Huebner and Pynnönen 1992). These results are particularly alarming because in natural populations only .0004% of released glochidia successfully encyst in fish hosts (McMahon 1991).**

Indirectly, Hg may contribute to the decline in mussel species by affecting population viability of the host fish that disperse their glochidia (Havlik and Marking 1987, Hardison and Layzer 2001). Fish are typically at the top trophic levels and so accumulate high levels of Hg and MeHg in their tissue (Downs *et al.* **1998, Boening 2000, Mason** *et al.* **2000). Indeed, concentration of MeHg in larval walleye (***Stizostedion vitreum***)** was positively correlated with MeHg concentration in eggs and so demonstrated maternal transference (Latif *et al.* **2001). Further, hatching success of walleye eggs was significantly negatively correlated with MeHg concentration in water (Latif** *et al.* **2001). Hg has also been shown to alter the processes that regulate the magnitude and specificity of fish immune response to environmental pathogens, decrease growth rate, and decrease prey capture ability (Nicoletto and Hendricks 1988, MacDougal** *et al.* **1996, Zhou** *et al.* **2001). Three known host fish (that is, largemouth bass** *Micropterus salmoides*, walleye *S. vitreum*, and bluegill *Lepomis macrochirus*) for two species of mussels federally listed as endangered in Mammoth Cave National Park rivers are known to accumulate Hg and MeHg (Pinkney *et al.* **1997, Olson and Leibfreid 1999, Gilmour** *et al.* **2000, Latif** *et al.* **2001). Finally, preliminary data from a study of the occurrence and distribution of Hg in Mammoth Cave National Park indicates** *M. salmoides* muscle tissue contained average Hg concentrations three times higher (that is, .6 milligrams per

gram or parts per million) than is recommended by the United States Environmental Protection Agency (Berryman *et al.* **2003).**

II. Subsurface aquatic biota

A. Decapod Crustaceans

Like other subsurface arthropods, the decapod crustaceans in Mammoth Cave (cave shrimp and cave crayfish) are long-lived and so likely bioaccumulate high concentrations of MeHg in their tissue over their lifetime. The author is aware of only one study that compared tissue concentrations of heavy metals between a troglophilic and a troglobitic crayfish (*Cambarus tenebrosus* and *Orconectes australis australis*, respectively); the study indicated heavy metal concentration was higher in *O. a. australis*' tissues (Dickson *et al.* **1979). The authors hypothesized that** *O. a. australis* and *C. tenebrosus* were exposed to heavy metals primarily by preying on isopods and amphipods and exposure to water (Dickson *et al.* **1979). The higher concentration of heavy metals in** *O. a. australis*' tissues, relative to *C. tenebrosus*, was attributed to its increased longevity (Dickson *et al.* **1979).**

Many contamination studies conducted on surface crustaceans rely heavily on Cambarid crayfish, a family that contains Mammoth Cave's troglobitic crayfish (*O. pellucidus*). The surface crayfish *Astacus astacus* demonstrated both biomagnification (via food intake) and bioconcentration (via gills and carapace) of Hg(II) and MeHg (Simon *et al.* **2000, Simon and Boudou 2001). Indeed, MeHg had a higher assimilation efficiency than Hg(II), and was accumulated in both the tail muscle and the green gland [(approximately 1000 nanograms/gram and 2500 nanograms/gram, respectively) (Simon** *et al.* **2000)]. Hg was also detected in the tail muscle (220 nanograms/gram) of crayfish (***O. virilis***)** inhabiting a prairie stream in Saskatchewan (Munro and Gummer 1980). Clearly, accumulation of MeHg in the crayfish tail muscle indicates biomagnification of MeHg is possible through predation and scavenging. In addition, accumulation of MeHg in the green gland may affect a crayfishes' ability to maintain fluid and solute balance. Finally, one study compared the ability of males and females in two species of crayfish (*Procambarus clarkii* and *Faxonella clypeata*) to withstand increasing concentrations of mercuric chloride to cause 50% mortality expressed in days (LC_{50} hour). The authors found significant differences between species and sexes exposed to relatively low concentrations of mercuric chloride [(Table 2) (Heit and Fingerman 1977)]. These data indicate significant variability both within and among species and so without further studies, some uncertainty exists as to the levels of bioaccumulation and toxicity of Hg and MeHg in subsurface species.

Contamination studies conducted on surface crustaceans have also utilized Palaemonid shrimp, a family that contains Mammoth Cave's troglobitic shrimp (*Palaemonias ganteri*). Palaemonid shrimp have been shown to bioaccumulate high concentrations of heavy metals in their tissue (Abdennour *et al.* 2000). In addition, of three metal salts tested for toxicity on the shrimp *Palaemon elegans*, LC_{50} levels (that is, concentrations needed to kill 50% of shrimp) for mercury were lowest relative to copper and cadmium; mercury toxicity also increased with time [(Table 3) (Lorenzon *et al.* 2000)].

