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Science of the Total Environment 363 (2006) 237–244

Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Humankind

www.elsevier.com/locate/scitotenv

Effects of perchlorate on earthworm (*Eisenia fetida*) survival and reproductive success

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Received 26 January 2005; accepted 25 May 2005
Available online 7 July 2005

Abstract

The effects of perchlorate on earthworm, *Eisenia fetida*, survival and reproductive success were evaluated using three types of tests: dermal contact (filter paper), sand, and artificial soil. All studies utilized a range of perchlorate concentrations in order to simulate levels that are likely to occur in the environment under different scenarios (typical soil levels vs. spill levels). The OECD filter paper contact test involved exposing earthworms to the test compound on moist filter paper for 14 days to evaluate earthworm survival under a worst-case dermal exposure scenario. A similar test involved exposing the earthworms to perchlorate-contaminated sand, where earthworm survival decreased as concentration of perchlorate increased, with no worms surviving 14 days at the highest treatment concentrations (>2000 μg/g). However, the perchlorate concentrations that affected the survival of *E. fetida* are likely to occur only under extreme conditions (e.g. spills). The effect of perchlorate on the reproductive success (cocoon production) of *E. fetida* over a 4-week test period in artificial soil and a 3-week test period in sand was also examined. Production of cocoons was observed in soil containing up to 100 μg/g perchlorate, with no production in the uppermost treatment groups (1000 μg/g). Cocoon production was highest in the control group, although overall cocoon production appeared to be low. In contrast to the acute toxicity tests, perchlorate did affect earthworm reproduction at environmentally relevant soil concentrations. In addition, preliminary data suggest that cocoons produced under perchlorate contamination did not hatch as well as cocoons produced in control soil despite incubation of both sets of cocoons in clean soil or sand.

Keywords: Earthworm; Perchlorate; Reproductive toxicology; *Eisenia fetida*

1. Introduction

Perchlorate (ClO_4^-) as ammonium, sodium, and/or potassium perchlorate has been used in pyrotechnics, explosives, and as an oxidizer in solid rocket propel-

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doi:10.1016/j.scitotenv.2005.05.020

lants. Perchlorate is quite soluble, extremely mobile in aqueous systems, and rather nonreactive (stable) in surface water and soil. For this reason, perchlorate as a contaminant can persist for many decades under typical ground and surface water conditions (Urbansky, 1998). With the improvement of analytical capabilities (Okamoto et al., 1999; Wirt et al., 1998; Jackson et al., 1999, 2000), widespread perchlorate contamination has been found, with confirmed releases in at least 20 states and manufacturers in 40 states (USEPA, 1998). As a result of perchlorate occurrence and persistence, there are potential human health and ecological concerns regarding the contaminant. In contrast to the abundance of human health data, few ecological data exist on the potential exposure and effects of perchlorate in the environment (Smith et al., 2001). There are especially few data regarding the presence in and effects of perchlorate on invertebrates, such as earthworms.

Earthworms are important in the later stages of soil formation, in maintaining soil structure and fertility (Edward and Lofty, 1977), and can be utilized as a tool to evaluate different transformations and impacts. Agricultural activities such as plowing, various tillage operations, fertilizing, and application of chemical pesticides have a dramatic influence on these animals. As such, the limited mobility of earthworms makes them an appropriate species for monitoring the potential impact of contaminants and changes in soil structure (Paoletti, 1999).

Ecologically, earthworms are near the bottom of the terrestrial trophic food chain and have many predators, such as birds. Earthworms are not only sensitive to chemicals but have a tendency to concentrate compounds such as organochlorine insecticides and heavy metals in their tissues (Edwards, 1983). Some of these chemicals may have little effect on earthworms directly but may either kill predators that consume the earthworms or be taken up into predator tissues, therefore affecting animals higher in the terrestrial food chains.

In addition to their key roles in soil fertility and trophic transfer, earthworms are common in a wide variety of soils, both in temperate countries and the tropics. These organisms are easily bred in the laboratory for toxicity testing and their longevity makes it unlikely that numerous worms would die during the period of a toxicity test in untreated media (OECD, 1984). In addition, they are relatively large in size, easy to handle, and can be readily collected and identified. From data collected in laboratory and field studies, earthworms are known to be affected by a number of organic and inorganic chemicals, which may also be taken up into their tissues and concentrated. Earthworms are capable of absorbing chemicals through respiratory exchange by diffusion through the skin, which is closely underlain by capillary networks. The capillaries allow the circulating blood to attain oxygen and eliminate carbon dioxide through the moist body surface (Gaddie and Douglas, 1975).

Eisenia fetida has gained recognition as a possible representative test organism for earthworms and other soil invertebrates (Roberts and Dorough, 1984). As such, studies have been conducted that have substantiated the toxicities of various chemicals to *E. fetida*. Inorganic compounds that are ionized in solution and similar to ammonium and sodium perchlorate, such as ammonium nitrate, methyl iodide, sodium chloride and sodium nitrate, were tested in 2-day filter paper contact tests and found to be moderately toxic (100–1000 μg/cm^2) to *E. fetida* (Roberts and Dorough, 1984). In a separate study, LC_{50}s for cadmium chloride, cadmium nitrate, copper chloride, copper nitrate, lead nitrate, nickel chloride, nickel nitrate, zinc chloride, and zinc nitrate tested in 2-day contact tests ranged from 5.63×10^{-4} to 6.32×10^{-3} mg/kg (Callahan et al., 1994). More recently, the toxicity of sodium perchlorate to *E. fetida* was evaluated in acute and sub-chronic tests in artificial soil. From these studies, estimated acute and sub-chronic LC_{50}s for sodium perchlorate to *E. fetida* were 4450 and 5071 μg/g, respectively (EA Engineering, 1998; Parsons, 2002).

The studies presented here are a representative selection of investigations that we performed on *E. fetida* to identify potential toxic effects of sodium and ammonium perchlorate on earthworm survival and the effect of sodium perchlorate on reproduction.

2. Materials and methods

2.1. Reagents and specimen

A 100 μg/mL certified perchlorate standard (Accustandard, Inc.) was used to prepare perchlorate