Abstract
Earthworms, Lumbricus terrestris, exposed in artificial soil to sublethal concentrations of technical chlordane (6.25, 12.5, and 25 ppm) and cadmium nitrate (100, 200, and 300 ppm) exhibited significant reduction in spermatozoa from testes and seminal vesicles. The onset time of reduction varied with exposure concentration, but absolute depression in sperm count was independent of exposure concentration or exposure duration after reduction was first manifested, demonstrating a threshold effect. Earthworm sperm counts show potential as a rapid-measurement endpoint biomarker for measuring sublethal effects of chemical pollutants on reproduction.

INTRODUCTION
The development of reproduction-based biomarkers using earthworms should benefit the ecological-assessment process for terrestrial hazardous-waste sites (HWS). Central to the process are measurement and assessment endpoints (Suter, 1989). Assessment endpoints are environmental attributes (e.g. wildlife populations) that are understood and valued by both the public and decision makers and can be assessed objectively. Measurement endpoints should be easily measured, toxicity-based biological parameters that correspond to and/or are predictive of toxic effects on assessment endpoints. Alterations in the reproductive process in individuals should both correspond to and portend effects at the population level. As such, they have potential as measurement endpoints for assessing sublethal toxicity of terrestrial pollutants.

Here we present data showing that sperm count in earthworms, Lumbricus terrestris, shows potential as a sensitive biomarker for measuring effects of inorganic and organic chemicals on reproduction. Earthworms are ecologically important soil organisms throughout the USA, and toxic effects on their gametes should correspond directly to their populations and, indirectly, to other wildlife. Our rationale, with data, for selecting earthworms to develop biomarkers for use as surrogates for vertebrates is assessing risks to public and environmental health is presented elsewhere (Goven et al., 1988; Venables et al., 1988; Rodriguez et al., 1989; Fitzpatrick et al., 1990; Eyambe et al., 1991; Chen et al., 1991; Venables et al., 1991; Fitzpatrick et al., in the press).

MATERIALS AND METHODS
Source and maintenance of earthworms
Adult L. terrestris, purchased from Carolina Biological Supply (Burlington, NC), were held for two weeks prior to experimentation. During this time, they were maintained in moistened peat moss and fed with high-protein baby cereal in an unlighted environmental chamber at 10°C. General earthworm health was assessed before experiments in terms of behaviour, overall condition, body mass, and a 48-h reference toxicity test involving the use of 2-chloroacetamide (Edwards, 1984).

Exposure of whole earthworms to chemicals
Earthworms were exposed to technical chlordane and cadmium nitrate (CdNO₃) by using a modification of the artificial-soil (AS) protocol described by Greene et al. (1989). The AS protocol enables earthworms to be exposed to chemicals or their mixtures in a manner that is more relevant to field conditions than other laboratory techniques (e.g. filter-paper exposure). Chlordane and cadmium nitrate (hereafter cadmium) were dissolved in acetone and water, respectively, and then mixed with AS by the method of Edwards (1984). Acetone was evaporated from AS under a hood before earthworms were introduced. Highest nominal-exposure concentrations of each chemical necessary to produce observable sublethal effects were determined through range-finding tests. Adult earthworms, in groups of nearly equivalent masses (4-6 g) were then exposed to chlordane (6-25, 12-5, and 25 ppm) or cadmium (100, 200, and 300 ppm) mixed with 100 g of AS in 250-ml
Effects of chemicals on sperm count
Sperm counts, body mass, and combined mass of seminal vesicles and testes were determined for earthworms removed periodically from the exposure media during a 16-d period. After weighing, earthworms were transferred into dissecting dishes, where seminal vesicles and testes were removed. Dissected organs, blotted with filter paper to remove excess fluids, were weighed and then placed into 20-ml glass vials containing 2 ml of sperm-counting fluid (5 g sodium bicarbonate : 1 ml neutral formalin : 100 ml distilled water). After sperm had been released into the fluid by teasing apart the organs, 8 ml of fluid were added to the vials, and the entire volume was filtered through a funnel lined with fine double gauze (375 µm) into fresh 20-ml vials. Two counts per earthworm were made in an improved Neubauer hemacytometer chamber. Sperm were allowed to settle for 2 min prior to counting. To obtain the number of spermatozoa per mg or per organ of each worm, the number of sperm estimated within 10 ml of solution was divided by the biomass of the respective organs. Results were expressed as (x ± SE) total sperm counts per mg or per organ for each earthworm and chemical-exposure concentration. Results were analyzed by using non-parametric two-way analysis of variance (ANOVA) and Dunnett’s non-parametric multiple-range test (MRT; α = 0.05) (SAS, 1985).

