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ScienceDirect
Ecotoxicology and Environmental Safety 68 (2007) 326–334
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Highlighted article

Effects of toxaphene on soil organisms

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Received 29 January 2007; received in revised form 12 April 2007; accepted 3 May 2007
Available online 26 June 2007

Abstract

The polychlorinated insecticide toxaphene belonged to the most used pesticides in the 20th century. Even recently, significant residues have been found in soils at various sites in the world. However, knowledge on toxicity to soil organisms is limited. In this study, the effects of toxaphene on soil invertebrates *Folsomia candida*, *Eisenia fetida*, *Enchytraeus albidus*, *Enchytraeus crypticus*, *Caenorhabditis elegans*, and microorganisms were investigated. Among the organisms tested, *F. candida* was the most sensitive. The 50% effect on survival and reproduction output (LC_{50} and EC_{50}) was found at concentrations of 10.4 and 3.6 mg/kg, respectively. Sensitivity of other organisms was significantly lower with effective concentrations at tens or hundreds of mg/kg. Our data on soil toxicity were recalculated to soil pore-water concentrations and good accordance with available data reported for aquatic toxicity was found. Since soil concentrations at some sites are comparable to concentrations effective in our tests, toxaphene may negatively affect soil communities at these sites.

Keywords: Toxaphene; Soil pollution; Soil organisms; Pesticides

1. Introduction

The polychlorinated insecticide toxaphene (CAS 800-35-2) is a complex mixture of several hundreds of congeners, mainly polychlorinated bornanes, with average chlorine content of 67–69% (de Geus et al., 1999). It belongs to the group of persistent organic pollutants (POPs) listed in international conventions (UN-ECE, 1998; UNEP, 2001) and was banned in many countries. Toxaphene shows ubiquitous occurrence in the environment as a result of extensive usage since 1945 until the mid-1980s, when it was one of the most widely used insecticides. Total global use of toxaphene between 1950 and 1993 was 1.3 million tons (Voldner and Li, 1993). Toxaphene distribution is worldwide and significant levels were also found in the arctic environment and biota (Bidleman et al., 1989).

Toxaphene has been shown to be biomagnified in the food chain (de Geus et al., 1999; HSDB, 2001). It was found to be highly toxic to aquatic organisms, especially to fish (Saleh, 1991). This chemical proved to induce toxic actions such as nephrotoxicity, hepatotoxicity, and reproductive effects (de Geus et al., 1999). Endocrine toxicity and mutagenicity were reported in *in vitro* studies (de Geus et al., 1999). As reviewed by Saleh (1991), the acute LD_{50} of toxaphene to laboratory mammals ranged from 5 to 1075 mg/kg body weights. Toxaphene was found to be highly carcinogenic in rat and mice (Saleh, 1991) and therefore it is probably also a human carcinogen (Group 2B; IARC, 2001).

Toxaphene field application rates in agriculture were usually from 1 to 4 kg/ha (FAO/WHO, 1973). The toxaphene content decreases with soil depth where total toxaphene residues between 85% and 95% were found in the upper 20 cm of soils (cultivated layer; HSDB, 2001; ATSDR, 1998). Similar to all POPs, toxaphene is strongly sorbed to soil particles, with $\log K_{oc}$ between 2.5 and 6

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0147-6513/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2007.05.009

(HSDB, 2001) and water solubility is max 3.3 mg/l. Transport in soil is very slow and leaching to groundwater improbable. Its half-life in soils was assessed to be between 100 days and 14 years depending on the soil type, climate, and possible degradation capabilities of soil microorganisms (HSDB, 2001; ATSDR, 1998). Losses of toxaphene from soils are due to volatilization, photodecomposition, chemical, and/or microbial degradation. The potential for volatilization increases if the soil matrix has a significant sand fraction (ATSDR, 1998). Aerobic degradation of toxaphene in soil is slow (Buser et al., 2000) while anaerobic degradation is faster because of reductive de(hydro)chlorination mediated by microorganisms (half-life from one to several months; Fingerling et al., 1998).

In all reports and studies published so far, toxaphene soil contamination was particularly considered as a possible source for water contamination or as a possible route of human exposure. There are much data on human toxicity or aquatic ecotoxicity, but effects on soil organisms have not been studied so far. In our study, the effects of toxaphene on soil invertebrates *Folsomia candida*, *Eisenia fetida*, *Enchytraeus crypticus*, *Enchytraeus albidus*, and *Caenorhabditis elegans* were investigated. Impact on soil microorganisms was also investigated, based on measurements of microbial biomass, respiration activity, ammonification, and nitrification. The results are discussed, compared with data reported on aquatic toxicity and with environmental levels that have been measured recently.

