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TECHNICAL REPORT

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LIST OF TABLES

TABLES CONTINUED

TABLES CONTINUED

ABSTRACT

Municipal solid waste (MSW) landfills have been found to contain many of the same hazardous constituents as found in hazardous waste landfills. Because of the large number of MSW landfills, these sites pose a serious environmental threat to groundwater quality. This study was conducted to assess the environmental hazards that materials leaching from four MSW landfills pose to groundwater supplies.

Four leachate and one upgradient groundwater samples were collected from landfills selected to be representative of landfills of differing ages and types of wastes. Each sample was tested through three genetic toxicity bioassays (The Aspergillus diploid assay, the Bacillus DNA repair assay and the Salmonella/microsome assay) to measure the ability of each sample to induce mutations in bacteria, bind to microbial DNA, or cause chromosome damage in diploid fungi. Genetically toxic chemicals may cause cancer, genetic disease, sterility, abortions, heart disease or a variety of other chronic effects. These chronic effects can be subtle and may not appear for decades after exposure.

In addition to the three genetic toxicity assays, each sample was tested in the Microtox test to measure acute toxicity. This assay is a measure of the ability of the sample to cause cell death. Organisms exposed to elevated levels of acute toxins may express the toxic effects through organ disfunction or the complete death of the organism.

Each sample was chemically analyzed using GC/MS techniques and the chemical concentrations were used to calculate a chemical based risk assessment which is an estimate of the potential carcinogenic health effects associated with the mixture of chemicals in the sample.

All four leachate samples exhibited acute toxicity in the Microtox test. Leachate from landfills representative of both an old unlined landfill which received residential waste and a new operating landfill receiving residential waste contained concentrations of some priority pollutants in excess of promulgated standards for drinking water. Chemical based risk assessments for these same two leachates showed them to have mean and 98th percentile cancer risks of 1 in a thousand (10^{-3}) which is greater than both leachate from a Superfund landfill and leachate from the Love Canal landfill.

The results of the acute and genetic toxicity bioassays, combined with the chemical analyses and associated cancer risk assessment clearly showed that leachate from municipal solid waste landfills is just as toxic as that which leaches from landfills where residential and hazardous wastes were co-disposed.

INTRODUCTION

Municipal solid waste (MSW) landfills have been and continue to be used for the disposal of nonhazardous solid wastes. A survey of operating landfills in the United States (Westat, Inc.; 1988) revealed that there are 3,847 planned and 6,585 active landfills. In 1988 the Office of Technology Assessment estimated that there are approximately 60,000 closed landfills in the nation. Approximately 40% of these landfills are unlined and many others are situated in floodplains, wetlands, or other areas with seasonally high water tables. Landfill leachate can potentially enter the groundwater in these areas (Westat, Inc., 1988).

A review of previous literature on the occurrence and concentration of organic chemicals in MSW landfill leachates concluded that toxic and carcinogenic chemicals were present in the leachate of all MSW landfills studied (Brown and Donnelly, 1988). The sources of these contaminants include municipal solid waste and its degradation products, illegally disposed hazardous waste, and legally disposed small quantity generator hazardous waste. Household MSW has also been shown to contain significant quantities of hazardous chemicals including pesticides, chlorinated and non-chlorinated hydrocarbons and solvents, and organic automotive products such as oil, lubricants, and cooling fluids (Bomberger et al., 1987; Gapiniski, 1988).

The use of landfill liners, both synthetic and compacted

clay, and leachate collection systems decreases the risk of groundwater contamination by landfill leachate. However, leachate collection systems may not always be pumped out frequently enough. In addition, landfill liners leak (an average of 14 holes per acre according to Laine and Miklas, 1989) either due to faulty design and/or construction and result in groundwater contamination (NUS Corp., 1988). Recent data indicate that volatile organic compounds commonly found in landfill leachate can rapidly migrate through synthetic flexible membrane liners even at dilute concentrations (Haxo and Lahey, 1988). Similarly, Johnson et al. (1989) has shown that organics can diffuse through compacted clay liners.

Many studies have been completed to determine and summarize the major organic constituents in municipal landfill leachate (Dunlap et al., 1975; Dunlap et al., 1979; DeWalle and Chian, 1981; Shuckrow et al., 1982; Cameron and Stewart, 1982; Kmet and McGinley, 1982; Sabel and Clark, 1984; Sawhney and Kozloski, 1984; Reinhard and Goodman, 1984; Stuart et al., 1984; Baxter, 1985; Booth and Vagt, 1986; Pohland and Harper, 1986; NUS Corp., 1987; and USEPA, 1988a). Thus far, no studies have been conducted using microbial bioassays to measure the carcinogenic potential of MSW landfill leachate. This study was undertaken to evaluate both the acute and genetic toxicity of MSW landfill leachate and to compare the measured toxicity to the chemical characteristics of the leachate.

