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Acute Toxicity of Organic Extracts of Municipal Sewage Sludge in Mice

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The disposal of sludge - the solid residue that is left after wastewater is treated to remove environmental contaminants - is a growing problem for more and more communities. Approximately 8 billion gallons of municipal waste containing some 17,000 dry tons of sludge are produced daily in the United States (NIEHS 1977). Sludge processing and disposal account for the largest single share of the total operation cost of the municipal wastewater treatment plant (MICHEL 1970). Disposal methods have included incineration, fresh water dilution, ocean dumping, disposal in landfills, and limited use on lawns, ornamentals, forests and agricultural land (STONE 1978; HARRINGTON 1978).

Estimating the potential hazards from these wastes requires that their toxic properties be determined. Once these characteristics are known, environmentally sound methods of disposal may be developed and employed. Many analytical surveys of the inorganic constituents of sludges have been performed (FURR et al. 1976, SOMMERS 1977; HEMKES et al. 1980). Additionally, the organic compounds in sludges, with particular reference to the health risks associated with land application, have been reviewed by BABISH et al. (1981). Estimates of risk of landspreading of sludges made by federal agencies have considered only the cadmium (Cd), lead (Pb) and polychlorinated biphenyl (PCB) content of sludges (FED. REG. 1978; USEPA 1981).

It is obvious from the surveys already conducted on both the organic and inorganic fractions of sludge that basing land application rates or assessing "cleanness" of a sludge on Cd or PCB content is inadequate. However, any analytical technique employed to examine the contents of sludges will suffer from a bias - it can only find what it is set up to detect. This procedure involves some pre-existing knowledge of possible contaminants.

As part of a continuing program of applying biological endpoints to assess the toxicity of environmental samples, this laboratory has examined the lethality of organic extracts of sludges from two cities. Since the purpose of this type of study was to examine the usefulness of a simple bioassay to characterize the toxicity of the organic fraction of sludges, modeling likely routes of exposure to humans (oral or dermal) was not intended. For these studies the intraperitoneal route was used; this route of administration avoids possible confounding due to bioavailability of the toxic components to the animals.

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EXPERIMENTAL

Collection and Preparation of Samples

In 1980, a letter and questionnaire were sent to municipal wastewater treatment plants in 65 cities. They were requested to participate by returning a representative sample of their sludge to us with the completed questionnaire describing the respective sewage treatment process. Responses were received from 35 cities. Upon arrival the sludge samples were spread on plastic sheets and allowed to air dry at ambient temperature. After grinding in a hammer mill, each sample was thoroughly mixed in a rotating mixer. Thirty gram subsamples were taken for extraction.

The subsamples were placed in a 45 x 123 mm cellulose extraction thimble, which had been washed 3 times with 100 mL of redistilled methylene chloride, covered with a glass wool plug and placed in a Soxhlet extraction apparatus containing 250 mL of redistilled methylene chloride. After 12 hr of refluxing in the Soxhlet apparatus, the methylene chloride was removed under a vacuum at 30°C. This procedure extracted approximately 10% of the starting material. This residue was dissolved in a minimal amount of dimehtylsulfoxide (DMSO) for testing in a bacterial mutagen assay system and median lethal dose (LD_{50}) determination in mice.

Dosing of Animals and Observations

Mice used in this study were male BALB/c obtained from a colony maintained at the NYS College of Veterinary Medicine. All animals were between 3-4 months of age and fed Agway RHM 3000 ad libitum. The light/dark cycle was maintained at 18/6 hr, respectively, with a room temperature of 72°F and relative humidity of 55%. They were housed 5 per cage in polycarbonate cages with softwood bedding chips.

All animals were fasted overnight prior to dosing. Sludge extracts were administered intraperitoneally at a constant volume of approximately 9.5 mL DMSO/kg (~0.2 mL per mouse). Concentrations of sludge extract ranged from 5.2 mg/mL DMSO to 45.9 mg/mL DMSO. Control animals received DMSO only. Observations were made on all animals continuously for the first 4 hr post dosing, 2 times per day for the next 3 days and 1 time per day for the following 10 days.