RESULTS
The mass of testes/semen vesicles did not change significantly during exposure for both controls and experimemtal (Dunnett’s parametric MRT, α = 0.05), which indicated that neither culture media nor the two chemicals affected tissue mass and/or water content of these reproductive organs. However, sperm counts varied during exposure to both chemicals but not in controls (Figs 1 and 2). Non-parametric two-way ANOVA indicated highly significant effects of exposure to both chlordane and cadmium (F = 14.94, p = 0.0001 and F = 17.09, p = 0.0001, respectively). Sperm count was depressed significantly, relative to initial controls, after a 7-d exposure to 12.5 and 25 ppm chlordane (Dunnett’s non-parametric MRT, α = 0.05). Continuation of exposure at 6.25 ppm for 21 d was necessary before a significant sperm-count depression occurred (not shown in Fig 1). Sperm count was depressed significantly after a 9-d exposure to 300 ppm cadmium, and after 16 d at 100 and 200 ppm (α = 0.05). Although the onset of sperm-count depression varied with exposure concentration, an absolute reduction in spermatozoa was not dependent on the concentration of either chlordane (except 6.25 ppm, which produced no observable effects) or cadmium. Sperm count was also not dependent on the exposure time to either chemical once the initial depression had occurred. Running controls (i.e. those at each exposure duration) were not significantly different from initial controls (Dunnett’s non-parametric MRT, α > 0.05).

DISCUSSION
The pattern of reduction in spermatozoa suggests that both chlordane and cadmium had threshold effects. Although depression occurred sooner in the higher-exposure concentrations of both chemicals, once manifested, a reduction in spermatozoa did not vary with either exposure time or concentration. Chlordane depressed the sperm count at exposure concentrations substantially lower than those of cadmium, and earlier (7 as compared with 9 d) at the highest concentrations (25 and 300 ppm). However, the magnitude of depression was similar for both chemicals. Since tissue concentrations were not determined, speculation on threshold level and the relative effects of chlordane and cadmium on earthworm sperm count is not possible. We are not aware of previous reports on decreased sperm counts in earthworms exposed to metals or
organochlorine pesticides. However, results reported here are in accordance with preliminary data from our work (see Venables et al., 1991) with Superfund HWS soil contaminated with chlordane, among other organochlorine pesticides and metals (see Callahan et al., 1991). *L. terrestris* exposed to a sublethal mixture of HWS soil (5%) and AS (95%) for 15 d showed significantly decreased sperm counts similar to those reported here: absolute counts (x ± SE = 1038 ± 270) were 39% of controls (2682 ± 386).

Long-term exposure to metals and organochlorine pesticides has been reported to affect cocoon production (Hartenstein et al., 1979; Malecki et al., 1982; Neuhauser et al., 1984; Ma Wei-chun, 1984), hatching success (Tomlin & Miller, 1980; Venter & Reinecke, 1985) and sexual development (Venter & Reinecke, 1985) in earthworms. Although these reproductive parameters show promise for assessing the effects of chronic exposure to environmental pollutants, sperm counts appear to offer a more rapid measurement endpoint biomarker for laboratory and in-situ field studies. For example, exposure to 100 ppm cadmium nitrate for 16 d resulted in a significant sperm count depression, whereas 20 weeks of exposure to the same concentration were required to reduce cocoon production in *E. foetida* (Malecki et al., 1982).

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**REFERENCES**