2. Materials and methods

2.1. Experimental soils

Artificial soil (OECD, 1984) was made of 70% fine quartz sand, 20% kaoline clay, and 10% finely ground peat proportionally mixed on a dry weight basis. pH_{KCl} was set to 6.0 ± 0.5 with $CaCO_3$ at the beginning of the tests and was found to increase to 6.5 ± 0.5 at the end of the tests. The water content of artificial soil was adjusted to 50% of maximal water holding capacity (WHC_{max}) at the beginning of the tests and evaporated water was replenished weekly, if necessary. All tests were performed in artificial soil except for the assay with *C. elegans*. This soil was found unsuitable for the *C. elegans* test because the peat floated on the surface during the extraction procedure and disabled counting of worms. Hence, a natural soil was used for the *C. elegans* tests. The same soil was used in the microbial experiment. This soil was collected from the top-soil layer (0–10 cm) in pristine area of South Moravia, Czech Republic. The soil was a loamy sand cambisol with the following particle size distribution: sand (>50 μm) 64.4%, silt (2–50 μm) 29.1%, and clay (<2 μm) 6.5%. The total cation exchange capacity was 16.4 meq/100 g and the pH_{KCl} was 6.5. Organic carbon and total nitrogen contents were 1.6% and 0.13%, respectively. Organic pollutants and heavy metals contents were comparable to the background levels according to the Czech Republic guideline (Ministry of Environment of Czech Republic, 1996). The soil was dried, sieved (2 mm), and then used for *C. elegans* tests. For microbial experiments, the fresh soil was sieved (2 mm) and stored at 4 °C in the field-moist condition (91.5% of dry matter).

2.2. Sample preparation

Toxaphene standard (product number PS79; ampoule of 1 g neat) was produced by Supelco and received from Sigma-Aldrich Ltd. (Czech Republic). The identical spiking procedure was used in all invertebrate tests. Toxaphene was dissolved in acetone (HPLC grade; Chromservis Inc., Czech Republic) and dilutions were prepared with the acetone solution to obtain required soil concentrations (specified for each test thereinafter). The soil surface in each test container was sprinkled with acetone solution to reach the appropriate chemical concentration. In all tests, spiking ratio was kept 1 mL of acetone per 10 g of soil (dry wt) for minimal effect of acetone on organisms. Pure acetone was used for control samples. Acetone was evaporated under the fume hood overnight after which each sample was thoroughly mixed and distilled H_2O was added to adjust soil moisture to 50% WHC_{max}. Spiked soil samples were left for 1 day to stabilize before organisms were introduced. The spiking procedure differed for the microbial experiment and is described thereinafter. Range-finding tests were performed (results not shown in this paper) to select concentrations for final tests.

2.3. Eisenia fetida test

A permanent culture of *E. fetida* was maintained in the mixture (1:1) of horse manure and peat. Water content was approximately 80% WHC_{max} and the pH was adjusted to 6–7. The temperature was 20 ± 2 °C. The performance of the test was based on OECD Guideline 222 (OECD, 2004a), but it differed from that guideline in terms of number of container repetitions for each exposure concentration and evaluated endpoint (number of cocoons). Nine exposure concentrations (1, 10, 25, 50, 100, 200, 400 and 800 mg/kg) and controls (water and solvent) were used. Test containers (1 l volume) per concentration with 500 g of soil (dry wt) were prepared and 10 adult worms with clitellum (300–400 mg) were introduced into each container. Food (5 g of dry ground horse manure) was added weekly under the soil surface. Survival and reproduction (number of cocoons) were evaluated after 4 weeks by manual counting. The test was kept in 20 ± 2 °C and under 16/8 light–dark cycle.

2.4. Enchytraeid tests

Permanent cultures of enchytraeids were maintained in a mixture of artificial soil and commercial garden substrate (50–60% WHC_{max}; pH 6–7) at 18 ± 2 °C and were fed with finely ground oat flakes. Worms were transferred to a Petri dish with water and examined under a microscope to find adults with well-developed clitellum that were used for tests. Tests with both species were performed according to OECD guideline no. 220 (OECD, 2004b). Concentration series of 10, 25, 50, 100, 250, 500, and 1000 mg/kg and 1, 10, 100, and 1000 mg/kg were prepared for *E. albidus* and *E. crypticus* tests, respectively. Five replicates of test containers were used for each concentration and controls. Twenty (*E. albidus*) or 10 (*E. crypticus*) grams of soil (dry wt) were prepared and 10 adult enchytraeids were added into each test containers after spiking. Containers were covered by lids and incubated at 18 ± 2 °C. Oat flakes were added at the beginning of the test and after that weekly. Animals were exposed for 42 days (*E. albidus*) and 28 days (*E. crypticus*). At the end of exposure periods, the worms were killed by 5 mL of ethanol applied to the soil and dyed by Bengal red. Survival of adults and number of juveniles were manually counted. Tests were kept under 16/8 light/dark cycle.

2.5. Folsomia candida test

Permanent culture of *F. candida* was maintained in plastic containers with a thin layer of plaster of Paris and charcoal mixture (9:1) at 20 ± 2 °C. Animals were fed with dried baker's yeast. Age synchronized juveniles (10–12 days) were used for the test. The test was performed according to ISO 11267 guideline (ISO, 1999). The test was carried out in glass containers with 30 g of soil (dry wt). Five replicates were designed for each concentration (1, 2, 4, 6, 8, 10, 20, and 40 mg/kg) and controls. Ten synchronized organisms were introduced into each container and about 10 mg of dried baker's yeast were added at the beginning of test and after 14 days. Test containers were closed with parafilm and incubated at