At the termination of the study, all surviving animals were killed by cervical dislocation and examined for gross lesions. Additionally, all animals dying during the observation period were subjected to a routine gross necropsy. Livers, kidneys and spleens were weighed.

Since many congeners of PCBs are potent inducers of hepatic cytochrome P-450 (NEBERT et al. 1981) and since PCB content of sludges is currently used to set standards for land application, the biological response of P-450 induction was measured in animals

treated with sludge extracts. In this way a comparison could be made as to the more sensitive indicator of the presence of problematic organic compounds, the response of P-450 induction or lethality. Hepatic cytochrome P-450 content was determined by the method of SCHOENE et al. (1972) and expressed per g liver, per mg 9,000 x g supernatant fraction protein and per total liver. Protein was determined by the method of SUTHERLAND et al. (1949). All determinations were made after the 14-day observation period when surviving animals were killed.

Selection of Samples for Lethality Testing

Respondents to the sludge survey included 35 cities. The decision as to which sludge extracts to compare in these studies was based on results of mutagenicity testing in the Salmonella/ mammalian microsome assay (AMES et al. 1975). The extract of the Dallas sludge was the only sample negative in the test for mutagenicity, and was chosen as a possible nontoxic sludge extract. However, the extract of the Boston (Deer Island) sludge was mutagenic with metabolic activation to tester strains TA1537 and TA100 with 106 and 86 revertants per mg extract, respectively; this sample was selected as an example of a relatively toxic sludge extract.

Data Analysis

The median lethal dose and its 95% confidence interval were calculated according to the method of WEIL (1952). Body weights, gross and relative organ weights were analyzed by one-way analysis of variance; differences among means were determined using Tukey's HSD test (SNEDECOR and COCHRAN 1971). The probability of a type I error was set at the nominal 5% level.

RESULTS AND DISCUSSION

Observations Post Dosing

Both treated and control animals exhibited signs of discomfort immediately after dosing. These signs were manifest as frequent examinations of the injection site and increased activity. However, 15 to 30 minutes after dosing, the Boston sludge treated animals began to show signs of effects on the central nervous system. Orientation was affected to some degree in all groups as animals rotated repeatedly in both clockwise or counter-clockwise directions; one animal (dosed at 32.9 mg/kg) turned over continuously for a period of 4-6 minutes. Additionally, motor coordination and gait were adversely affected by the Boston sludge extract in nearly all the animals. Animals dosed with the Dallas sludge extract showed few of the signs of toxicity of the Boston sludge extract groups and were more nearly like control animals.

All signs of excessive activity had diminished in all animals within the first 4 hr. By 24 hr post-dosing, decreased activity and decreased muscle tone were observed in all sludge groups, while

TABLE 1

Body weights, Food Consumption, Percent Lethality and Mean Time-to-Death for Mice Receiving Municipal Sewage Sludge Extracts Intraperitoneally in 0.2 mL of Dimethylsulfoxide

