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Mutagenic Activity (Ames Test) of Wood-Preserving Waste Sludge Applied to Soil

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In both laboratory (McGinnis et al. 1988; Aprill et al. 1990) and field soil treatability studies (Brown et al. 1985a), the majority of hazardous constituents present in creosote/pentachlorophenol (PCP) bottom sediment sludges have been rendered less toxic after undergoing degradation or transformation. However, the present investigators, utilizing the Ames test to monitor potential detoxification of creosote/PCP sludge constituents during field land treatment studies, observed a persistent mutagenic residue remaining in surface soil a year or more after the sludge was applied (Donnelly et al. 1987; Barbee et al. 1992). Not surprisingly therefore, surface runoff from soils amended with creosote/PCP bottom sediment sludge also displayed significant levels of Ames test mutagenic activity even 360 days after sludge application (Davol et al. 1989). It was estimated that three or more years would be required for the mutagenic activity of runoff water discharging from land treated sludge to return to background levels. In addition, after one year of treatment water soluble fractions and leachate extracted from a creosote sludge/soil mixture produced a toxic response in the Microtox™ assay when treated in laboratory batch reactors (Aprill et al. 1990). These studies indicate mutagenic constituents from wood-preservative wastes may not only persist, but also migrate in the soil environment, which could have a significant impact on human health and the environment. The present study was undertaken to better assess the persistence and mobility of mutagenic chemicals in surface soil and soil leachate from a land treated creosote/PCP bottom-sediment sludge by monitoring mutagenic activity with the Ames *Salmonella* microsome mutagenicity assay.

MATERIALS AND METHODS

The creosote/pentachlorophenol (PCP) bottom-sediment sludge (EPA waste code K001; EPA 1994) from a surface impoundment at an active

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wood treatment facility was collected for study. Creosote is comprised of approximately 80-85% polycyclic aromatic hydrocarbons (PAH), 10% phenols, and 5% N-, S-, and O-heterocyclics (azarenes) (McGinnis et al. 1988). PAHs, and other large molecular weight n-heterocyclics, are the chemicals of concern in creosote since they are responsible for most of its mutagenic and carcinogenic activity. Commercial grade PCP consists of 85-90% PCP, 4-8% 2,3,4,6-tetrachloro-phenol, 2-6% other chlorophenols, and trace quantities (~0.1%) of chlorinated dibenzodioxins and dibenzofurans. PCP is not mutagenic in the Ames test and does not cause teratogenesis. However, PCP has moderate acute oral toxicity and can be highly embryotoxic to rats. Chlorophenols have been shown to promote carcinogenesis (McGinnis et al. 1988) and trace levels of chlorinated dioxins and furans have caused acute toxicity and carcinogenesis (Safe 1986).

To model the fate of sludge constituents in undisturbed soil, nine barrel-size lysimeters from the Weswood soil were collected for use in a field experiment. The Weswood soil is a deep silty clay loam alluvial soil (Fluventic Ustochrept) formed on flood plains adjacent to the Brazos River (Brazos County, Texas). In brief, the barrel lysimeters were collected by gently pushing a modified 55 gal. drum into the soil to collect an undisturbed core to a depth of approximately 80 cm. The procedure is described in detail by Brown et al. (1985b). To collect leachate from the bottom of a lysimeter, and to maintain a hydraulic gradient within the soil, three porous ceramic cups (Coors Model 7001:P6C) were imbedded in soil at the bottom, to which a continuous 21 kPa vacuum was applied. To prevent short-circuiting of leachate down barrel sidewalls, the lysimeters were fitted with sidewall flow barriers. The hydraulic integrity of each lysimeter was determined prior to use by leaching a 200 mg/L KBr solution through the soil and plotting the Br- breakthrough curve (BTC). If the BTC was symmetrical, and preferential Br migration down sidewalls was not observed, the lysimeter was used in the study.

To assess the level of background mutagenic activity in the sludge and unamended soil, a 2.3:1 (v/v) dichloromethane:methanol (MeCl2:MeOH) solvent extract of each was assayed in the Ames test. The extracts were collected by Soxhlet extraction of 25g of sludge or soil for six hours, according to recommended procedures for the Ames bioassay (EPA 1985). To determine which solvent was most responsible for extracting mutagenic compounds, two additional extracts were obtained by extracting a 25g sludge or soil sample sequentially with dichloromethane (MeCl2) and methanol (MeOH) on a Tecator Soxtec™ apparatus. The Tecator extraction involves steeping the sludge or soil in approximately 50mL of heated solvent for five minutes followed by soxhlet-like perfusion

for fifty minutes. These extracts were also assayed for mutagenic activity in the Ames test.

