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Legacy Chlordane in Soils from Housing Areas Treated with Organochlorine Pesticides

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Abstract: Chlordane was used for termite prevention in Air Force housing areas, where it was sprayed heavily on the ground around building exteriors. Base closure regulations require the Air Force to assess residual chlordane in the soil. The USACE-ERDC team examined two soil types containing chlordane residuals; a Silty Sand and a Sand soil type from housing areas at McGuire AFB, NJ, and Davis-Monthan AFB, AZ, respectively. Using these two soils, chlordane leachability was evaluated; the potential for chemical or biological degradation of the chlordane was evaluated; the potential toxicity of the chlordane was assessed using plant and earthworm bioassays. Leachability was tested using four different procedures. The potential for chemical remediation was evaluated by alkaline hydrolysis and persulfate oxidation. The potential of biodegradation was evaluated by composting with spent mushroom waste. The soil toxicity and uptake of chlordane into plants and earthworms was also assessed. The results of these investigations indicated that aged chlordane in the soil was not likely to desorb and migrate under either landfill or physiological conditions. Chlordane in solution was susceptible to photodegradation and alkaline hydrolysis, chlordane in soil was not. Chlordane in soil was also not susceptible to persulfate oxidation. Composting did show promise as a long-term ex situ remediation strategy. Biouptake results indicated that chlordane did not adversely affect seed germination, root length, or shoot length. The plants did uptake chlordane from the soil and translocate it to the shoots. The presence of chlordane did not affect earthworm mortality, but did affect weight loss and reproductive success.

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Preface

This report presents the results of a study funded by MIPR number F1JFAA7249G001 from the Air Force Center for Engineering and the Environment (AFCEE) under the direction of Samuel L. Brock.

This study was directed by Dr. Victor F. Medina and Scott A. Waisner, Environmental Engineering Branch (EP-E), Environmental Processes and Engineering Division (EPED), Environmental Laboratory (EL), US Army Engineer Research and Development Center (ERDC). They were assisted by Agnes B. Morrow, Environmental Chemistry; Afrachanna D. Butler, EP-E; Dr. David Johnson, Environmental Risk Assessment Branch; Allyson Harrison, SpecPro, Inc.; and Catherine C. Nestler, Applied Research Associates, Inc. Deborah Felt and Christopher Griggs provided in-house review.

This study was conducted under the direct supervision of W. Andy Martin, Branch Chief, EP-E; and under the general supervision of Dr. Richard E. Price, Division Chief, EPED; and Dr. Elizabeth C. Fleming, Director, EL.

At the time of this study, COL Gary E. Johnston was Commander and Executive Director of ERDC. Dr. James R. Houston was Director.

Unit Conversion Factors

Multiply	By	To Obtain
degrees Fahrenheit	$(F-32)/1.8$	degrees Celsius
feet	0.3048	meters
foot candles (ft-c)	0.0929	lux
inches	0.0254	meters
ounces (mass)	0.02834952	kilograms
pounds (mass)	0.45359237	kilograms
square feet	0.09290304	square meters
square inches	6.4516 E-04	square meters

Acronyms

AFB	Air Force Base
AFCEE	Air Force Center for Engineering and the Environment
ATSDR	Agency for Toxic Substances and Disease Registry
DIWET	Distilled Water-Waste Extraction Test
EL	Environmental Laboratory
ERDC	Engineer Research and Development Center
FDA	Food and Drug Administration
FMC	field moisture capacity
FRTR	Federal Remediation Technology Roundtable
GC-ECD	gas chromatography with electron capture detection
IRIS	Integrated Risk Information System
MCL	maximum contaminant level
ng/Kg	nanogram of analyte per kilogram of soil (or tissue), ppt
ng/L	nanogram of analyte per liter of solution, ppt
NIOSH	National Institute for Occupational Safety and Health
OSHA	Occupational Safety and Health Administration
PBET	physiologically based extraction test
PEL	permissible exposure limit (OSHA)
ppb	part(s) per billion
ppm	part(s) per million
ppt	part(s) per trillion
r ²	coefficient of determination
SPLP	synthetic precipitation leachate procedure
TCLP	toxicity characteristic leachate procedure
TWA	time-weighted average
µg/Kg	microgram of analyte per kilogram soil (or tissue), ppb
µg/L	microgram of analyte per liter of solution
USEPA	United States Environmental Protection Agency

Chemical Compounds

DNT	2,4-/2,6-dinitrotoluene
PCB	polychlorinated biphenyl
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
TNT	2,4,6-trinitrotoluene

1 Introduction

Chlordane

Commercial chlordane, technical grade, is a mixture of more than 140 compounds, including the chlorocyclodiene compounds (Dearth and Hites 1991). The *cis*- and *trans*- isomers of chlordane (Figure 1) make up 60-85% of the commercial mixture depending on the manufacturing process (Buchert et al. 1989). The use of chlordane in the present report refers to both isomers. Commercial chlordane was used worldwide as an effective insecticide but has been banned in the United States since the late 1980's and worldwide since 2004 due to its toxicity and persistence in the environment. Although banned for 20 years, chlordane is still being detected in air, soil, and groundwater samples around the world (Bidleman et al. 2004).

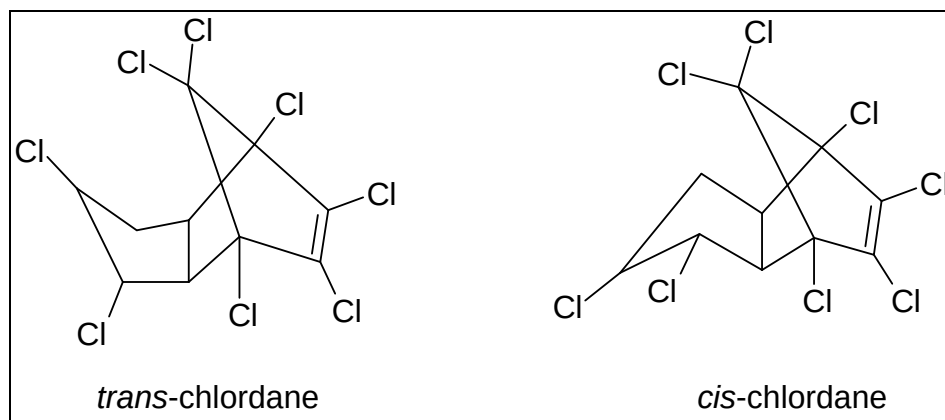


Figure 1. Structural isomers of chlordane.

People are exposed to chlordane through ingestion, dermal exposure, or inhalation of vapors. Chlordane primarily affects the nervous and digestive systems causing headaches, irritability, confusion and vision problems, as well as vomiting and stomach cramps (Agency for Toxic Substances and Disease Registry (ATSDR 1994)). Chlordane has been classified for carcinogenicity in humans as B2, a probable human carcinogen based on animal studies (U.S. Environmental Protection Agency/Integrated Risk Information System (USEPA/IRIS) 2008). The EPA has set a maximum contaminant level (MCL) in drinking water of 2 ppb (USEPA 2006). The Food and Drug Administration (FDA) limits chlordane in fruits and vegetables to <300 ppb, and <100 ppb in animal fat and fish (ATSDR 1994).

The time-weighted average (TWA) exposure limit in the workplace environment is limited to 0.5 mg chlordane/m³ for skin (National Institute for Occupational Safety and Health (NIOSH 2005). The Occupational Safety and Health Administration (OSHA) has set a similar workplace TWA permissible exposure limit (PEL) of 0.5 mg/m³ for skin (NIOSH 2005).

The *cis*- and *trans*- isomers of chlordane are non-superimposable mirror images and have identical physico-chemical properties (Table 1). These physical and chemical properties are often difficult to specify for technical grade chlordane because it is a complex mixture (USEPA/IRIS 2008). For example, the vapor pressure of the mixture will change over time since the more volatile components will be removed faster, changing the composition of the mixture. However, the ratio of chlordane isomers will remain the same, since these processes are not selective for a particular isomer. This is true for all the physical processes affecting chlordane such as volatilization, hydrolysis, or photodegradation (Müller et al. 1997). In contrast, biological processes are isomer specific and will change the ratio of *cis*- to *trans*- isomers in the soil, providing a reliable test for occurrence of biodegradation (Eitzer et al. 2003; Li et al. 2007; Meijer et al. 2003b). Different rates of degradation and transport among the constituents of the mixture may result in compositional changes over time. These compositional changes need to be considered when selecting a remediation option. Chlordane is highly hydrophobic and binds strongly to organic carbon, clay, and silt in anaerobic sediments (Nakano et al. 2004). Chlordane in surface soil undergoes a two-step desorption process. The initial “fast” desorption results in volatilization into the atmosphere (Meijer et al. 2003a). Scholtz and Bidleman (2007) have prepared a long-term predictive model of the fate of chlordane isomers in soil. The model predicts that chlordane is immobile in soil. Field data, which support this prediction, have determined that leaching is not a transport mechanism for chlordane. However, volatilization is an important route of loss of soil residues. The rate limiting factor is soil binding, not atmospheric concentration.

Table 1. Physical and chemical properties of chlordane.

Parameter	Value	Reference
Chemical formula	C ₁₀ H ₆ Cl ₈	
Molecular weight (pure chlordane)	409.76	
Color	Colorless to amber	Windholz 1983 Hawley 1981
Physical state (technical product)	Viscous liquid	Windholz 1983
Solubility in water	0.056 mg/L at 25 °C for cis:trans (75:25)	Sanborne et al. 1976
Solubility in organic solvents	Miscible with hydrocarbon solvents	Worthing and Walker 1987
Partition coefficients Log K _{ow} Log K _{oc}	5.54 (estimated, pure chlordane) 3.49-4.64	USEPA 1986 Lyman 1982
Vapor pressure cis-chlordane (crystal) trans-chlordane (crystal)	3.0x10 ⁻⁶ mmHg 3.9 x 10 ⁻⁶ mmHg	Foreman and Bidleman 1987
Henry's law constant (25 °C)	4.8 x 10 ⁻⁵ atm·m ³ /mol	Cotham and Bidleman 1991

Remediation strategies

Chemical remediation

Chlordane undergoes photodegradation in the environment and UV irradiation has been applied experimentally to remediate contaminated groundwater. Irradiation of chlordane has been studied in 2-propanol, water, and ethanol, water (Shimizu et al. 2005), or ethanol (Buser and Müller 1993; Yamada et al. 2008) to promote degradation. Yamada et al. (2008) also reported that the chlorine balances changed during treatment, an aspect of the chemistry that will affect various types of remediation. All the chlorine atoms in chlordane were eventually mineralized.

