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Terrestrial toxicity evaluation of decabromodiphenyl ethane on organisms from three trophic levels

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ABSTRACT

Decabromodiphenyl ethane (DBDP-Ethane) was evaluated for its potential to effect sewage sludge respiration, soil nitrification, survival and reproduction in *Eisenia fetida*, and seedling emergence and growth in *Zea mays*, *Lolium perenne*, *Glycine max*, *Allium cepa*, *Lycopersicon esculentum*, and *Cucumis sativa*. The no observed effect concentrations (NOECs) were identified at the limit concentration level for sewage sludge respiration (> 10 mg DBDP-Ethane/kg dry soil), > 2500 mg/kg dry soil for soil nitrification, > 3720 mg/kg dry soil for earthworm survival, and > 6250 mg/kg dry soil for seedling emergence and growth in *Z. mays*, *L. perenne*, and *G. max* . Treatment-related effects were identified for *E. fetida* reproduction, *C. sativa* survival, and *L. esculentum* and *A. cepa* height and dry weight. The most sensitive endpoints were decreased height and dry weight for *A. cepa* and decreased reproduction for *E. fetida* with NOECs of $1563_{nominal}$ ($1540_{measured}$) and $2210_{nominal}$ ($1907_{mean\ measured}$) mg/kg dry soil. The NOEC for soil nitrification and the lowest NOEC identified for soil (i.e., *A. cepa*) were used to derive predicted no effect concentrations (PNEC) values of 2500 mg/kg for sewage sludge and 156 mg/kg for soil. The calculated PNECs indicate DBDP-Ethane presents little risk to organisms in the sewage sludge and soil compartments.

1. Introduction

Decabromodiphenyl ethane (DBDP-Ethane; CASRN 84852-53-9; Fig. 1) is a brominated flame retardant used primarily in styrenic polymers, engineering resins, and wire and cable insulation (ALB, 2001). Because of its efficacy, load levels of only 12% are needed for thermoplastic resins to achieve Underwriters Laboratories (UL) UL 94V-0 fire safety rating. The test requirements for achieving a UL 94V-0 rating include exposing the base of a plastic coupon to an open flame for 10 s, followed by a second open flame exposure for 10 s. The plastic must stop burning within 10 s from either of the two flame applications (e.g., self-extinguish) with no dripping of flaming particles (IDES, 2009).

Evaluation of the chemicals' potential to affect human health and environment are equally important. DBDP-Ethane has low inherent mammalian toxicity with no adverse effect levels of 1000 mg/kg body weight in repeated dose studies (Hardy et al., 2010, 2002). Environmental modeling estimates suggest DBDP-Ethane will adsorb strongly to particulates, partition in the environment primarily to soil and sediment, and, if present in the influent of a sewage treatment plant (STP), settle to the sewage sludge (EPA, 2009a, 2009b). Experimental support of DBDP-Ethane's fate and behavior in a STP have been reported; less than 1% of DBDP-Ethane left a STP *via* the effluent, with the remainder being present in the digested sludge (Ricklund et al., 2009).

In the agricultural setting, sewage sludge is routinely used to replenish the nutritional content of the topsoil (Banasik et al., 2009). If DBDP-Ethane is present in sludge used for this purpose, sludge may contain elevated levels of organic matter if, for example, DBDP-Ethane disrupted aerobic digestion inhibiting bacterial respiration. Further, sewage sludge containing DBDP-Ethane may lead to exposure and potentially deleterious effects on organisms that populate topsoil, e.g., nitrifying bacteria, earthworms, or plants. Thus, DBDP-Ethane's possible presence in sewage sludge and soil represent areas of

Abbreviations: a.i., active ingredient; AF, assessment factor; BOD, biochemical oxygen demand; DBDP-Ethane, decabromodiphenyl ethane; DBM, dibromomethane; DO, dissolved oxygen; GLP, Good Laboratory Practice; log K_{OW}, logarithm of the n-octanol–water partitioning coefficient; log K_{OC}, logarithm of the organic carbon–water partitioning coefficient; NIF, nitrification inhibitor formula; OECD, Organization for Economic Co-operation and Development; NOAEL, no adverse effect level; NOEC, no effect concentration; %CV, percent coefficient of variation; PAR, photosynthetically active radiation; PEC, predicted environmental concentration; PNEC, predicted no-effect concentrations; %RH, relative humidity; STP, sewage treatment plant; K_d, soil–water partitioning coefficient; THF, tetrahydrofuran; TSS, total suspended solids; UL, Underwriters Laboratories; VWD, variable wavelength detector; WHC, water holding capacity