The sludge was applied at a rate of 1.64 kg per lysimeter, which was 1.5% (wet w/w) of the zone of incorporation (ZOI) soil weight. This low application rate was chosen due to the sludge's strong mutagenicity (Table 1). Sludge application was accomplished by removing 10.2 cm of surface soil from a lysimeter, mixing the sludge and soil, and then evenly redistributing the sludge/soil mixture on the lysimeter surface. The persistence of mutagenic chemicals in the ZOI was assessed by sampling the sludge/soil mixture immediately (Day 0), 180, and 350 days after sludge application. The ZOI soil samples were extracted for six hours using the 2.3:1 dichloromethane: methanol solvent. All extracts were assayed in the Ames test for mutagenicity.

A yearly rainfall equivalent of about 110 cm was applied to the lysimeters to potentially leach organic constituents through the soil column from the ZOI. Rainfall applications were controlled so that unsaturated soil conditions were maintained in the lysimeters throughout the 350 day study. Soil-pore water leaching to the bottom of each lysimeter was continuously collected in refrigerated (4°C) bottles. Leachate was collected at 90 day intervals from 0 to 350 days after sludge application.

Leachate collected from the bottom of each lysimeter was passed through a packed bed of amberlite (American Scientific Products) XAD-2 and XAD-7 (2Occ each) resins contained in glass Econo-Columns™ (Bio-Rad) in order to extract organic chemicals from the leachate. The maximum volume of leachate passed through each column was 40 L. After leachate passage, the XAD resins were rinsed with 1.2 L of distilled water (-30 bed volumes) to remove residual histidine that might be present. Most excess water was removed from the resin bed by blowing purified N_2 gas through it. Sorbed organic chemicals were then extracted from the resins by rinsing them four times with 40mL of acetone (160mL). Residual water in the acetone was removed with anhydrous Na_2SO_4, and the acetone extract was concentrated to 10mL by rotary evaporation (Brinkman-Bucci, Model R). The organic chemicals remaining were concentrated by evaporating the acetone under a gentle stream of purified N,. The organic residue was weighed, diluted with dimethylsulfoxide (DMSO), and assayed in the Ames test.

The Ames *Salmonella* microsome bioassay (Ames et al. 1975), with suggested modifications (Maron and Ames 1983), monitored the persistence and mobility of mutagenic chemicals in ZOI soil and soil leachate. The *Salmonella* TA98 tester strain with and without metabolic activation (±S9) was used since this strain is most sensitive to frameshift

mutagens present in creosote/PCP sludge (Donnelly et al. 1987). Each soil or leachate extract was tested on duplicate plates in two independent experiments in the standard plate incorporation assay at four dose levels (0.1, 0.25, 0.5, 1.0 mg/plate). On each test date, the TA98 strain was calibrated with a positive control (2-nitrofluorene) and negative control (DMSO). Benzo(a)pyrene was used to verify the functioning of the S9 metabolic activation system. Bioassay data were analyzed and interpreted using the modified two-fold rule (Chu et al. 1981) where a mutagenic response, expressed as a mutagenic ratio (MR), is considered positive if the average mutagenic response for at least two consecutive dose levels was greater than twice the average response for the concurrent DMSO solvent control. Thus, if MR is greater than two, the sample is considered mutagenic.

RESULTS AND DISCUSSION

All solvent extracts of the unamended Weswood soil with and without metabolic activation (±S9) were nonmutagenic in the Ames test (Table 1). A previous investigation (Brown et al. 1985c) showed the Weswood soil had weak indirect (±S9) mutagenicity (strain TA98) in the Ames test. A GC-MS scan of the soil extracts collected by Brown et al. revealed only 13 alkanes (C_{14} to C_{22}) were present in the unamended soil. Undetected mutagenic contaminants, from sources such as tractor exhaust (PAHs) and/or pesticides aerially applied to adjoining cropland, were suggested as possible reasons for the weak mutagenic response of the Weswood soil.