Several case studies on chemical remediation of chlordane to treat soil/sediment have been reported on the website of the Federal Remediation Technology Roundtable (FRTR) (2008). Two of the sites employed thermal desorption of the pesticide, the FCX Superfund Site in Washington State and the Arlington Blending and Packaging Superfund Site. Thermal desorption volatilizes the chlordane from the soil and these residues must be scrubbed from the emissions in a separate treatment train. The Washington Superfund Site met a cleanup goal of total pesticide equal to 1 mg/kg. The Arlington site met a cleanup goal for chlordane of 3.3 mg/kg.

A third case study, Parsons Chemical/ETM Enterprises Superfund Site, involved vitrification of the contaminated soil. The cleanup goal for chlordane was 1 mg/kg at 25 lb soil/hour. Initial testing of the melt cells indicated that the goals were met.

Other research has reported on chemical remediation of related pesticides, the hexachlorocyclohexanes (HCH) and various aldicarb species including Temik, and diuron, in aqueous systems. Ngabe et al. (1993) used base hydrolysis to remove HCH in a system simulating cold, deep lake or ocean water. Miles and Delfino (1985) studied base hydrolysis of aldicarb in groundwater. Miles (1991) studied the effect of chlorination of drinking water on fate of aldicarb. Chlorination resulted in faster degradation rates than base hydrolysis but complete mineralization wasn't achieved and the degradation intermediates (including dichloromethylamine) are possibly toxic as well. Zhang et al. (2008) used gamma ray treatment to degrade diuron in aqueous solution. The reduction in diuron concentration was a result of reaction with the hydroxyl ion and free electrons produced by the irradiation of the solution. Reaction kinetics were affected by pH, as well as the presence of carbonate, nitrite, nitrate, and humic acids.

Biological remediation

Conventional physico-chemical remediation approaches for the remediation of organochlorine pesticides such as discussed above can be difficult and costly to apply to soil and often produce toxic intermediates. Current research also considers biological remediation methods as a possible alternative approach. The difficulties inherent with a biological approach are the toxicity of the compounds to the microbial population, the complex mixtures of compounds to be degraded, the hydrophobicity of the compounds, and a lack of knowledge concerning the biodegradation pathways. These issues have been reviewed by Gadd (2004) and Paul et al. (2005).

Because the organochlorine pesticides exist in an oxidized state, they are generally not amenable to aerobic oxidation processes, except in the case of co-metabolism. Therefore, research has been directed toward anaerobic reduction processes. Hydrogen Release Compound (HRC) has been studied for many years as an amendment to encourage biological remediation of groundwater, including chlordane (Fennell et al. 2003). Groundwater has also been treated at the Ft. Pierce, FL, Orkin facility with hydrogen peroxide and nutrients to stimulate biodegradation of chlordane (FRTR 2008). Phytoremediation of a related pesticide, dieldrin, concluded

that, while not biodegraded, uptake of dieldrin into the roots of poplar and willow trees was a potentially useful tool for removing the pesticide from groundwater (Skaates et al. 2005).

Bacterial strains tolerant/resistant to organochlorine pesticides were screened from wastewater sediment (Benimeli et al. 2003). These authors reported isolation of four aquatic streptomycete strains capable of growth on organochlorine pesticides. Previously, it was felt that the actinomycetes species were capable of initial transformations but not mineralization (Liu et al. 1990, 1991). Hirano et al. (2007) reported anaerobic biodegradation of chlordane in river sediment that was accompanied by methane production and a drop in ORP potential. The biodegradation rate was higher for *trans*-chlordane than *cis*- and was favored by high organic content in the sediment.

The potential of fungi to aerobically degrade/transform alkyl halides such as chlordane has been explored using *Phanerochaete chrysosporium*, the white rot fungus, and reviewed by Gadd (2004). In one study, approximately 23% of chlordane was mineralized in 30 days in liquid culture and 60 days in solid culture (Kennedy et al. 1990). Anaerobic biodegradation, however, has been reported to produce chlorobenzenes and polymerization to α -hexachlorocyclohexane in some related pesticides (Benezet and Matsumura 1973).

Environmental issues

Chlordane was first produced in 1947 as an insecticide for agricultural crops and livestock, for lawns and gardens, and also for underground treatment of building foundations for termite treatment/prevention. The USEPA canceled above-ground uses in 1978 because of concern about cancer risks and canceled all uses after 1988 (USEPA/IRIS 2008). Therefore, all chlordane present in soil in Air Force housing areas is 20 to 61 years old. The Air Force Center for Engineering and the Environment (AFCEE) has indicated that chlorinated pesticides, including chlordane, are troublesome contaminants at decommissioned Air Force sites. Issues include the need for inexpensive soil treatment or disposal and concerns on potential migration of chlordane into new structures through vapor intrusion (Mr. Samuel Brock, AFCEE, personal communication). Prior to the transfer of Air Force base housing to private management, the chlordane soil residuals must be addressed, either by cleanup or by establishing that it is of minimal risk to leave in place.

Objectives

The project had the following objectives:

- Evaluate chlordane leachability from two soil types using four different leaching tests:
 - o Toxicity Characteristic Leaching Procedure
 - o Synthetic Precipitation Leaching Procedure
 - o Distilled water-Waste Extraction Test
 - o Physiologically-based Extraction Test
- Evaluate chemical remediation strategies:
 - o Lime treatment
 - o Persulfate treatment
- Evaluate mushroom waste compost as a biological remediation strategy
- Assess plant toxicity and uptake of chlordane
- Assess earthworm toxicity.

2 Materials and Methods

Soil collection and preparation

The AFCEE arranged the collection of both uncontaminated (control) and contaminated (test) soils: Sandy Silt soil type from McGuire Air Force Base (AFB) in New Jersey and a Sand soil type from Davis-Monthan AFB in Arizona. The soils were shipped to the U.S. Army Engineer Research and Development Center (ERDC), Environmental Laboratory (EL), in Vicksburg, MS. The soil from each container was manually homogenized, mixed by mechanical tumbling, and then analyzed for total chlordane. Chlordane analysis showed that the soil samples collected as “clean or control” soils were actually contaminated with very low concentrations of chlordane. In the absence of other clean soils from these sites, these lightly contaminated soils were set aside for use as the “control” soils. Samples (1 kg) were separated out from the containers that had the highest and lowest chlordane concentrations. The contaminated soils from the remaining containers from each site were thoroughly mixed to form a single soil sample, called “mixed” for both sites (Table 2).

Table 2. Soil sample identification.

Site	Sample
McGuire AFB	Control soil
	Low concentration soil
	Mixed soil (medium concentration)
	High concentration soil
Davis-Monthan AFB	Control soil
	Low concentration soil
	Mixed soil (medium concentration)
	High concentration soil

Field moisture capacity of the soils was determined by controlled water addition (Figure 2).



Figure 2. Measurement of field moisture capacity (FMC) by controlled water addition.

The control, mixed, low concentration, and high concentration soils from both McGuire and Davis-Monthan AFBs were extracted in triplicate for total chlordane. Additionally, samples of each of the different concentration soils underwent the Synthetic Precipitation Leaching Procedure (SPLP), the Toxicity Characteristic Leaching Procedure (TCLP), the Distilled Water-Waste Extraction Test (DIWET), and the Physiologically Based Extraction Test (PBET).

Analysis methods

Total chlordane was extracted from soil samples using SW-846 Method 3545A for Accelerated, or Pressurized, Solvent Extraction for chlorinated pesticides (USEPA 1999). This extraction is designed to efficiently extract all the contaminant from the soil sample to give an accurate and repeatable total concentration. The soil extracts were analyzed in all cases by SW846 Method 8081A, which uses gas chromatography with an electron capture detector (GC-ECD) (USEPA 1999).

Extraction procedures

Synthetic Precipitation Leaching Procedure (SPLP)

The Synthetic Precipitation Leaching Procedure, or SPLP, is designed to assess relative leaching of the contaminant when exposed to a slightly acidic rainwater simulant. Soil samples were leached using SW846 Method 1312 (USEPA 1999). The East Coast Extraction Solution was chosen, as it is more acidic. The SPLP leachates were extracted with hexane and analyzed for chlordane via EPA SW 846 Method 8081A (USEPA 1999) using a gas chromatograph equipped with an electron capture detector (GC-ECD).

Toxicity Characteristic Leaching Procedure (TCLP)

The Toxicity Characteristic Leaching Procedure, or TCLP, is an extraction method intended to simulate leaching resulting from acidic reactions expected in landfill disposal of the soil. The TCLP is an 18-hr extraction using a buffered acetic acid solution (USEPA 1999, Method 1311).

Distilled Water-Waste Extraction Test

The Distilled Water-Waste Extraction Test, DIWET, is designed to identify any acidic neutralization reactions that can compromise the SPLP and TCLP tests. If DIWET extraction concentrations are greater than those of SPLP/TCLP, then these reactions could be occurring. The DIWET is based on the California Waste Extraction Test, which is a 40-hr extraction. In the DIWET, the buffered citric acid solution is replaced with deionized water.

Physiologically Based Extraction Test (PBET)

This in vitro test is intended to simulate conditions in an adolescent's gastrointestinal tract. The method described in Ruby et al. (1996) was used.