OBJECTIVES

This study evaluated the acute toxicity, genetic toxicity and chemical characteristics of landfill leachate; the data were then used to estimate the carcinogenic risk associated with municipal solid waste landfill leachate. The specific objectives of the study were as follows:

1) To collect leachate samples from several representative municipal solid waste landfills.

2) To utilize the Salmonella/microsome mutagenicity bioassay to characterize the genetic toxicity of the organic extract of the leachate samples.

3) To utilize the Bacillus subtilis DNA repair bioassay to characterize the genetic toxicity of the organic extract of the leachate samples.

4) To utilize the diploid Aspergillus nidulans chromosome damage bioassay to characterize the genetic toxicity of the organic extract of the leachate samples.

5) To utilize the Microtox bioassay to characterize the acute toxicity of the organic extract of the leachate samples.

6) To conduct a chemical analysis on the leachate samples.

7) To estimate the carcinogenic risk of the leachate samples using the analytical data.

8) To correlate the results of the bioassays with the analytical data and estimated carcinogenic risks of the leachate samples.

MATERIALS AND METHODS

SAMPLE COLLECTION

Four landfill leachate samples and one upgradient groundwater sample were collected for the study. The four landfill leachate samples were selected to represent the various types of leachates likely to be produced. These include older, closed landfills subject to co-disposal of hazardous waste, newer landfills where mixed industrial and residential waste streams are disposed, and recently closed and currently operating landfills with primarily residential municipal waste. An upgradient groundwater sample was collected for use as a negative control in the study.

Leachate sample D001 was collected from a landfill in the midwestern U.S. This landfill was open from the 1940's until it was closed in 1974. The waste stream entering the landfill was primarily composed of residential refuse, along with smaller quantities of incinerator ash and industrial wastes. The leachate generated by this landfill should be representative of older landfills that received co-disposal of municipal and hazardous waste streams and were closed before, or as result of, regulations preventing co-disposal of hazardous waste in municipal waste landfills.

Orange-brown liquid leachate emanated from a seep in the side of the landfill, flowed through a ditch along the side of

the landfill, into a culvert which crossed under a road, and emptied into a ravine on the side of the road opposite the landfill seep. Approximately, 98 L of leachate were collected with clean plastic pails from the culvert as it emptied into the ravine. The pails were emptied into a clean plastic barrel, which was tightly covered with a lid and ring clamp. The sample was transported to an EPA office for extraction with XAD resin (Applied Science Laboratory, State College, PA) columns. A separate sample of D001 leachate was collected for later solvent extraction and analysis for semi-volatile organic compounds.

Three landfill leachate samples and one upgradient groundwater sample were collected from a state in the eastern United States. Leachate 001 and Leachate 002, and Groundwater 003 were collected at a state-managed municipal solid waste landfill facility. Leachate 004 was collected at another state-managed landfill facility and transported to the central facility by a state employee.

Leachate 001 was collected from a leachate collection system which drains an old, unlined cell in the central facility landfill which was opened in 1982 and closed in 1988. The central facility receives primarily residential waste with very little industrial waste. Leachate 001 is representative of leachates produced by landfills with a waste stream composed of primarily residential municipal waste.

Approximately 98 L of leachate were collected. The oily,

frothy liquid was collected from a manhole opening in the system, using a clean plastic pail. The pail was lowered into the leachate collection basin, filled, and raised using a clean plastic rope. The leachate was emptied into a clean plastic barrel, which was tightly covered with a lid and ring clamp. The sample was transported to an on-site state-managed laboratory for extraction with XAD resin columns. Three separate Leachate 001 samples were collected in hermetically sealed 40 mL glass VOA (volatile organic analyte) vials for analysis of volatile and semi-volatile organic compounds.

Leachate 002 was collected from a cell in the central facility landfill. The cell and its leachate collection system were opened in October, 1988. The cell was constructed using a single flexible membrane liner over 60 cm of native sandy clay soils. Leachate 002 is representative of leachate produced by an open and operating landfill receiving primarily residential municipal waste.