Table 1. Specific and weighted mutagenic activity of the Weswood soil and wood-preserving waste sludge

Sample	Solvent	Specific Activity (revertants / mg extract)				Weighted Activity (revertants / g solid)	
		+S9	MR	-S9	MR	+S9	-S9
Soil	MeCl2:MeOH	9	0.2	13	0.5	3	19
	MeCl2	11	0.3	11	0.4	2	2
	MeOH	11	0.3	32	1.1	14	42
Sludge	MeCl2:MeOH	71	2	0	0	2028	0
	MeCl2	0	0	0	0	0	0
	MeOH	293	8	17	0.6	2209	128

MR = Mutagenic Ratio

The methanol (MeOH) extract of the sludge with (+S9) metabolic activation produced a very strong mutagenic response (MR = 8) in the Ames test (Table I), but did not produce a mutagenic response without

metabolic activation (-S9). The dichloromethane (MeCl2) extract of the sludge did not produce mutagenic activity, neither with nor without metabolic activation. The intermediate mutagenic response from the dichloromethane:methanol (MeC12: MeOH) extract reflects the diluting effect of the nonmutagenic dichloromethane fraction. These results indicate that the wood-preserving sludge exhibits strong indirect mutagenic activity (i.e., requires metabolic activation) and polar compounds are primarily responsible.

The necessity of using different, sequential solvent extractions to isolate mutagenic constituents in media containing complex chemical mixtures is demonstrated by the above results. This may be particularly important when microbial transformations impact the soil chemical detoxification process since biotransformation and/or biodegradation frequently produce intermediate breakdown products which are more polar, mutagenic, and mobile than the parent compound (Abbott and Sims 1989).

Immediately (Day 0) after application to the field lysimeters, the strong indirect (+S9) mutagenicity (MR = 8) of the sludge was reduced considerably (MR = 3) in the ZOI, but still produced positive mutagenic activity (Fig. 1). This reduction of indirect mutagenic activity was most likely due to dilution by the soil. However, 180 days after soil application, the ZOI soil produced a four-fold increase (MR = 12) in indirect mutagenicity (Fig. I), and at day 350 the ZOI soil still had greater indirect mutagenic activity (MR = 4) than the ZOI soil immediately following sludge application (MR = 3).

The ZOI sludge/soil mixture did not produce direct mutagenicity (-S9) immediately after sludge application (Fig. I), which is consistent with the response for the unamended sludge before land treatment (Table 1). However, 180 days after sludge application, strong direct mutagenicity (MR = 9) was observed in the ZOI soil which persisted at least 350 days (MR = 6). These results indicate that soil treatment of the wood-preserving sludge can cause a significant increase in both its indirect and direct mutagenicity over at least 350 days.

The relative changes in direct and indirect mutagenic activity of ZOI soil during the 350 day study (Fig. 2) may be demonstrated by the weighted mutagenic activity (TA98 revertants per gram of soil) of the waste-amended soil. These changes may be the result of additive and/or synergistic effects between direct and indirect acting mutagens and could be attributed to the biotransformation of PAHs and related heterocyclic compounds by soil micro-organisms. Such compound's breakdown products have been shown to be potent direct and indirect acting mutagens.

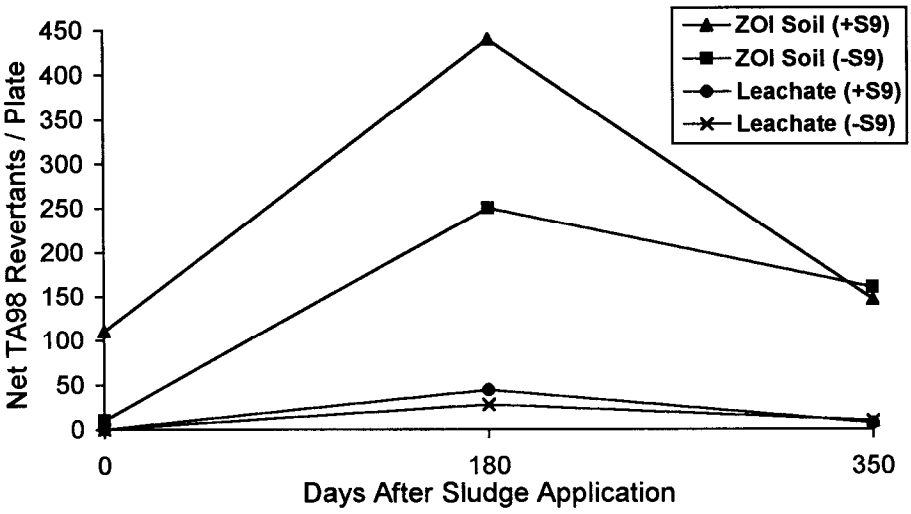

Figure 1. Specific mutagenic activity of ZOI soil and soil leachate in the wood- preserving sludge amended lysimeters.

The first 90 days after sludge application, the concentration of organic compounds in leachate from waste-amended soil was nearly 10 times that from the unamended Weswood soil (data not shown). This difference was statistically significant ($P < 0.05$). However, residue extracts of leachate from the 0 to 90 day interval did not produce a mutagenic response (Fig. 1). After 90 days, there was no significant difference between organic compound concentrations in leachate from the sludge amended and unamended soil. However, organic compound concentrations in the waste-amended soil leachate remained about 1.7 times that of the unamended soil until at least day 350.