Chemical remediation

Materials

Chlordane standards prepared in hexane were purchased from Restek U.S. (Bellefonte, PA).

Lime titration for alkaline hydrolysis

The first step in evaluating an alkaline soil treatment is determining the mass of lime to be added to the soil to achieve the required pH (lime titration). Previous work with lime treatment demonstrated that a pH of 11 was sufficient to result in the removal of 2, 4, 6-trinitrotoluene (TNT), 2,4-/2,6-dinitrotoluene (DNT), hexahydro-1, 3, 5-trinitro- 1, 3, 5-triazine (RDX), and polychlorinated biphenyl (PCB) compounds (Davis et al. 2006, 2007; Waisner et al. 2008). A soil pH over 11 was selected as the alkaline goal for chemical degradation of chlordane based on this earlier research. Lime titration was conducted to determine the lime dosage necessary to increase the soil pH over 11. Soil (15 g) was added to each of nine, 50-mL plastic centrifuge tubes. A different mass of hydrated lime was added to each tube. The mass of lime ranged from 0 to 2% of dry soil mass in increments of 0.25%. Deionized water (30 mL) was added to each tube. Tubes were sealed and placed on a laboratory tube rotator turning at approximately 20 rpm for two days.

After two days, the tubes were removed and the pH of the slurry in each tube was measured and recorded. The slurry pH was plotted versus the mass of lime added. The level of lime addition necessary to achieve a pH of 11.5 or greater was determined from a curve fit through the data.

Lime treatment of aqueous chlordane and effects of light

Preliminary studies as well as a literature review (Buser and Müller 1993, Shimizu et al. 2005, Yamada et al. 2008) indicated that fresh chlordane can be volatile and can be susceptible to photo reactions. Therefore, alkaline hydrolysis was tested in combination with photosensitivity. A 50-µg/L chlordane solution was prepared in water by mixing a hexane-based chlordane standard in alcohol, evaporating the hexane, then mixing the alcohol/chlordane solution in water. These were placed in 20-mL clear screw-top septum vials and filled to the rim to create a zero headspace condition. The following conditions were studied:

- Control (no lime) incubated in the dark
- Control (no lime) incubated in the light
- Lime-treated (0.1% CaOH₂ in solution) incubated in the dark
- Lime-treated (0.1%) incubated in the light.

Slurry reactor

Chemical treatment tests were conducted in 50-mL glass centrifuge tubes with Teflon-lined caps. Each tube contained 15 g of air-dried soil and 30 mL of solution. A total of 18 tubes were prepared for each test, 15 for treatment and 3 for control. They were placed on laboratory rotators and turned at approximately 20 rpm. Three tubes were removed for analysis at elapsed times of 1 hr, 6 hr, 1 day, 3 days, and 7 days. The three control tubes were removed after 7 days. Following treatment, the tubes were centrifuged to separate water and soil for analyses, and soil samples were analyzed for chlordane.

Alkaline hydrolysis

To produce alkaline conditions, 0.15 g of hydrated lime ($\text{Ca}(\text{OH})_2$) and 30 mL of deionized water was added to each active tube. Following treatment and prior to centrifugation, the pH of each tube was recorded and 2 mL of 1-M KH_2PO_4 (monobasic potassium phosphate) was added to each active tube to neutralize any remaining lime. The tubes were placed back on the rotator for approximately 2 hr to allow the neutralization step to complete. The pH of the slurry was measured to ensure that it was below 10. The final pH of the neutralized slurry was typically between 8 and 9.

Heat-activated persulfate oxidation

To introduce persulfate, 24 mL of deionized water and 6 mL of 0.5-M sodium persulfate ($\text{Na}_2\text{S}_2\text{O}_8$) solution were added to each active tube. The laboratory tube rotator was operated inside an incubator maintained at 50°C. Following treatment, the tubes were placed in ice to slow/stop the thermal activation of persulfate and then centrifuged. A sample of the supernatant was analyzed for persulfate and the soil was analyzed for chlordane using the method described above.

Biological remediation

A treatability test was conducted incubating both the McGuire AFB and Davis-Monthan AFB mixed soil with spent mushroom substrate.

Materials

Mushroom substrate (Figure 3) was supplied by personnel from McGuire AFB. The substrate consisted of wood mulch that was previously used for the commercial growth of mushrooms.



Figure 3. Mushroom substrate (exhibiting vigorous fungal growth).

Method

Tests were conducted by adding 2 L (2986-g air-dried McGuire-mix; 3031-g air-dried Davis-Monthan-mix) of soil and 1 L (364 g) of used mushroom substrate to an aluminum pan. The soil and mushroom substrate were thoroughly mixed with a hand garden cultivating tool. Sufficient deionized water (440 mL for McGuire, 690 mL for Davis-Monthan) was added to elevate the soil moisture to the estimated field moisture capacity (14.7% w/w McGuire, 22.6% Davis-Monthan), and the water was thoroughly mixed into the soil/substrate mixture with the cultivating tool (Figure 4). The pans were covered with aluminum foil to minimize water evaporation and kept in the laboratory at room temperature (20-25°C).

Three discrete soil samples were collected from each pan at 23 and 49 days following initiation of the test. Soil samples were chopped to break up the mushroom substrate, homogenized, and analyzed for chlordane concentration and moisture content. The results of the chlordane analysis were corrected for the estimated dilution, by weight, of the soil by the mushroom substrate addition. The dilution factors were 1.12 and 1.10 for the McGuire and Davis-Monthan soils, respectively.



Figure 4. McGuire AFB soil with used mushroom substrate.

Phyto-uptake and translocation

A phyto-uptake study was conducted to evaluate the effect of chlordane on the germination, growth, and appearance of two grass species and one sedge species.

Materials

The grass species used in this study were *Poa pratensis* L. (Kentucky Bluegrass) and *Lolium perenne* L. (Perennial Ryegrass). One sedge was also tested, *Cyperus esculentus* L. (Yellow Nutsedge). Plants were grown from seeds obtained from Ernst Conservation Seeds, Meadville, Pennsylvania.

Germination and uptake procedures

This study used method ASTM E1963-98 (ASTM 1998). Grass ($n = 10$) and sedge ($n = 5$) seeds were planted in McGuire AFB soils at 1 to 2 cm depth and grown for 26 days in 16-hr cycles at 24.5 ± 4.5 °C. Cool white fluorescent lamps provided illumination at an average light intensity of 12 lux.

Germination was calculated based on the number of germinated seeds in each pot and expressed as a percentage of the number of seeds added. At the end of the growing period, the plants (shoot plus root) were harvested, washed with deionized water, air dried at room temperature for 48 hr and weighed. Shoot and root measurements were also recorded. Results were analyzed by Student t-test, $p < 0.05$.

Chlordane effects on terrestrial invertebrates

Earthworm soil avoidance tests

Behavior is an effective, ecologically-relevant indicator of whether or not ecological receptors will remain in environmental media contaminated with chemicals (Hund-Rinke and Wiechering 2001, Natal-da-Luz et al. 2004, Martinez et al. 2006). Earthworm avoidance behavior was evaluated in fully clitellate earthworms according to methods described by Environment Canada (2004). McGuire and Davis-Monahan AFB low, medium, and high chlordane concentration soils were hydrated to field moisture capacity and allowed to equilibrate overnight. Dual exposure chambers were filled with uncontaminated soil on one side and one concentration of an experimental soil on the other. Earthworms ($n = 10$, 1 replicate) were added to the chambers, allowed free movement between the soils and maintained for 48 hr at $20 \pm 2^\circ \text{C}$. After 48-hr exposures, earthworms were counted in control or chlordane soils.

Earthworm lethality and reproductive effects

Earthworm lethality and reproductive effects were evaluated in fully clitellate earthworms according to methods described by Inouye et al. (2006). Briefly, McGuire and Davis-Monahan AFB soils were hydrated to field moisture capacity and allowed to equilibrate overnight. Earthworms ($n = 10$, 5 replicates) were added to control, low, medium, and high chlordane concentration soils and allowed to stay in the soils for 28 days at $20 \pm 2^\circ \text{C}$. Earthworms were also added to culture medium (hydrated peat moss) for 28 days as an experimental condition control. Food (2 g) was added once on day zero. At the end of the experiment, surviving earthworms and cocoons were counted and weighed. Data were analyzed by Kruskal-Wallis one-way ANOVA on ranks with Dunn's post-hoc test ($p < 0.05$).

3 Results and Discussion

Initial soil concentrations

Total chlordane

The results of total chlordane extractions and measurements are summarized in Table 3. Results are provided as an average $\pm$ the standard deviation. The concentrations of total chlordane for the McGuire AFB soils are similar to those reported by previous studies of this site and provided by S. Brock (AFCEE). The spectra were examined and there was no evidence of any other pesticides (such as dieldrin) in these soils.

Table 3. Results of soil extraction and analysis for total chlordane.

Site	Sample	Replicates	Concentration ($\mu\text{g/Kg}$)
McGuire AFB	Control soil	3	110 $\pm$ 23
	Low concentration soil	3	1,122 $\pm$ 210
	Mixed soil	5	2,683 $\pm$ 575
	High concentration soil	3	5,526 $\pm$ 658
Davis-Monthan AFB	Control soil	3	123 $\pm$ 18
	Low concentration soil	3	4,280 $\pm$ 116
	Mixed soil	5	13,832 $\pm$ 568
	High concentration soil	3	20,106 $\pm$ 887

Soil extraction tests

The results of the soil extraction tests are summarized in Table 4. All of the extraction tests were below the laboratory reporting limit of 0.25 $\mu\text{g/L}$, indicating that aged chlordane is very tightly bound and would probably not leach (transport) from these soils. The PBET is designed to mimic digestive acids. These results suggest that soil bioavailability is also not an issue with the aged chlordane.

Table 4. Concentration of chlordane reported by the SPLP, TCLP, DIWET, and PBET extraction tests.