Approximately 98 L of leachate were collected. The gray-orange, frothy liquid was collected by gravity flow from a tanker truck that had just been filled with leachate pumped from the leachate collection system for the cell. The leachate was collected in a clean 113 L plastic barrel, which was tightly covered with a lid and ring clamp. The sample was transported to an on-site laboratory for extraction with XAD resin columns. Three separate Leachate 002 samples were collected in hermetically sealed 40 mL glass VOA vials for

analysis of volatile and semi-volatile organic compounds.

Groundwater 003 was collected from a groundwater monitoring well upgradient from the central facility landfill. Approximately 98 L of groundwater were collected using a 12-volt battery-powered pump to purge the well and collect the sample. The sample was enclosed in a clean plastic barrel with a lid and ring clamp, and transported to an on-site laboratory for extraction with XAD resin columns. Three separate Groundwater 003 samples were collected in hermetically sealed 40 mL glass VOA vials for analysis of volatile and semi-volatile organic compounds.

Leachate 004 was collected from another state-managed municipal solid waste landfill facility. The sample was collected from a manhole, where all leachate collection systems for the landfill (old and new cells) come together. The landfill is lined with a 75 cm layer of high clay content dredge spoil. The landfill is in a more industrialized area of the state, and receives both residential and industrial waste. Leachate 004 is representative of leachate produced by operating landfills with closed and open cells which are receiving both residential and industrial wastes.

Approximately 98 L of leachate were collected from the manhole by a state employee, put in a clean plastic barrel, covered tightly with a lid and ring clamp, and transported to the central facility laboratory for extraction on XAD resin columns.

SAMPLE EXTRACTION

Leachate D001 was extracted in an office near the landfill. Leachate 001, Leachate 002, Groundwater 003, and Leachate 004 were extracted in an on-site laboratory at the central facility landfill. The organic constituents of the leachate samples were extracted and concentrated on a column of XAD-2 and XAD-7 resins as described by Brown and Donnelly (1984). A mixed bed of non-polar XAD-2 and moderately polar XAD-7 resins ensures the collection of most organics of various polarities (Rappaport et al., 1979).

The U.S. EPA has suggested this extraction method for preparing environmental samples for mutagenicity testing using the Salmonella/microsome assay (USEPA, 1985). Grabow et al. (1981) noted that the XAD resin method may be more efficient than a liquid-liquid extraction for low concentrations of organic mutagens. Dressler (1979) determined that the combination of XAD-2 and XAD-7 resins provided the most efficient concentration and extraction for a large range of organic compounds.

The XAD-2 and XAD-7 resins were prepared before use in the columns by washing and decanting three times sequentially with 10 volumes of acetone, methanol, and distilled water. After washing, the prepared resins were packed into glass econo-columns (Bio-Rad,Richmond, CA) with 2×10^{-3} m^3 (6.3×10^{-3} kg) of XAD-7 resin packed above 2×10^{-3} m^3 (4×10^{-3} kg) of XAD-2 resin.

As recommended by Rappaport et al. (1979), a glass wool plug was placed above the resin bed to trap particles in the samples which could have clogged the resin beds below. The columns were flushed with 1.2 L of distilled water prior to passing the leachate sample through the columns by gravity flow at a rate of approximately 50 mL/minute.

Several variations were made in the Brown and Donnelly (1984) XAD resin extraction procedure. The samples were extracted promptly in air-conditioned laboratories, due to the absence of 4°C cold rooms. Also, multiple econo-columns (cut in half for ease of transport) were packed with the specified amounts of XAD-2 and XAD-7 resins and used to extract each sample. The use of multiple columns for each sample hastened the process of extracting the large samples, which would have been excessively slow due to low flow rates and clogging resulting from the use of single columns. The faster extraction time helped decrease the time until the samples could be stored at 4°C, and minimized the effects of organics being extracted at approximately 20°C rather than 4°C. The samples were passed through the columns by gravity flow at a rate of approximately 50 mL/minute.

Sample D001 was extracted using three resin columns. A total of 91 L (of the 98 L of raw leachate collected) was extracted. Leachate 001 was extracted using a total of seven resin columns. A total of 57 L (of the 98 L of raw leachate collected) was extracted. Leachate 002 was extracted using a

total of six resin columns. A total of 68 L (of the 98 L of raw leachate collected) were extracted. Groundwater 003 was extracted using a total of four columns. A total of 91 L (of the 98 L of raw sample collected) was extracted. Leachate 004 was extracted using a total of three columns. A total of 79 L (of the 98 L of raw leachate collected) were extracted. The resin columns used to extract samples D001, Leachate 001, Leachate 002, Groundwater 003, and Leachate 004 were packed in ice and shipped back to the Texas A & M University Environmental Mutagenicity Laboratory. The columns were stored in a walk-in cold room at 4°C until further extraction of the adsorbed organics.