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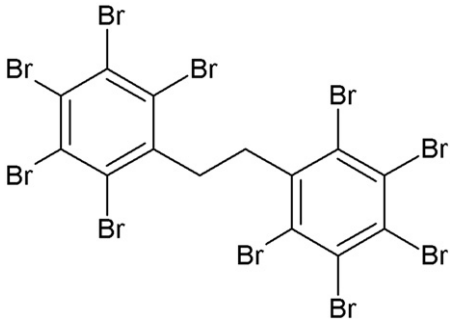

Fig. 1. Chemical structure of DBDP-Ethane.

uncertainty with regard to its potential toxicity to organisms that occupy these compartments.

The objectives of the studies conducted and reported herein were two fold. First, a series of studies were performed for use in the regulatory arena evaluating DBDP-Ethane's potential effects on sewage sludge respiration inhibition, soil bacteria nitrification, earthworm survival and reproduction, and seedling emergence and growth in six terrestrial plants. These organisms were chosen because they span three trophic levels, i.e., decomposers, consumers, and producers. Second, these data were used to perform provisional ecological risk estimations for the sewage sludge and soil compartments.

2. Materials and methods

2.1. Chemicals

The commercial DBDP-Ethane product, obtained from differing manufacturing lots, was used as the test article in all studies. Each lot was characterized under Good Laboratory Practice (GLP) standards. No adjustments for purity were made in any of the studies. 3,5-Dichlorophenol was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Nitrification inhibitor formula (NIF) 2523 was purchased from Hatch Company (Loveland, CO) and consisted of 2-chloro-6-(trichloromethyl) pyridine coated on a sodium sulfate substrate. All other chemicals were of reagent grade quality or better.

2.2. Experimental matrices

The activated sewage sludge inoculum for the respiration inhibition test was collected from the Denton Wastewater Treatment Plant (Denton, MD). The Denton facility receives waste from predominantly domestic sources. The nitrification test used a natural sandy loam soil collected from a tree farm in Grand Forks County, North Dakota by Agvise Laboratories, Inc. (Northwood, ND). No pesticides or fertilizers were applied in the previous year. Alfalfa obtained from Hatfield Pet Specialties (Bentonville, AR) was used to amend the soil.

The earthworm study used artificial soil that was prepared to approximate a sandy loam soil by mixing the following ratios of constituents: 70% silica sand, 20% kaolin clay, and 10% finely ground sphagnum peat (dry weight equivalents). After preparation with the dry constituents, the air-dry soil was stored at room temperature. Additional details on the sludge and the soils used in the nitrification and earthworm studies are found in the Supplemental Material (SM).

The plant study used artificial soil representative of loam soil and composed of kaolinite clay, industrial quartz sand, and peat. Crushed limestone was added to buffer the pH. A slow-release fertilizer was added to provide nutrients essential for plant growth. Agvise Laboratories, Inc., analyzed a representative sample of the soil for particle size distribution and organic matter content and reported the following: 50% sand, 28% silt, and 22% clay, with an organic matter content = 2.7% and pH of 6.5.

2.3. Test species

The sewage aerobic bacteria and soil nitrifying bacteria used for the respiration inhibition and nitrification inhibition studies, respectively, were those naturally present in sewage sludge and sandy loam soil. The earthworms, *E. fetida*, were obtained from a commercial supplier (Vicker's Farms, Orlando, FL). The animals were from a synchronous population where the animals were no greater than four weeks apart in age and were approximately nine months of age with a developed clitellum at the time of testing. The common name and species/variety for the plant species tested were as follows: corn (*Z. mays*)/Mandan Bride and soybean (*G. max*)/Black Jet, Johnny's Selected Seeds (Albion, ME); onion (*A. cepa*)/Texas Grano, Henry Field's Seed and Nursery (Aurora, IN); ryegrass (*L. perenne*)/Manhattan 3 and tomato (*L. esculentum*)/Rutgers, Meyer Seed Co. (Baltimore, MD); and cucumber (*C. sativa*)/Parks Whopper, Park Seed Wholesale (Greenwood, SC). Seeds were not treated with fungicides, insecticides, or repellents prior to test initiation.