Between 90 and 180 days, leachate from the waste-amended lysimeters produced weak direct (MR = 2.3) and indirect (MR = 2.5) mutagenic responses (Fig. 1). Such direct (-S9) and indirect (+S9) mutagenic responses correspond with the increased direct and indirect mutagenic activity of ZOI soil during the same time interval. Similarly for the ZOI soil, however, by 350 days the leachate was again non-mutagenic (MR < 2.0).

The wood-preserving sludge consists of approximately 10-15% “higher” chlorophenols and cresols which have intermediate to high mobility in soil. Such chemicals may be responsible for the initial surge of organic compounds, yet without mutagenic activity, detected in leachate from waste-amended soil collected the first 90 days after sludge application. A GC-MS screen of leachate collected the following 90 to 180 day interval

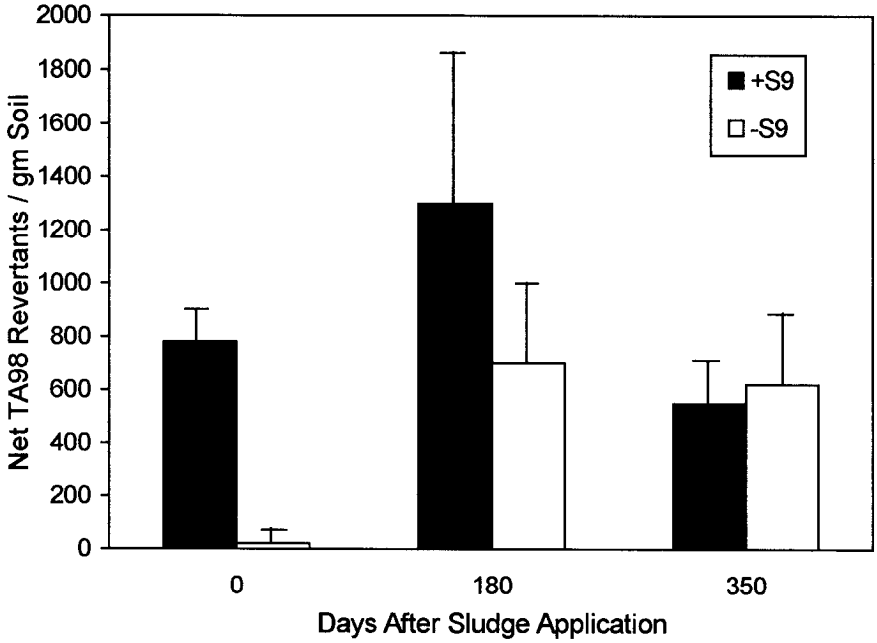

Figure 2. Weighted mutagenic activity of ZOI soil in the wood-preserving sludge amended lysimeters (mean ± SD).

revealed n-alkanes and several mutagenic PAHs were present. These PAHs, and/or their metabolites, may have been responsible for the weak mutagenic activity of the waste-amended soil leachate.

In a long-term laboratory soil column leachability study, leachate toxicity (Microtox™) was observed after 10 pore volumes of column effluent had been collected from a wood-preserving sludge-amended soil (Aprill et al. 1990). In the same study, the soil/waste mixture could still produce toxic leachate after nearly one year of soil incubation. Since many PAHs and nitro-PAHs are water soluble enough to be extracted from a wood-preservative sludge using water and/or become more water soluble due to biotransformation, such mutagenic constituents should have sufficient persistence and mobility in soil to migrate through the vadose zone to groundwater.

The present study demonstrated that mutagenic constituents from a land treated wood-preservative sludge persisted in surface soil at least 350 days after sludge application and were able to leach to at least 70 cm below the soil treatment zone. The greatest levels of mutagenicity were

detected in both the soil in the ZOI and the leachate in the samples collected 180 days following waste application. The mutagenicity of both soil and leachate decreased but did not reach background by 350 days after application. Thus, long-term engineering controls (e.g., dikes, liners, underdrains) are needed to protect surface and groundwaters associated with soil used to treat wood-preserving bottom sludges (EPA K001).

The present study also demonstrated that the Ames **Salmonella** microsome bioassay can be effective in monitoring the fate of mutagenic constituents in the soil environment. Alone, or with a battery of complimentary bioassays (Abbott and Sims 1989; Athey et al. 1989; Aprill et al. 1990), the Ames test could provide an effective means for monitoring environmental mutagens and evaluating human and ecological health risks associated with exposure to them.

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