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Toxicity of Diisopropyl Methylphosphonate (DIMP) to Aquatic Organisms

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Diisopropyl methylphosphonate (DIMP) is a by-product of the manufacture and detoxification of the nerve gas isopropyl methylphosphonofluoridate (GB or Sarin). It was first introduced into the environment at Rocky Mountain Arsenal (RMA), Colorado, as a consequence of the storage of effluents generated from the production and stockpiling of GB during the mid 1950s. Detoxification and destruction of these stockpiles began in 1973 and was extensive for at least a decade, generating widespread concern over the presence of substantial quantities of DIMP in the alluvial aquifer underlying RMA. Environmental fate studies have shown that DIMP is highly stable, not readily photolyzed or biodegraded, and resistant to volatilization (Gordon and Hartley 1992).

In 1995, DIMP contamination of subsurface soils and surficial groundwater was found at the Edgewood Area of Aberdeen Proving Ground (APG), Maryland (Jacobs Engineering Group Inc. 1995). The source at APG was a former wastewater discharge from a building which had been operated as a pilot production, storage, and destruction facility for GB from 1952 to 1978. A plume of DIMP is currently migrating in a surficial aquifer from the site toward Kings Creek, a tributary of the Bush River, Maryland. As a result, the U.S. Environmental Protection Agency recommended that the toxicity of DIMP to aquatic organisms be evaluated. A review of the literature showed that acute toxicity data were available for several aquatic organisms; however, no data for chronic toxicity were available. The current study was initiated to confirm the acute toxicity of DIMP reported in the literature, to provide chronic toxicity, and to conduct a preliminary risk assessment for aquatic organisms.

MATERIALS AND METHODS

DIMP (CAS No. 1445-75-6), which was >99% pure, was obtained from the U.S. Army Center for Environmental Health Research, Fort Detrick, Maryland. Stock solutions prepared in distilled water were quantified by GPL Laboratories, LLLP, Gaithersburg, Maryland. Nominal test concentrations were prepared from the stock solutions and corrected based on the measured concentrations of the stock solutions.

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The acute and chronic toxicity of DIMP was evaluated using an array of four bioassays which included a number of endpoints. The following toxicity tests were conducted: 96-h green algal (*Selenastrum capricornutum*) growth test, 7-d cladoceran (*Ceriodaphnia dubia*) survival and reproduction test, 7-d larval fathead minnow (*Pimephales promelas*) survival and growth test, and 96-h frog embryo (*Xenopus laevis*) teratogenesis assay.

DIMP toxicity to the green alga (*S. capricornutum*) was determined by the EPA short-term chronic toxicity procedure given in Lewis et al. (1994). Stock algal cultures were reared in 2.5 L Pyrex culture flasks containing 1 L of sterilized algal assay medium. The stocks were maintained at 25°C on a shaker table oscillating at 100 rpm under constant light (3,200 lux at the surface of the test vessels). Log growth cells were used to start the test. Triplicate algal test solutions (100mL) prepared by addition of DIMP stocks were dispensed into 250 mL Delong flasks and inoculated with *S. capricornutum* cells to achieve a density of approximately 10^4 cells/mL. The flasks were incubated on a shaker table under the culturing conditions described above for 96 h. Growth measurements (algal cell densities) were determined from 1 mL aliquots with a Model ZBI Coulter counter (Coulter Electronics, Inc., Hialeah, Florida). The instrument was calibrated with each use via hemocytometer counts.

The chronic toxicity of DIMP to the cladoceran *C. dubia* was determined by the EPA 24-h static renewal method given in Lewis et al. (1994). The cladoceran was cultured at 25 ±1 °C in 600 mL glass beakers filled with 400 mL of 20% Perrier:80% reverse osmosis water. All neonates used in the 7-d survival and reproduction test were <4 h at the start of the test. The test was conducted in 50 mL beakers containing 25 mL of test solution at 25 ±1 °C under a 16-h light:8-h dark photoperiod (650-915 lux at the surface of the test vessels).

The chronic toxicity of DIMP to fathead minnow (*P. promelas*) was determined by the EPA static renewal method given in Lewis et al. (1994). All larvae used in the 7-d test were <24 h old at the start of the test. The exposures were conducted in 600 mL glass beakers containing 400 mL of test solution. The dilution water was a 20% Perrier:80% reverse osmosis water. All test organisms were fed brine shrimp (*Artemia* sp.) nauplii <24 h old daily at each 24-h renewal. The test was conducted at 25 ± 1°C under a 16-h light:8-h dark photoperiod (650-915 lux at the surface of the test vessels). Dry weight was determined by drying at 100°C for a minimum of 12 h.