All of the resin columns were extracted to remove adsorbed organics following the general procedures outlined by Hooper et al. (1978) and Rappaport et al. (1979) as modified by Brown and Donnelly (1982). The columns were first eluted by gravity flow with 120 mL (3 resin bed volumes) of distilled water. This process was followed to ensure that any residual histidine in the columns would be removed. Residual histidine would cause false positive results in the Ames bioassay because of the nature of the test, which identifies mutagenic compounds when they cause reversion of the Salmonella strains back to the histidine-independent state. Any histidine present in the samples could allow the Salmonella strains to grow without being reverted by a mutagenic substance.

After elution of the columns with distilled water, they

were dried with a stream of dry nitrogen gas to remove any residual water. The columns were then extracted with 160 mL (4 resin bed volumes) of acetone to remove adsorbed organics.

The standard procedure described by Brown and Donnelly (1982) calls for 40 mL (1 bed volume) to be added to the resin column, with vortexing to remove air pockets and allow maximum contact between the resins and acetone. Due to the use of half-length econo columns, this procedure was modified. Two 20 mL (1/2 bed volumes) of acetone were added to each column, with vortexing and hand-shaking following each addition to remove air pockets and achieve maximum acetone-resin contact. After the two 20 mL volumes had been added and collected, the final 120 mL (three bed volumes) of acetone was added by gravity flow to the columns and collected along with the initial 40 mL used in the two 1/2 bed volume treatments. The acetone extracts from the columns from each sample were combined and collected in clean, glass stoppered roto-evaporator flasks in preparation for dry down on the rotoevaporator.

The standard procedure (Brown and Donnelly, 1982) for dry down of the acetone extract calls for filtering of the extract through anhydrous Na_2SO_4 to remove any aqueous phase prior to final dry down of the acetone extract by rotoevaporation. Since several columns were used to extract organics from each sample, the acetone extract volume from each sample was several times larger than the 160 mL sample that would result

from a single column extraction. The procedure was modified by first placing the large acetone extracts on a Brinkman-Bucci Model R rotoevaporator to dry down the acetone extract to a more manageable volume. The reduced volume of acetone extract was then passed through Whatman No. 42 filter paper in a glass funnel, filled approximately 2/3 full with anhydrous Na_2SO_4, to remove the residual aqueous phase. The Na_2SO_4 was rinsed with acetone to ensure that all of the acetone extract was removed. Samples were collected in another clean rotoevaporator flask for final dry down.

The final dry down of the acetone extracts to approximately 10 mL was achieved with the rotoevaporator set at 60°C. Boiling chips were added to the flask to aid in the dry down of the extracts. After the final dry down, the extracts were transferred to several clean culture tubes to which additional anhydrous Na_2SO_4 was added. The tubes were shaken and allowed to stand several hours to allow the Na_2SO_4 to precipitate. The supernatent acetone extract was then pipetted off into clean, autoclaved, weighed culture tubes for final dry down with dry nitrogen gas.

Before final dry down, the acetone extracts were thoroughly mixed by vortexing, and 1.9 mL aliquots of each extract were taken for chemical analysis. The fractions to be used for chemical analysis were filtered with 0.2 micron chemical resistant filters and collected in clean 2 mL crimp top vials and stored at 4°C until analyzed. The remaining

volume of each sample to be used in the bioassays was measured, and then reduced to a dry residue with a stream of dry nitrogen. The dry residue weight of each sample was recorded, and then the samples were redissolved in dimethyl sulfoxide (DMSO, Grade 1, Sigma, St. Louis, MO) in preparation for the bioassays.