Site	Sample	SPLP ($\mu\text{g/L}$)	TCLP ($\mu\text{g/L}$)	DI-WET ($\mu\text{g/L}$)	PBET ($\mu\text{g/L}$)
McGuire	Low concentration	<0.25	<0.25	<0.25	<0.25
	Mixed	<0.25	<0.25	<0.25	<0.25
	High concentration	<0.25	<0.25	<0.25	<0.25
Davis Monthan	Low concentration	<0.25	<0.25	<0.25	<0.25
	Mixed	<0.25	<0.25	<0.25	<0.25
	High concentration	<0.25	<0.25	<0.25	<0.25

Chemical remediation

Lime titration

The lime titration curve (Figure 5) indicated that a 1% lime addition increased the soil pH to over 11 (to 11.5). Therefore, 1% lime was selected as the additive for the alkaline hydrolysis treatment.

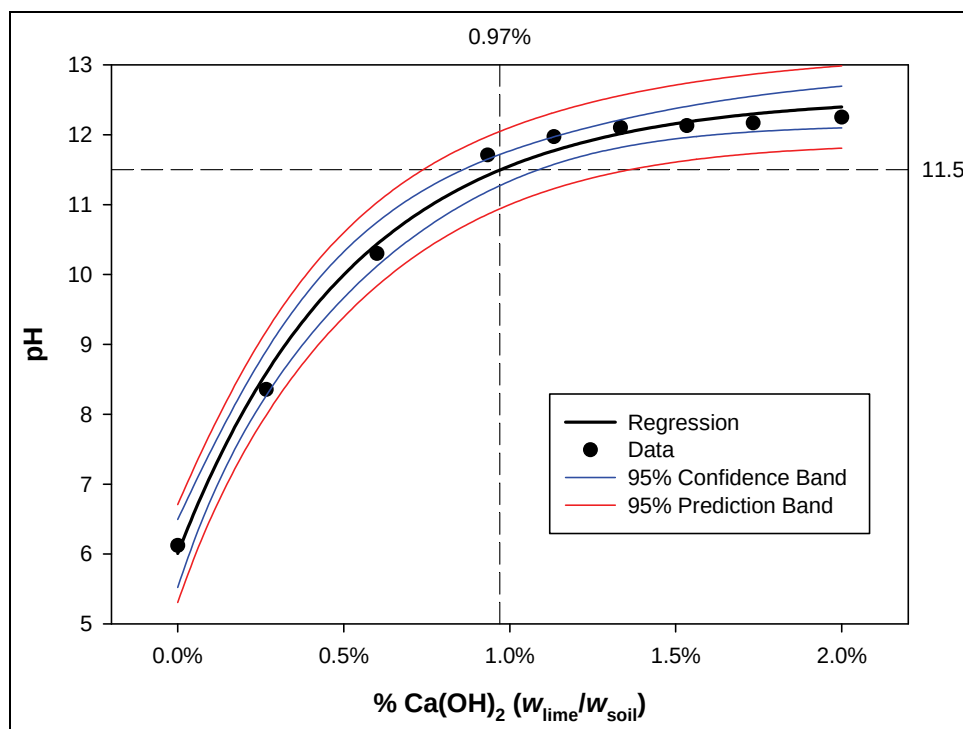


Figure 5. Results of lime titration of McGuire AFB soil.

Lime treatment of aqueous chlordane and effects of light

Table 5 summarizes the results of alkaline treatment of chlordane in an aqueous solution. In 26 hr of incubation, there was no chlordane removal in the dark-incubated control. Removal (27%) was found in the light-incubated control, suggesting the occurrence of photo reactions. A similar amount of removal (26%) was found in the dark-incubated lime treatment. The light-incubated lime treatment had approximately 40% removal, suggesting combined removal from chemical and photo reactions.

Table 5. Results of alkaline treatment and light on concentrations of aqueous chlordane.

Treatment condition	Chlordane concentration (µg/L)	Change ¹ (%)
Control - Dark	57.2	—
Control - Light	42.0	-26.6
Alkaline - Dark	42.4	-25.9
Alkaline - Light	34.5	-39.7

¹ Change expressed as a percentage of the dark-incubated control's final concentration.

Slurry reactor

Alkaline hydrolysis

The application of lime to McGuire soil for alkaline hydrolysis indicated that there was no chlordane removal over the 7-day sampling period (Figure 6). The final concentration was not statistically different than the control concentration.

Persulfate treatment

Figure 7 shows the results of persulfate treatment of chlordane in the mixed McGuire AFB soil. During the 7-day experiment, the extractable chlordane concentration did not decrease. The final concentration of chlordane in the treated reactor was actually slightly higher than that of the control. Persulfate concentrations decreased at the expected rate during the experiment (Figure 8), indicating that thermal activation of the persulfate was occurring.

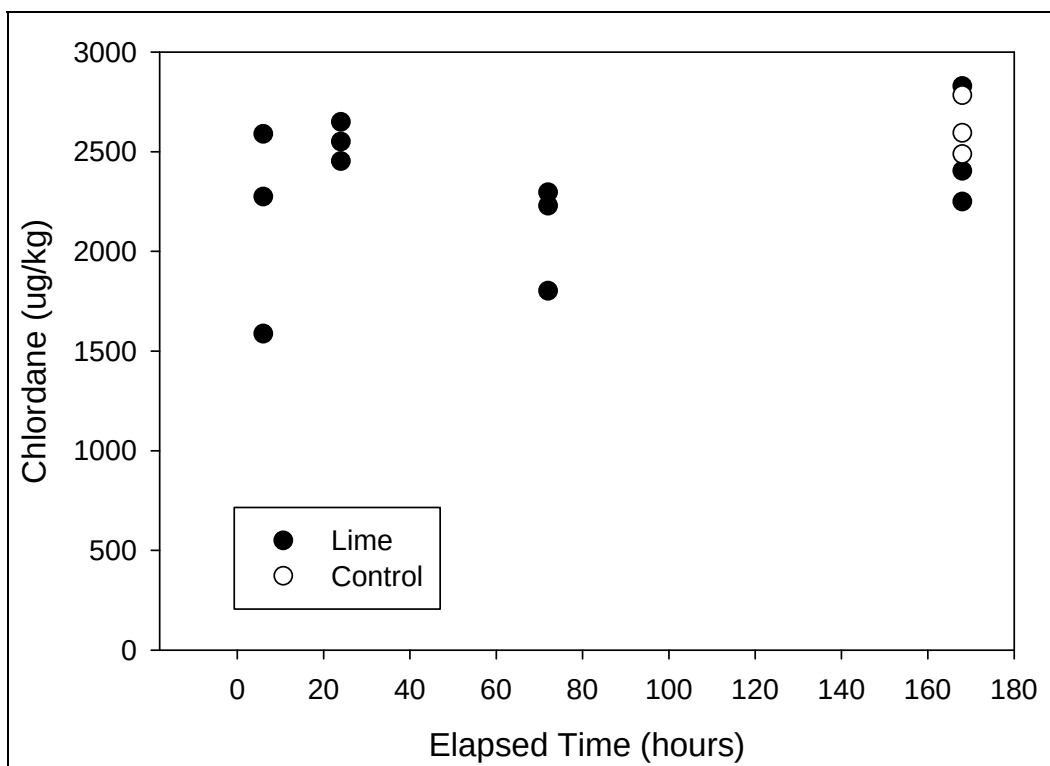


Figure 6. Treatment of chlordane-contaminated soil from McGuire AFB by alkaline hydrolysis.

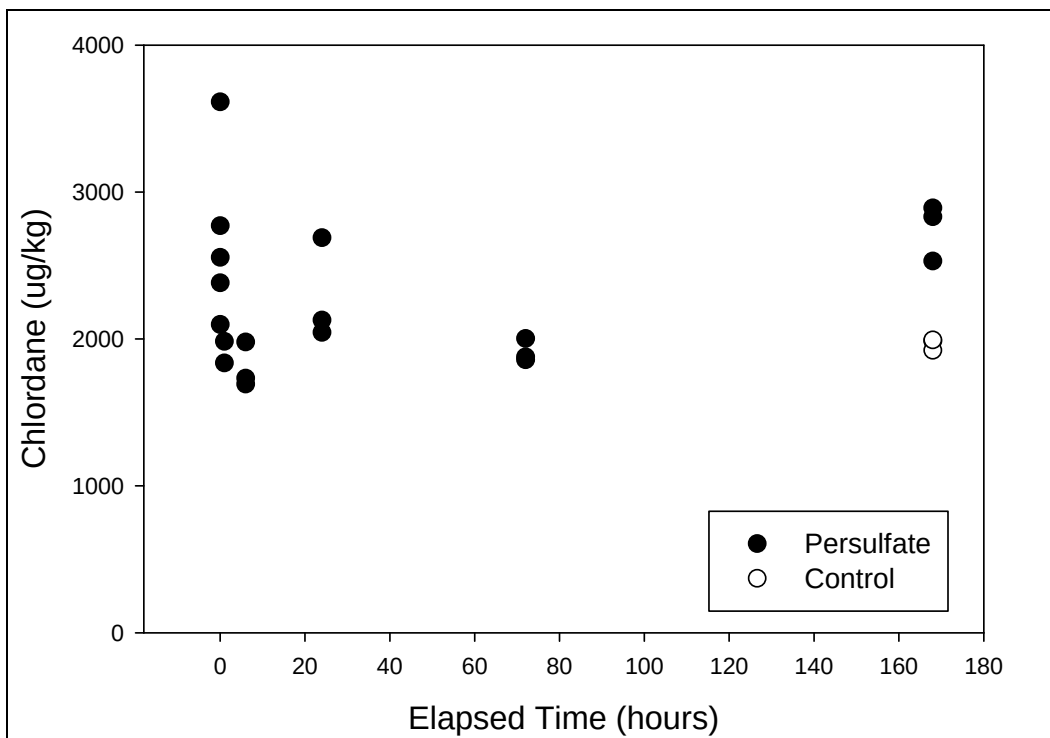


Figure 7. Chlordane concentrations over time during persulfate treatment.