2.4. Experimental design

2.4.1. Sewage sludge respiration inhibition

The study was performed in compliance with GLP standards and according to the Organization for Economic Co-operation and Development (OECD) test guideline 209 and the European Communities (EC) test guideline C.11. (EC, 1988; EPA, 1989b; MAFF, 1999; OECD, 1984a, 1997). The study consisted of control, reference, and treatment groups prepared in 1000 mL Erlenmeyer flasks. The controls were used to determine the background respiration rate of the sludge and were not exposed to the reference or test substances. The reference groups were dosed with the known respiration inhibitor 3,5-dichlorophenol (97.0% purity) at 3, 15, and 50 mg/L. The test sample contained DBDP-Ethane (97.64% purity) at a nominal concentration of 10 mg/L.

Text mixtures were prepared at 15 min intervals starting with the first control, which contained 16 mL of synthetic sewage, 200 mL of inoculum, and enough municipal water to bring the total volume up to 500 mL. The mixtures were incubated at 20 ± 2 °C and aerated for 3 h at a rate sufficient to provide aerobic conditions and keep the solids in suspension. Subsequent mixtures contained 16 mL of synthetic sewage, 200 mL of inoculum, the appropriate amounts of reference or test substance, and enough municipal water to bring the volume to 500 mL. Finally, a second control sample was prepared.

After incubation, and beginning with the first control, the samples were transferred to fill 300 mL biochemical oxygen demand (BOD) bottles and dissolved oxygen (DO) measurements were made every 10 s over a 10 min period or until DO concentrations fell below 1.0 mg/L (YSI Model 50B Dissolved Oxygen Meter) for the first control vessel. The respiration rate in subsequent vessels was determined in an identical manner at 15 min intervals over a total of 3 h. The criteria for a valid test required 2 control respiration rates that were within 15% of each other, and the EC_{50} (3 h) of 3,5-dichlorophenol be in the accepted range of 5–30 mg/L.

2.4.2. Soil bacteria nitrification inhibition

The study was performed in compliance with GLP standards and according to OECD test guideline 216 (OECD, 1997, 2000c). Test chambers consisted of 0.5 L French-square glass bottles with perforated aluminum foil lids to allow for circulation of air. Six of these test chambers (2 replicates/test condition) were filled with 353 g of moist sandy loam soil (equivalent to 300 g of dry soil) and acclimated to test conditions for 5 days, prior to study initiation. Just prior to dosing, the moisture content of the soils was adjusted to approximately 50% WHC, and each test chamber was amended with 1.5 grams of dried, ground alfalfa. The study samples consisted of a control group (3.0 g of untreated quartz sand; $n=2$ chambers), a treatment group (3.0 g of quartz sand containing DBDP-ethane (97.64% purity) to yield a nominal concentration of 2500 mg DBDP-Ethane/kg dry soil; $n=2$ chambers), and a negative control group treated with a nitrification inhibitor (1000 μL of the inhibitor solution resulting in a nominal concentration of 500 mg NIF 2533/kg dry soil; $n=2$ chambers). After dosing, soils were thoroughly homogenized using stainless steel spatulas. The samples were incubated in the dark under aerobic conditions at 20 ± 2 °C for a total of 28 days. The moisture content of the soil samples was adjusted to approximately 50% WHC after sampling on days 0, 7, 14, and 21. The weights of the test chambers were measured before and after adjustment for %WHC. Subsamples of soil were removed for analysis of nitrate concentration immediately after dosing and on day 28. Nitrate analysis was performed using a Dionex DX-500 Ion Chromotagraphy System. The criteria for a valid test required that the control mean nitrate concentrations at each sampling interval were within 15% of each other.

2.4.3. Earthworm survival and reproduction

The study was performed in compliance with GLP standards and according to OECD test guideline 207 and the U.S. Environmental Protection Agency (EPA) test guideline 850.6200(EPA, 1996a; OECD, 1984b, 1997, 2000b). The study consisted of a negative control of 8 replicate chambers and five treatment groups of four replicates each. Test chambers were 1.8 L glass jars. Each replicate contained ten earthworms for a total of 80 control earthworms and 40 treatment earthworms per concentration level. Nominal soil concentrations were 313, 625, 1250, 2500, and 5000 mg DBDP-Ethane (98.3% purity)/kg dry soil. Soils were mixed in a rotary mixer for at least 24 h after addition of the test article, hydrated to 60% of the WHC with deionized water, and re-mixed to a uniform consistency. Approximately 615 g of soil (i.e., 500 g dry soil) was added to each replicate, which resulted in a soil depth of approximately 8 cm.

Adult earthworms were acclimated to artificial soil and invertebrate diet slurry six days prior to study initiation. No mortality was observed during acclimation. At initiation, 10 earthworms were randomly collected, rinsed in deionized water, blotted dry, and weighed as a group. Each group was randomly assigned to a