Dose (mg/kg)	Body Weights[a] Pre-dose (g)	Body Weights[a] At Termination (g)	Estimated Food Consumption[b] (g/mouse/day)	Lethality (No. dead/No. dosed)	Time-to-Death[a] (days)
Control	23.2±0.8	24.8±0.6	3.7	0/10	ND[c]
Boston Sludge					
4.3	22.0±1.7	23.6±0.5	3.6	0/5	ND
6.5	19.7±0.3	21.3±0.6	3.2	2/5	4.5±1.5
9.7	20.9±0.3	22.4±0.3	3.0	0/5	ND
14.6(e)	19.1±0.9	21.2±1.0	-(d)	2/5	3.0±0.0
22.0(e)	21.2±0.6	20.5±1.6	2.4	0/5	ND
32.9(e)	19.5±1.0	—	—	5/5	1.8±0.6
49.3(e)	25.2±0.7	—	—	5/5	1.8±0.4
Dallas Sludge					
11.2	20.9±0.9	23.2±0.6	3.8	0/5	ND
16.9	18.8±0.4(f)	20.6±0.5(f)	4.0	0/5	ND
25.3(e)	23.2±1.2	23.3±0.5	4.0	0/5	ND
38.1(e)	22.8±0.6	21.8±1.5	2.3	2/5	3.0±0.0
57.3(e)	22.9±0.7	16.2±2.4(f)	—	3/5	1.8±0.2
86.0(e)	16.4±0.5(f)	—	2.0	5/5	3.0±0.0
129.0	24.1±0.4	—	1.2	5/5	3.2±0.2
193.5	22.6±0.7	—	2.4	5/5	1.4±0.2
290.3	23.9±0.5	—	1.3	5/5	1.2±0.2
435.4	21.0±1.1	—	—	5/5	1.0±0.0

(a) Each value is mean ± SEM of 2 to 5 observations; (b) estimated from weighing back food not consumed and dividing by surviving animals in cage; (c) no deaths observed; (d) value could not be determined; (e) morality response used in computing LD_{50} value; (f) value is significantly different from control ($p<0.05$) by Tukey's HSD test.

control animals appeared normal. Deaths were recorded at the highest doses for both sludge extracts. Within 6 da, all moribund animals at all dose levels had either recovered or died.

As seen in TABLE 1, mean time-to-death for Boston sludge extract ranged from 4.5 to 1.8 da, decreasing with increasing dose. The same decrease in mean time-to-death with increasing dose was observed for Dallas sludge extract, although the range of 3.0 to 1.0 da was somewhat shorter.

Body Weights and Food Consumption

Body weights were adversely affected in only one group with sufficient survivors to allow for a statistical comparison. Animals receiving the Dallas sludge extract at 57.3 mg/kg lost 30% of their initial weight. Food consumption data were lost for this group; however, from an examination of TABLE 1, it appears that consumption of 2.3 g feed/da/mouse was sufficient to prevent a significant reduction in mean body weights during the observation period. High dose groups for both Boston and Dallas sludge extracts did not consume any food before dying.

Median Lethal Doses

For the Boston sludge extract the median lethal dose was 23.4 mg/kg and the 95% confidence interval was 17.8 - 30.9 mg/kg. These figures were calculated from responses at 14.6, 22.0, 32.9, and 49.3 mg extract/kg.

The median lethal dose for the Dallas sludge extract was 46.5 mg/kg, with a 95% confidence interval of 35.1 to 61.6 mg/kg. Responses at doses of 25.3, 38.1, 57.3 and 86.0 were used in the calculations of the LD_{50} and its 95% confidence interval.

Although the Boston sludge was more toxic, as demonstrated by an LD_{50} approximately one-half the Dallas sludge, both of these sludge extracts would be considered extremely toxic (KLAASSEN and DOULL 1980). This rating is applied to compounds with LD_{50} values below 50 mg/kg.

Necropsy Observations

No gross lesions were observed in any animals which could be associated with the test materials. Additionally, no treatment effects were seen in gross or relative organ weights. Hepatic cytochrome P-450 levels were not significantly different from control values (data not shown). These results indicate that PCB content of these sludges was probably low. In fact, analysis of PCB content of these sludges indicated relatively low levels of 0.84 and 1.00 ppm for Boston and Dallas sludges, respectively.

Overally, this study demonstrates that an organic extract of municipal sewage sludge can be extremely toxic. This toxicity did not correlate with PCB content or mutagenicity; although the

mutagenic sample was more toxic, the quantitative difference was not qualitatively significant.

The relative hazard of the toxic component(s) of these sludges is difficult to assess from this study. However, it is clear that setting standards of sludge application and classification based on analytical measurements of 1 or any number of compounds is inadequate for protection of public health. An effective sludge monitoring and classification program must consider a combination of analytical and biological assays.

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