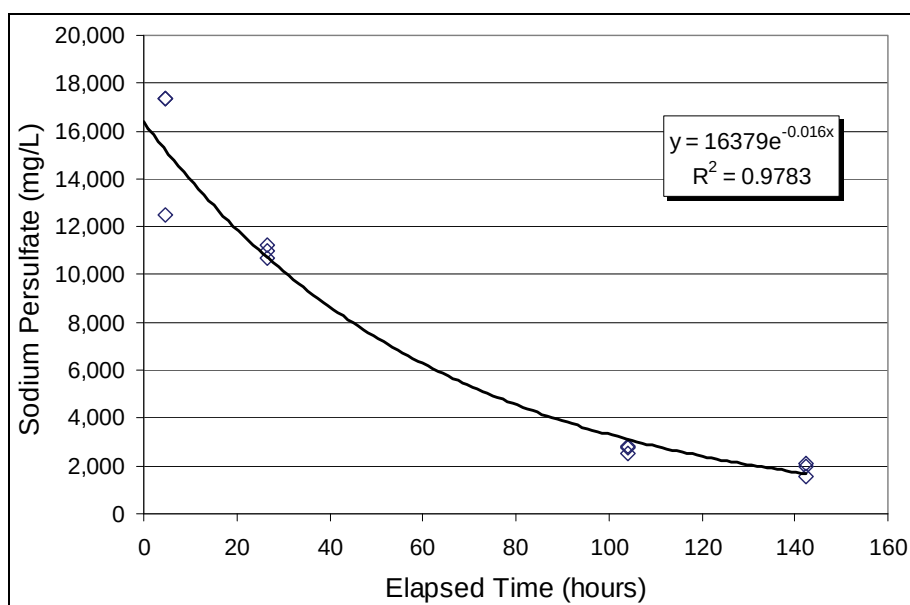


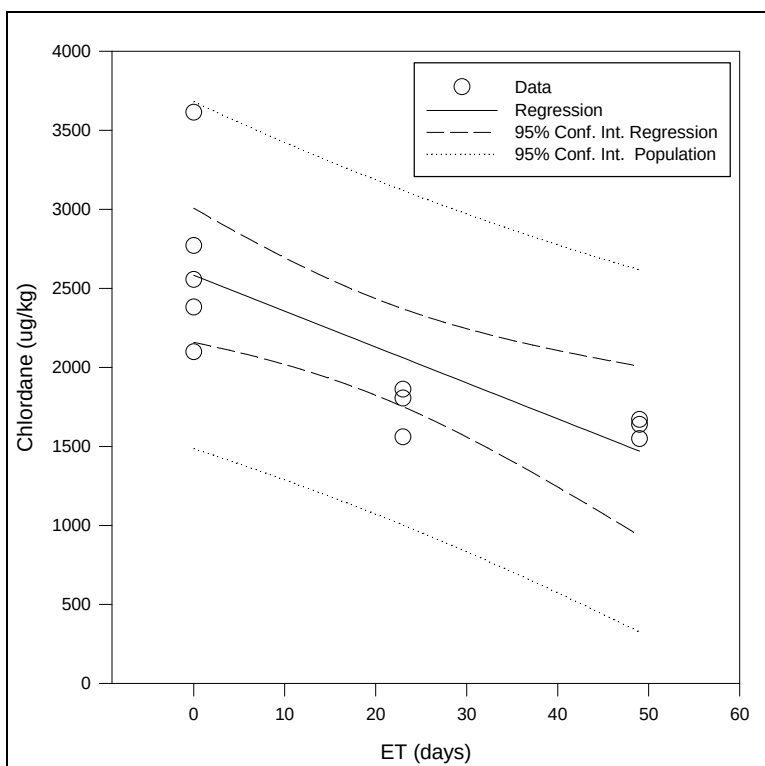
Figure 8. Persulfate concentration in slurry during treatment of McGuire AFB soil.

Biological remediation

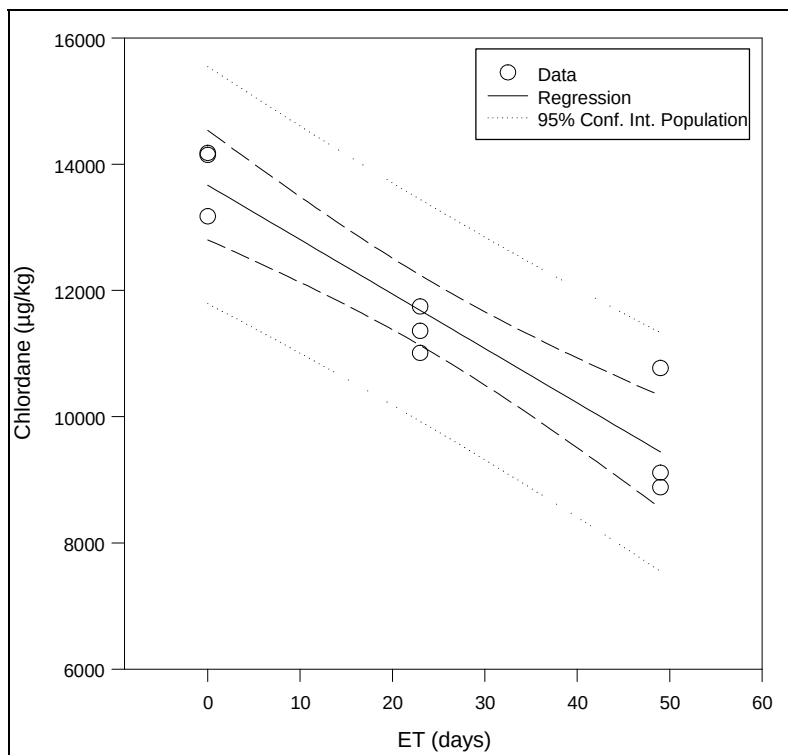
The results (Table 6) of incubating soils with used mushroom substrate showed significant reductions of chlordane levels in the soils over the 7-week test. The McGuire AFB soil exhibited a reduction of approximately 40% from initial chlordane concentrations. A linear regression of the results (Figure 9a) produced an adjusted correlation coefficient (R^2_{adj}) of 0.517. The low correlation appears to be due to a tapering of the reduction rate between the 23-day and 49-day sampling events. The Davis-Monthan AFB soil exhibited a reduction of approximately 31% from initial chlordane concentrations. A linear regression of the results (Figure 9b) exhibited a very good correlation (R^2_{adj}) of 0.869, which indicates that significant reductions may still occur. These reductions may be actual degradation of the chlordane or, as found with TNT, be the result of soil sorption of breakdown intermediates (Pennington et al. 1995).

Table 6. Change in chlordane concentration in two soils as a result of incubating with used mushroom substrate.

Soil	Average Concentration ($\mu\text{g/Kg}$)		Change (%)
	Initial (n = 5)	Final (n = 3)	
McGuire AFB (mix soil)	2,683 $\pm$ 575	1,619 $\pm$ 63	-39.7 $\pm$ 31.4
Davis-Monthan AFB (mix soil)	13,832 $\pm$ 568	9,586 $\pm$ 1030	-30.7 $\pm$ 9.8



a. McGuire AFB mix soil



b. Davis-Monthan AFB mix soil

Figure 9. Linear regressions of chlordane concentration vs. time.

Phytoremediation

Effect of chlordane on seed germination

The effect of chlordane contamination on germination of the studied grasses and sedge is shown in Figure 10. After 26 days of growth, the germination of Kentucky Bluegrass and Yellow Nutsedge was slightly lower in the chlordane-contaminated soils compared to the control, but the difference was not significant ($p>0.05$). For Perennial Ryegrass, the germination percentage increased in the chlordane-contaminated soil compared to the control, and this difference was statistically significant ($p<0.05$).

Figure 11 compares germination percentage over time. The germination patterns differed for each of the plant species studied. The seeds of Kentucky Bluegrass in the uncontaminated soil germinated earliest. After the first 9 days, there was nearly 30% germination in the control and none in the contaminated soils. However, after this initial period, the rate of germination in the contaminated soils increased, so that by the end of the experiment (28 days), the growth differences were not statistically significant. In a similar manner, there appears to be a retardation in germination in the chlordane-contaminated soils over the first nine days for the Perennial Ryegrass. Afterwards, Perennial Ryegrass germination increased in contaminated soils and, by 21 days, had passed that of the control soils. The germination percentage over time for the Yellow Nutsedge of both the contaminated soils and the controls were virtually identical. There were no obvious visible physiological symptoms in the plants during the 26-day study (Figure 12).

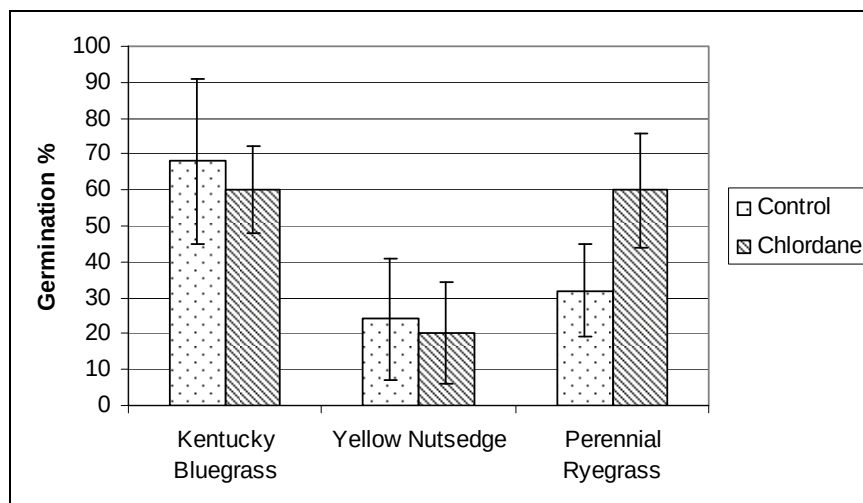


Figure 10. Seed germination percentage of plant species in McGuire AFB soils.