Crustaceans' physiological processes (for example, molting, limb regeneration, blood glucose levels, and reproduction) are often coordinated by hormones and exposure to heavy metals can induce rapid changes in hormone levels that interfere with these processes (Fingerman *et al.* **1998). Experimental data show Hg decreased fecundity in Red Swamp Crayfish (***Procambarus clarkii***)** through inhibition of maturation in ovaries (Reddy *et al.* **1997). Freshwater prawn (***Macrobrachium kistenensis***)** exposed to Hg exhibited high variations in blood glucose

Species	Sex	LC_{50} hr (20 µg/L)	LC_{50} hr (10 µg/L)	LC_{50} hr (.2 µg/L)
P. clarkii	Male	6	24	72
	Female	48	72	—
F. clypeata	Male	48	48	72
	Female	24	72	—

Table 2. *Determination of LC_{50} hr (ability of crayfish to withstand increasing concentrations of mercuric chloride to cause 50% mortality expressed in days) for males and females in* P. clarkii *and* F. clypeata. *Note at .2* µg/L *females of both species were apparently healthy for the duration of the 30-day experiment (taken from Heit and Fingerman 1977).*

	24-hour , LC_{50} (mg/L)	48-hour, LC_{50} (mg/L)	96-hour, LC_{50} (mg/L)	n
$HgCl_2$	9.54	3.54	0.67	20
$CdCl_2$	49.77	8.91	1.46	20
$CuCl_2$	249.46	12.79	3.27	20

Table 3. *LC_{50} levels (that is, concentrations needed to kill 50% of shrimp) in* P. elegans *of both sexes. Hg was the most toxic metal in the 96-hour assay, followed by Cd and Cu. The order of toxicity did not change during the experiments (taken from Lorenzon et al. 2000).*

which indicated a stress response (Lorenzon *et al.* **2000). Finally, Hg has also been found to inhibit limb regeneration and molting in the horseshoe crab [(***Limulus polyphemus***)** (Itow *et al.* **1998)]. Undoubtedly the potential exists for deleterious effects on subsurface crustaceans due to bioaccumulation of Hg and MeHg.**

III. Terrestrial Biota

A. Indiana and Gray Bats

Bats are vulnerable to Hg and MeHg bioaccumulation because they are small, mobile, long-lived, and generally consume 40-100% of their body mass in prey each night (Hickey and Fenton 1996). In addition, bats are also exposed to contaminants through the placenta, nursing, breathing, and drinking water (Keating *et al.* **1997, Straube 1998, Clark and Shore 2001). Insectivorous bats that feed heavily on emerging aquatic insects (for example, Trichoptera), which spend their larval stages in contaminated sediments, are particularly susceptible to biomagnification of Hg and MeHg (Miura 1978, Massa and Grippo 1999, O'Shea** *et al.* **2001). This is worrisome because two insectivorous bats in Mammoth Cave National Park are federally listed endangered species (that is,** *Myotis grisescens* and *M. sodalis*). Non point-source contamination was responsible for Hg levels in bat hair (that is, *M. lucifugus*, *M. septentrionalis*, *M. leibii*, and *Eptesicus fuscus*) that exceeded the threshold (that is, 10 milligrams/kilogram) at which deleterious effects are detected in humans (Hickey *et al.* **2001). High levels of Hg have also been found in guano deposits beneath** *M. grisescens* colonies (Ryan *et al.* **1992).**

The toxic effects of Hg and MeHg on bats are not well researched. However, bats exposed to other nonessential heavy metals (for example, cadmium) have been found with damage to their heart, kidneys, and lungs (Clark and Shore 2001). In addition, exposure to heavy metals has been associated with reproductive failure, neurological disorders, and death in bats (Clark and Shore 2001). Heavy metals may also indirectly affect insectivorous bats by affecting their prey's behavior, prey populations, and composition of prey communities (Winner *et al.* **1980, Cain** *et al.* **1992, Kiffney and Clements 1993, Beltman** *et al.* **1999, Groenendijk** *et al.* **1999). Given the high toxicity of Hg and MeHg relative to well-studied heavy metals, the sensitivity of small carnivorous mammals (for example, minks) to Hg, and the relative paucity of toxicological data on Hg and MeHg with respect to bats, a research and monitoring program must be initiated (Keating** *et al.* **1997).**

Conclusion

The data presented in this briefing paper are based on an extensive literature search and represent the best available knowledge on Hg and MeHg bioaccumulation and toxicity in aquatic and terrestrial biota. All studies indicated long-term exposure to Hg and MeHg produces deleterious effects and even death in affected organisms. Most of the cave and karst taxa discussed in this briefing paper are long-lived and so particularly vulnerable to the deleterious effects produced by long-term exposure to Hg and MeHg. However, data on Hg and MeHg bioaccumulation and toxicity for taxa of special concern are either sparse (for example, bats and mussels) or practically nonexistent (for example, decapod crustaceans). Consequently, baseline data are required on Hg and MeHg levels in cave and karst surface and subsurface ecosystems. Further, long-term monitoring of Hg and MeHg levels in cave and karst surface and subsurface species that may be affected. In addition, results were highly variable in those bioaccumulation and toxicity studies that examined species comparable to cave and karst species of concern. Therefore, Hg and MeHg bioaccumulation and toxicity studies must be performed on cave and karst ecosystems to determine which species are affected. Thus, valuable resources and mitigation efforts will not be "wasted" on cave and karst species less or unaffected by Hg and MeHg, if any.

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