Developmental toxicity was determined using the 96-h frog embryo teratogenesis assay - *Xenopus* (FETAX). The assay was conducted using the 24-h static renewal test method Designation E 1439-91 of the American Society for Testing and Materials (ASTM 1998). Embryo lethality (96-h LC50) and malformations (96-h EC50) were evaluated; growth retardation was not evaluated. The identification and interpretation of malformations in the embryos at 96 h were made via the atlas of

Bantle et al. (1998). Embryos between normal stage 8 blastulae and normal stage 11 gastrulae were obtained from an in-house *X. laevis* breeding colony. The embryos were de-jellied in a 2% L-cysteine solution (2 g of L-cysteine per 98 mL of FETAX solution). Once de-jellied, the embryos were rinsed and re-suspended in FETAX solution (ASTM, 1998). The embryos were tested in glass petri dishes containing 10 mL of solution. Two replicates of 25 embryos/replicate were used for each test treatment. The test was conducted at 24 ± 0.2°C under a 16-h light: 8-h dark photoperiod (~810 lux at the surface of the test medium) in a constant temperature environmental chamber.

The 96-h EC50 for algal growth was estimated by the probit statistic (Stephan 1978). The 48-h and 7-d LC50s with cladocerans, 96-h and 7-d LC50s with fathead minnows, and 96-h LC50 and 96-h EC50 for embryo survival and malformations in the FETAX assay were determined by the Trimmed Spearman-Karber method (U.S. EPA 1993). The no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) for green algal growth were determined by Dunnett's test. The NOAEL and LOAEL for the adult cladoceran raw survival data assay were estimated by Fisher's Exact test. An arc-sine square root transformation was made on the fathead minnow percent survival raw data before further data analyses were performed to estimate the NOAEL and LOAEL. The raw data for cladoceran neonate production and fathead minnow larval growth were not transformed before the NOAEL and LOAEL statistics were performed. Dunnett's statistic was used to determine the NOAELs and LOAELs.

The NOAEL and LOAEL for the FETAX percent embryo survival and percent embryo malformation data were estimated by Dunnett's method following arc sine square root transformation with the following exception. The data were assumed to be normally distributed, with homogeneity of variance after arc-sine square root transformation. The transformed data could not be tested for normality and homogeneity of variance because two replicates were used in the assay as required by the ASTM test protocol (ASTM 1998). Estimates of the NOAEL and LOAEL are not recommended endpoints in the FETAX protocol; however, we estimated the two metrics in order to compare the frog NOAEL and LOAEL with the other three test species. The statistical tests were performed using WEST and Gulley (1994) at a minimum probability level of 0.05.

RESULTS AND DISCUSSION

The results of the current study are summarized and compared to other data where appropriate in Table 1. The acute 96-h EC50 (inhibition of growth) for *S. capricornutum* was 3,185 mg/L. A 96-h EC50 of 2,623 mg/L was established by Bentley et al. (1976) for the same algal species. Van Voris et al. (1987) found that a concentration of 500 mg/L, which was the highest concentration studied, had no effect on *S. capricornutum* during their logarithmic growth phase. Bentley et al. (1976) found similar 96-h EC50s (inhibition of growth) for three other species of freshwater algae which ranged from 2,234 to 6,107 mg/L (Table 1).

Table 1. SUMMARY OF THE DIMP TOXICITY DATA BASE FOR AQUATIC ORGANISMS

Species	Endpoint	Current Study (mg/L)	Other Studies (mg/L)
	ACUTE TOXICITY		
Algae:			
Selenastrum capricornutum	96-h EC50[a]	3,185	2,623[b]
			>500[c]
Microcystis aeruginosa	96-h EC50[a]		2,234[b]
Anabeana flos-aquae	96-h EC50[a]		6,107[b]
Navicula pelliculosa	96-h EC50[a]		2,345[b]
Chlorella pyrenoidosa	96-h EC50[a]		>500[c]
Invertebrates:			
Ceriodaphnia dubia	48-h LC50	610	
Daphnia magna	48-h LC50		267[b]
Gammarus fasciatus	48-h LC50		494[b]
Asellus militaris	48-h LC50		2,160[b]
Chironomous tentans	48-h LC50		1,720[b]
Fish:			
Pimephales promelas	96-h LC50	604	479[b]
Ictalurus punctatus	96-h LC50		285[b]
Lepomis macrochirus	96-h LC50		406[b]
Oncorhynchus mykiss	96-h LC50		631[b]
Amphibian:			
Xenopus laevis	96-h LC50	1,543	
	96-h EC50[d]	1,225	
	NOAEL[e]	398	
	LOAEL[e]	569	

Table 1. (Continued)

Species	Endpoint	Current Study (mg/L)	Other Studies (mg/L)
	CHRONIC TOXICITY		
Alga:			
Selenastrum capricornutum	NOAEL[a]	711	
	LOAEL[a]	1,423	
Invertebrate:			
Ceriodaphnia dubia	7-d LC50	375	
	NOAEL[f]	142	
	LOAEL[f]	285	
Fish:			
Pimephales promelas	7-d LC50	381	
	NOAEL[g]	142	
	LOAEL[g]	285	
Lepomis macrochirus	14-d Bioconcentration		>167[h]

[a] Test endpoint- reduction in growth (cell density).
[b] Bentley et al. (1976).
[c] 500 mg/L DIMP highest concentration studied (Van Voris et al. 1987).
[d] Test endpoint- increase in malformations.
[e] Test endpoint- mortality.
[f] Test endpoint- reduction in neonate production.
[g] Test endpoint- reduction in growth.
[h] 167 mg/L DIMP highest concentration studied; no apparent stress or bioconcentration of DIMP occurred during a 14-d exposure (Bentley et al. 1976).