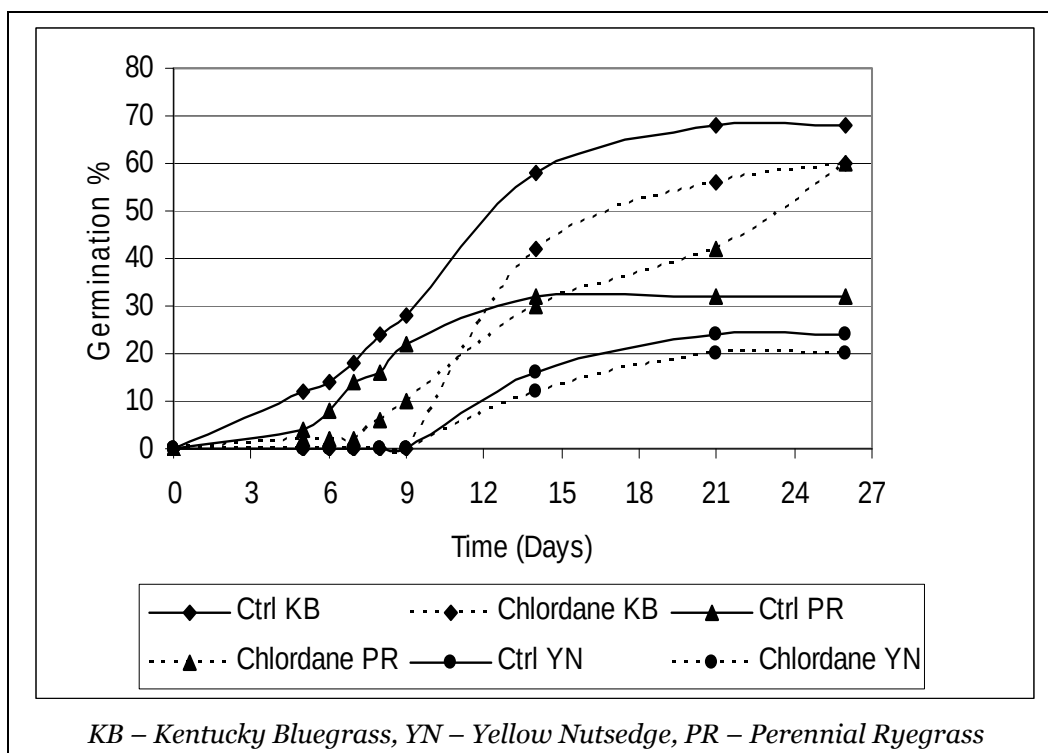


Figure 11. Seed germination percentage over 26 days.

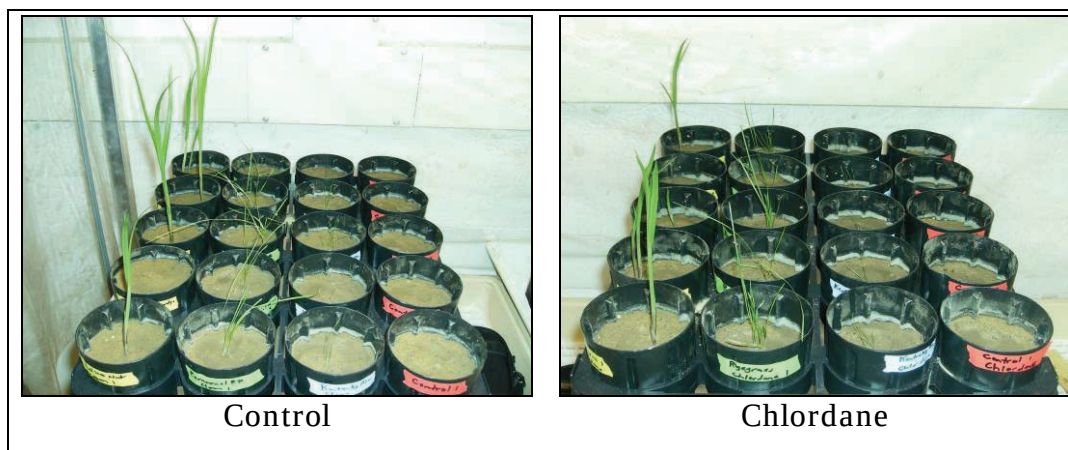


Figure 12. Plants grown in control (uncontaminated) and chlordane-contaminated soils over 26 days.

Effect of chlordane on shoot and root growth

Plant growth of the two grasses and sedge is shown in Figures 13 and 14. No significant ($p > 0.05$) difference was observed in the shoot and root heights of Kentucky Bluegrass, Perennial Ryegrass, and Yellow Nutsedge, compared to their controls. The large variation observed in the Yellow Nutsedge was due to no plant growth in one replicate of the control and chlordane soils.

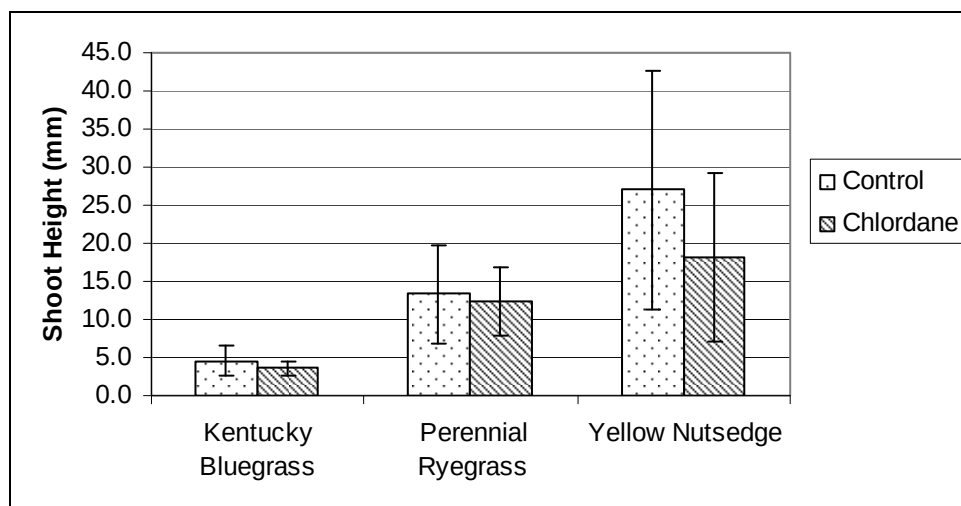


Figure 13. Plant shoot growth in control and chlordane-contaminated soils.

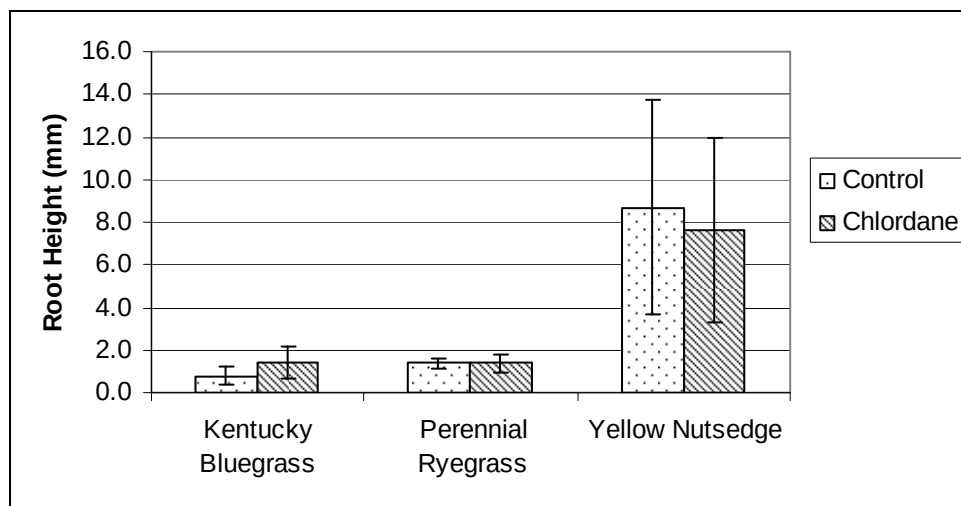


Figure 14. Plant root growth in control and chlordane-contaminated soils.

Plant uptake of chlordane

As part of a screening test, plants were grown in the mixed McGuire AFB soil for 26 days. Following harvest, using the procedures described above, the shoots and roots were separated and both plant sections were extracted and analyzed for chlordane concentration. The results are summarized in Figures 15 and 16. In each case, the plants had taken up chlordane from the soil, translocated the compound, and accumulated it in the shoots. The root concentrations were similar to the initial soil concentration of 2,683- $\mu\text{g}/\text{kg}$. Since the control soils were actually contaminated with low concentrations of chlordane (Table 1), chlordane was also found in the plant material that was grown in the control soils.

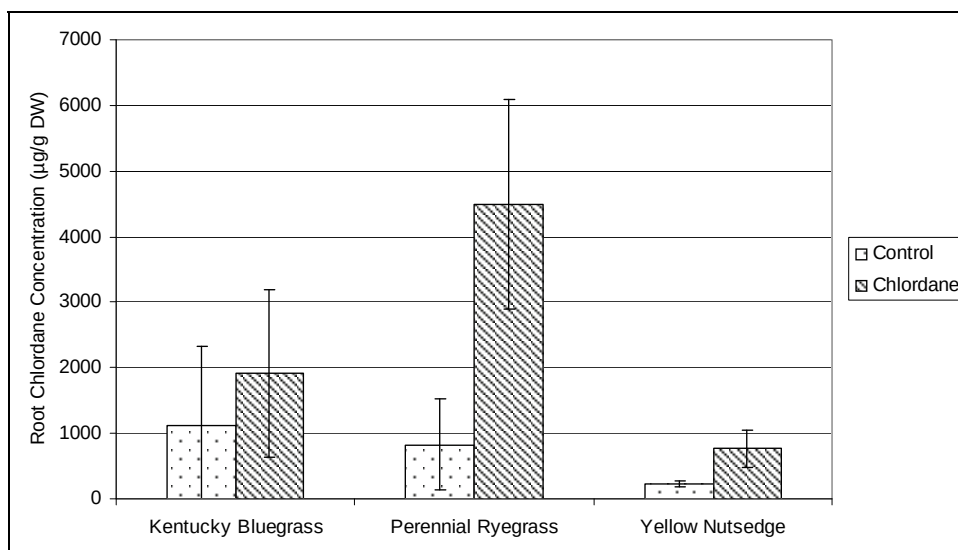


Figure 15. Concentration of chlordane in roots of plants grown in McGuire AFB soil.

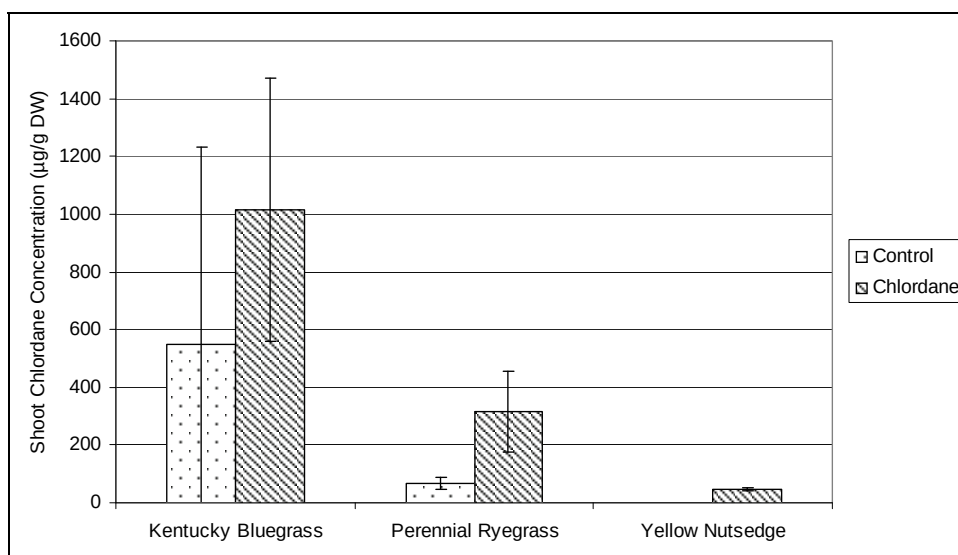


Figure 16. Concentration of chlordane in shoots of plants grown in McGuire soil.

Chlordane effects on terrestrial invertebrates

Organochlorine pesticides are known to affect earthworm mortality and reproduction. Callahan et al. (1991) demonstrated that a 7-day exposure of earthworms (*Lumbricus terrestris*) to field soil from the Baird and McGuire Superfund Site (Holbrook, Massachusetts), a former pesticide mixing site, produced highly significant correlations between soil chlordane levels and earthworm chlordane concentrations, as well as between soil and earthworm chlordane concentrations and earthworm responses (i.e., death and swelling). Chlordane has also been shown to reduce sperm count in earthworms (*Lumbricus terrestris*) (Cikutovic et al. 1993). The

onset of sperm count reduction varied with concentration, but the absolute sperm count drop (approximately 40% of control) demonstrated a threshold effect.

Earthworm avoidance

As shown in Table 7, after 2 days of exposure, in all three McGuire AFB soils, earthworms preferred control soils versus chlordane-containing soils (60–70% vs. 30–40%, respectively). In Davis-Monthan AFB soil, earthworms preferred soil with low chlordane concentration, but at higher chlordane concentrations, the earthworms preferred the control soil. This trend, the attraction of earthworms to low concentrations of contaminant and avoidance of higher contaminant concentrations has also been reported for organophosphate insecticides (Hodge et al. 2000), lead nitrate and mancozeb (Reinecke et al. 2002), and the fungicides benomyl and carbendazim (Garcia et al. 2008). The conclusion drawn by these researchers was that the sensitivity of the test depended on the specific chemical being tested as well as the soil type.

Table 7. The results of earthworm avoidance tests of chlordane-contaminated soil from McGuire and Davis-Monthan Air Force Bases.

Site	Earthworm population	
	Soil	Earthworm (% of total)
McGuire AFB	Control	70
	Low concentration	30
	Control	60
	Mixed soil concentration	40
	Control	60
	High concentration	40
Davis-Monthan AFB	Control	40
	Low concentration	60
	Control	60
	Mixed soil concentration	40

Like the McGuire AFB soils, these data suggest that earthworms will avoid soils contaminated with higher chlordane concentrations. Although these data are preliminary ($n = 1$), it appears that earthworms do not like burrowing in chlordane-contaminated soils from either McGuire or Davis

Monthan AFB. If this is the case, then it reduces the likelihood that earthworms will be exposed to chlordane through this pathway, thus reducing or eliminating this route of exposure from conceptual models of ecological risk assessments for these two AFB sites.

Earthworm survival

After 28 days, there was nearly 100% survival of earthworms in all of the McGuire and Davis-Monthan AFB soils under the different chlordane concentration regimes. McGuire AFB soil earthworms burrowed a quarter of the depth of the soil. At lower chlordane concentrations, Davis-Monthan soil earthworms also burrowed a quarter of the depth of the soil, but at higher chlordane concentrations, Davis-Monthan soil earthworms only burrowed in a small area underneath the food. All McGuire AFB soil earthworms looked healthy with only the low concentration chlordane soil statistically differing from controls in earthworm weight loss (Figure 17). Davis-Monthan AFB soil earthworms also looked healthy but were shorter and thicker (Figure 18). Weight loss in Davis-Monthan soil earthworms increased statistically in a concentration-dependent manner (Figure 17). This trend was most likely due to the increased concentration of chlordane in the soil; however, it is also possible that there were other confounding factors that generated the dose-response pattern.

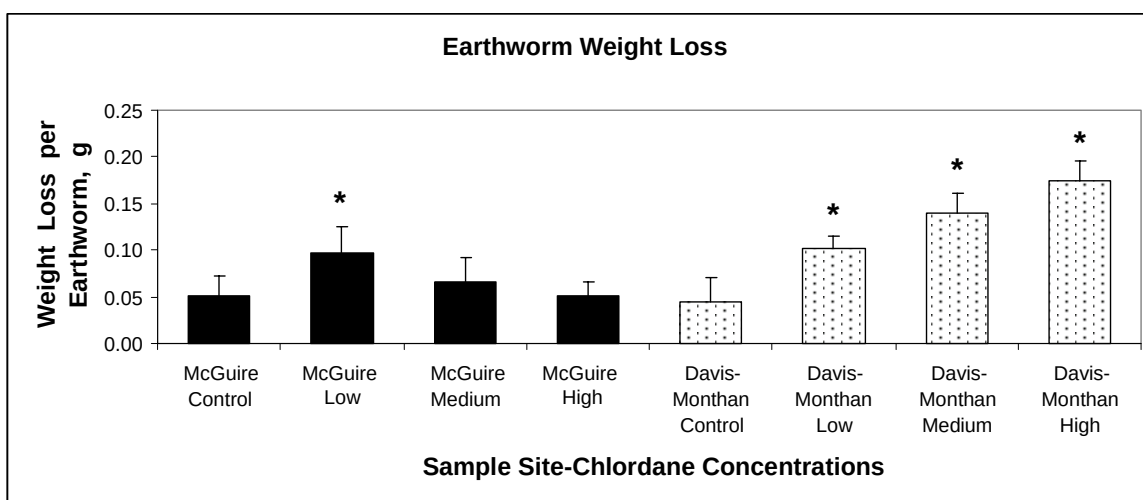


Figure 17. Earthworm weight loss after 28 days in chlordane-contaminated McGuire and Davis-Monthan AFB soils.



Figure 18. Comparison of earthworms exposed to Davis-Monthan (left) and McGuire (right) AFB soils for 28 days.

Earthworm reproduction

The effects of 28-day exposure to chlordane-contaminated soils on reproductive success are shown in Figure 19. Both McGuire AFB and Davis-Monthan AFB control soil earthworms showed healthy cocoon production. In McGuire soils, both low and medium concentration chlordane-contaminated soils significantly reduced earthworm reproductive success. Contrary to the norm, the chlordane in McGuire AFB soil had a negative dose-response, with the biggest effect on cocoon production seen with the low dose and the lowest effect on cocoon production seen with the high dose. In Davis-Monthan soils, all chlordane-contaminated soils stopped earthworm cocoon production. One possible explanation for the differences in the effects of the two soils is the presence of clay fines in the McGuire AFB soil, which may sorb the hydrophobic chlordane and lower its bioavailability to the earthworms.