The acute 48-h LC50 for the invertebrate *C. dubia* was 610 mg/L. A 48-h LC50 of 267 mg/L was found by Bentley et al. (1976) for *Daphnia magna*, another species in the same family as *C. dubia* (Table 1). As shown in Table 1, Bentley et al. (1976) also established 48-h LC50s for the scud (*Gammarus fasciatus*), sowbug (*Asellus militaris*), and midge (*Chironomous tentans*) which were 494, 2,160, and 1,720 mg/L DIMP, respectively. The acute toxicity (96-h LC50) of DIMP to the larval fathead minnow (*P. promelas*) was 604 mg/L in the current study. Bentley et al. (1976)

obtained a 96-h LC50 of 479 mg/L for the same species. Bentley et al. (1976) found similar 96-h LC50s for three other species of freshwater fish (Table 1).

The 96-h LC50 for frog embryos exposed to DIMP was 1,543 mg/L. The 96-h NOAEL and LOAEL for mortality were 398 and 569 mg/L, respectively. The 96-h EC50 for malformations was 1,225 mg/L. The predominant malformations were multiple edema (50% of the embryos) and gut coiling (28%) followed by notochordial abnormality (9%) and severe axial/edema (9%); cardiac, face, and eye abnormalities each accounted for ~1% of the malformations. A NOAEL and LOAEL for malformations could not be determined because significant mortality occurred at exposure concentrations of 569 mg/L and above. The teratogenic index (TI), which by definition is the 96-h LC50 divided by the 96-h EC50 (malformations), provides an estimate of the teratogenic risk associated with a material (Dumont et al. 1983). TI values of 1.5 to 2.0 indicate that material may be a potential teratogen. Materials with TI values >2.0 should be considered for further teratogenicity testing. The TI in the current study was ~1.3; thus, a low potential exists that DIMP is a developmental hazard.

The chronic toxicity of DIMP to the green alga, cladoceran, and fathead minnow are summarized in Table 1. With the exception of a 14-d bioconcentration study by Bentley et al. (1976), no other chronic DIMP data were found in the literature for aquatic organisms. According to the authors, the bluegill appeared normal, fed readily, and generally showed no signs of stress during the study.

Bioconcentration of DIMP by aquatic organisms would not be expected to occur when one considers that the log K_{ow} for DIMP is 1.03 (Krikorain et al. 1987). Bioconcentration of a material up to 100-fold above background (bioconcentration factor or BCF = 100) normally does not occur until log K_{ow} = 3 (Williamson et al. 1993). Bentley et al. (1976) showed that DIMP did not bioconcentrate in bluegill exposed to 167 mg/L ^{14}C-DIMP for 14 d. Toxicokinetic studies have shown that no storage of DIMP occurs in the tissues of mammals (ATSDR 1998). Since DIMP is not anticipated to bioconcentrate, no food chain bioaccumulation or transfer pathways are expected to be important in the fate and transport of the compound in the environment.

The acute and chronic toxicity data indicate that DIMP is not a potential risk to aquatic organisms at either the Rocky Mountain Arsenal or Aberdeen Proving Ground. Computer simulation model runs in the early 1980s of the alluvial aquifer at RMA predicted that the DIMP concentration would decrease from several mg/L at the source to ~0.002 mg/L as the plume discharged to the South Platte River. Measurements of DIMP which entered a creek, an adjacent lake, and its canal tributary, showed that 0.0025 mg/L was present in the aquatic environment (Gordon and Hartley 1992). The highest concentration observed at APG in the surficial groundwater in 1998 was 6.05 mg/L (Burton and Turley 1999). Based on the NOAELs of 711, 142, 398, and 142 mg/L for the alga, invertebrate, fish, and frog

in this study, and the assumption that the highest concentration of 6.05 mg/L in the aquifer enters the aquatic environment at Kings Creek, Superfund screening-level risk assessment hazard quotients for the four organisms would be 0.008, 0.042, 0.015, and 0.042, respectively. A hazard quotient <1 indicates that a contaminant has a negligible potential for ecological impact (U.S. EPA 1997). Thus, the plume of DIMP migrating in the surficial aquifer at APG and the concentrations present in the environment at RMA should have minimal impact on the aquatic ecosystems.

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