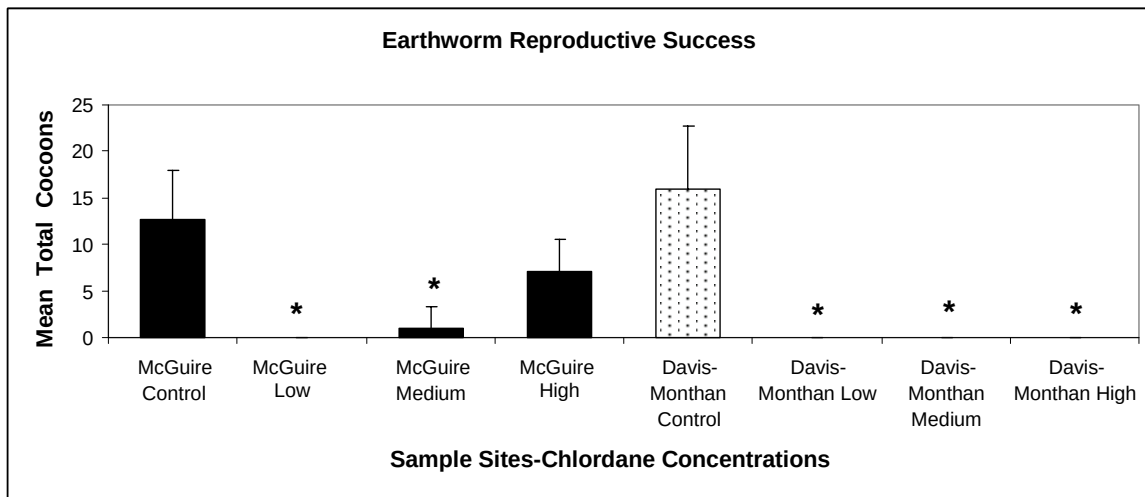


Figure 19. Reproductive effects in earthworms after 28 days in chlordane-contaminated McGuire and Davis-Monthan AFB soils.

4 Conclusions

The first conclusion resulting from this study is that chlordane leaching from the two soils tested (McGuire and Davis Monthan AFBs) is minimal, at least as defined by the TCLP, SPLP, and by DI water extraction (DIWET) tests. In all of these tests, the chlordane levels leached from the soils were less than the detection limit of $0.25 \mu\text{g L}^{-1}$. The TCLP in particular has a regulatory function, as soils that have concentrations below key thresholds can be defined as non-hazardous and can be disposed of in a standard, municipal waste landfill. It is not particularly surprising that leaching was minimal, as it is well-established that soil weathering (aging) generally results in residual contamination becoming highly bound to the soil matrix (Cline et al. 1994; Gong and Kolluru 1996; Loehr et al. 2001; Shirer et al. 2003; Fendorf et al. 2004; Stanforth et al. 2005).

At the same time, it is critical to recognize the shortcomings of these extraction tests. One particular difference is the time frame involved. The TCLP and SPLP are 18-hr extractions while the DIWET is a 40-hr extraction. These are very short time periods compared to the long-term exposure of these soils (≥ 20 years since first exposure). Somewhat longer studies can be conducted, including multiple extraction procedures, column leaching studies, and rainfall lysimeter studies (Larson et al. 2007).

A second conclusion is that chemical treatment may not be effective at treating the chlordane in the soil. Chemical treatment of aqueous solutions of chlordane suggested that lime treatment can result in removal of chlordane if the chlordane is dissolved in an aqueous solution. However, the soil slurry studies indicated that chemical treatments (both lime and persulfate) were not effective in treating the contaminants in the McGuire and Davis-Monthan AFB soils. Presumably, aging processes have strongly sorbed the chlordane to the soil, limiting its ability to react with the chemicals. Preliminary desorption of the chlordane from the soil may improve chemical remediation results.

Biological treatment, stimulated by the addition of used mushroom substrate, showed promise as a treatment approach, particularly if treatment time is not a limiting factor. Like chemical treatment, biological processes are believed to be limited to contaminants that are dissolved in the liquid phase. It seems, therefore, a bit surprising that the biological treatment worked better than the chemical treatment because it, too, should be limited by contaminant transfer in the liquid phase. It appears that the biological process must make the contaminant more available for treatment as opposed to abiotic, chemical treatment.

Spent mushroom substrate was chosen because of the recognition of the versatile enzymes that fungi can have that enable them to degrade a wide range of recalcitrant contaminants (Boonchan et al. 2000; Johannes and Majcherczyk 2000; Milstein et al. 2001; Szewczyk et al. 2003; de Boer et al. 2005; Takagi et al. 2007). Fungi are also recognized for their ability to exude exoenzymes, or enzymes that are active outside of their cell membranes (Jabra-Rizk et al. 2004; de Boer et al. 2005; Takagi et al. 2007). It is possible that the fungal organisms in the waste have exoenzymes capable of desorbing the chlordane from the soil making it more susceptible to degradation.

The use of spent composting material to simulate bioremediation has been successful for the treatment of many xenobiotic compounds (Semple et al. 2001). These materials are rich in nutrients and have diverse microbial floras, including bacteria, actinomycetes, and fungi. Semple et al. (2001) describe successful applications for pesticides, such as stimulated treatment of 2,4-D with grass clippings and degradation of diaznon, chlorpyrifos, and pendimethalin stimulated by the addition of garden waste compost.

Chlordane did not result in any obvious toxic effects on plants, but may have had some sub-toxic effects. Chlordane did not adversely affect seed germination of the plants tested compared to the controls. Nor did chlordane affect the final root and shoot lengths of the plants. However, chlordane appeared to have delayed initial germination of the Kentucky Bluegrass and Perennial Ryegrass.

In a similar manner, chlordane-laden soil did not cause earthworm lethality, but did result in sub-lethal effects on earthworm growth and reproduction. Although data are preliminary, chlordane appeared to cause a

concentration-dependent avoidance response in McGuire AFB but not in Davis-Monthan AFB soil. This difference may be due to reactions between components of the Davis-Monthan soil geochemistry and the chlordane. The sub-lethal effects on earthworm weight gain and reproduction, if validated at larger scale, could have repercussions on population-level loss of adult and juvenile earthworms due to weight loss and reproductive failure, respectively.

The studies also indicated that chlordane in the test soils was available to the organisms (grasses and earthworms) tested. The plants tested accumulated the chlordane from the soil and translocated the compound to the shoots. Similarly, appreciable concentrations of chlordane were found in the digested earthworm tissue. The biota-soil accumulation factor for McGuire AFB soil was 2.35, 1.38, & 2.11 for the low, medium, and high concentration soils, respectively. These data indicate that the earthworms accumulated more chlordane than is found in the soil.

Earthworms, being organisms that have evolved to live on organic materials extracted from soil, are, not surprisingly, very efficient at extracting contaminants from soils. In a study conducted by Gevoa et al. (2001), earthworms could uptake small (0.02 to 2%), but measurable, pesticide residuals that were “non-extractable” following a series of Soxhlet extractions (which are much more rigorous than the leaching/extractions used in this study to evaluate the stability of the chlordane).

The results suggest that the earthworms themselves may desorb the chlordane from the soil matrix increasing its bioavailability. In contrast, each leachability test (TCLP, SPLP and DIWET) had chlordane concentrations that were below detection. Even the PBET, which aims to mimic acids and conditions found in the human digestive system, did not result in detectable chlordane extraction. The ineffectiveness of the chemical treatment for the contaminated soils was consistent with the non-detect extraction levels. However, the biological treatment had more effectiveness at removal of the chlordane. Further, chlordane was detected in both plant tissue and in digested earthworms. Perhaps these organisms have enzymes, exudates, or organic acids that are effective at chelating the chlordane, and making it more available for uptake and for biodegradation (Juwarkar et al. 2007).

It is possible that different extraction methods should be used when evaluating persistent organic chemicals in soil. For example, semipermeable membrane devices (SPMDs) have been used to extract hydrophobic organic contaminants from water, sediment/soil, and air (Petty et al. 2000). SPMDs are made of lipids, such as triolein, that mimic the lipid content of organisms as well as the passive diffusion-mediated uptake mechanism of organic chemicals (Petty et al. 2000). This not only allows for detection of organic chemicals down to the part per trillion level, but also better represents the bioavailability and bioaccumulation potential of organic chemicals in organisms. This is especially true for organic chemicals with high octanol-water partitioning coefficients (K_{ow}), an indication of high hydrophobicity and high persistence in environmental media, such as soils. SPMDs have been shown to detect persistent organic chemicals, such as polyaromatic hydrocarbons (PAHs), organochlorine pesticides (e.g., chlordane) and polychlorinated biphenyls (PCBs) in laboratory- and field-based experiments (Petty et al. 2000; Norrgren et al. 2000; Verweij et al. 2004; Charlestra et al. 2008).

These results also indicate that effects on organisms are greater than one might expect from simply the leachability/extraction tests. Although general toxicity was not increased, specific chronic toxicity effects were noted; i.e., germination for some plants was delayed, and growth and reproductive effects were found with the worms. Many studies show a strong correlation between health effects and various extraction and leachability tests (Nakajima et al. 2005; Simpson et al. 2006; Furman et al. 2006). However, there are cases where there are differences (Schultz et al. 2004; Wiklund and Broman 2005; Puglisi et al. 2007). Understanding the conditions that create these discrepancies between extraction data and live organism studies could be an interesting area for further research.

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14. ABSTRACT Chlordane was used for termite prevention in Air Force housing areas, where it was sprayed heavily on the ground around building exteriors. Base closure regulations require the Air Force to assess residual chlordane in the soil. The USACE-ERDC team examined two soil types containing chlordane residuals; a Silty Sand and a Sand soil type from housing areas at McGuire AFB, NJ, and Davis-Monthan AFB, AZ, respectively. Using these two soils, chlordane leachability was evaluated; the potential for chemical or biological degradation of the chlordane was evaluated; the potential toxicity of the chlordane was assessed using plant and earthworm bioassays. Leachability was tested using four different procedures. The potential for chemical remediation was evaluated by alkaline hydrolysis and persulfate oxidation. The potential of biodegradation was evaluated by composting with spent mushroom waste. The soil toxicity and uptake of chlordane into plants and earthworms was also assessed. The results of these investigations indicated that aged chlordane in the soil was not likely to desorb and migrate under either landfill or physiological conditions. Chlordane in solution was susceptible to photodegradation and alkaline hydrolysis, chlordane in soil was not. Chlordane in soil was also not susceptible to persulfate oxidation. Composting did show promise as a long-term ex situ remediation strategy. Biouptake results indicated that chlordane did not adversely affect seed germination, root length, or shoot length. The plants did uptake chlordane from the soil and translocate it to the shoots. The presence of chlordane did not affect earthworm mortality, but did affect weight loss and reproductive success.							
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