BIOASSAYS

Salmonella/Microsome Assay

The Salmonella/microsome assay as developed by Ames et al. (1975) and modified by Maron and Ames (1983) was used to determine the mutagenicity of the organic extracts of the leachate and groundwater samples. The Salmonella tester strains were kindly supplied by Dr. B.N. Ames (University of California, Berkeley, CA). The standard plate incorporation assay utilizing strain TA 98 with and without various levels of metabolic activation was used to test all of the samples. The metabolic activation mixture (S-9) consisted of 0.3 mL of a 9000 x g supernatent from homogenized, Aroclor-induced rat liver (Organon Teknika, West Chester, PA) and 0.7 mL of cofactor supplement. The cofactor supplement consists of 11.4 mM $MgCl_2$, 47 mM KCl, 7.1 mM glucose-6-phosphate, 5.7 mM NADP, and 140 mM potassium phosphate buffer at pH 7.4. This S-9 mixture is used to test samples at high levels of metabolic activation. For testing samples at medium and low levels of metabolic activation 0.1 mL of rat liver supernatent per 0.9

mL of cofactor supplement and 0.04 mL of rat liver supernatent per 0.96 mL of cofactor supplement were used, respectively.

Leachate D001 was tested using strains TA 97 and TA 1538 with high levels of metabolic activation; Leachate 001 was tested using strains TA 97, TA 100, TA 102, and TA 1538 with high levels of metabolic activation. Tester strains TA 97, TA 98, TA 100, TA 102, and TA 1538 were used as they are recommended for general mutagenicity testing by Maron and Ames (1983). Strains TA 97, TA 100, TA 102, and TA 1538 did not appear to be more sensitive in detecting mutagenicity than the TA 98 strain with the D001 and leachate 001 samples; thus, the more commonly used TA 98 strain was utilized for the bulk of the testing.

In the plate incorporation assay (Maron and Ames; 1983) all of the samples were tested at a minimum of five consecutive dose levels, ranging from 1 mg/mL to 200 mg/mL. The plate incorporation procedure involves adding 0.1 mL of (approximately 10^9 cells/mL) and 0.05 mL of the sample dose to an autoclaved culture tube containing 2 mL of minimal top agar (containing agar, NaCl, 0.5 mM histidine/biotin solution, and distilled water). The tube is capped and vortexed to thoroughly mix the contents. The contents are poured onto a petri plate containing minimal glucose agar (containing agar, 50 X VB salts, 20% glucose solution, and distilled water). The plate is swirled gently to mix the test mixture with the plate agar. This procedure was followed for each sample at

the various dose levels, and was replicated by duplicate plates on each test date. Each sample was also retested with duplicate plates on at least one other independent test date, using at least five consecutive dose levels.

If the test was to include metabolic activation to test for promutagens, 0.5 mL of the S-9 mix (at the low, medium, or high level) was added to the culture tube along with the bacteria and sample before vortexing and plate incorporation. A 0.05 mL volume of a sample dilution was added to the agar plate, and the dose per plate (for both -S-9 and +S-9 tests) was calculated using the following equation:

$$\text{mg/plate dose} = \begin{matrix}\text{mg/mL} \\ \text{sample dilution}\end{matrix} \times \begin{matrix}\text{mL of} \\ \text{sample dilution}\end{matrix}$$

The volume of original aqueous sample required to supply the dosage in mg/plate was calculated using the following equation:

$$\begin{matrix}\text{sample dose in L of} \\ \text{original sample}\end{matrix} = \frac{(\text{mg/plate})(\text{L of sample extracted})}{\text{mg of total organic extract}}$$

The plates were incubated for 72 hours at 37°C and evaluated for revertants using a Biotran II Automated Colony Counter (New Brunswick Sci., Edison, NJ). A test was considered positive and the sample to be mutagenic if the average response for at least two consecutive dose levels was greater than twice the response for the corresponding negative

and solvent control, as recommended by Chu et al. (1981). All of the tester strains were calibrated with positive and negative controls on each independent test date. The positive control for TA 98, TA 102 and TA 1538 was 25 ug of 2 nitrofluorene/plate (Aldrich, Milwaukee, WI). One microgram of sodium azide/plate (Sigma) was used as a positive control for strains TA 97 and TA 100. The functioning of the metabolic activation system was checked using benzo(a)pyrene (Sigma) for all tester strains. All reagents and extracts were checked for sterility and DMSO was used as a solvent control. The negative control for all strains consisted of only the tester strain and the top agar, without any added DMSO, sample, or positive control.

All strains of the Salmonella typhimurium were checked monthly for the nutritional markers histidine and biotin, along with other genetic characteristics, such as sensitivity to crystal violet, ampicillin, and ultraviolet light.

Bacillus Subtilis Assay

The DNA repair deficient strains used in the Bacillus subtilis repair assay included excision repair-deficient (Exc^-) and recombination-deficient (Rec^-) strains. The Exc^- strain used was hcr-9. The Rec^- strains used were recA8 and mc-1. Strain fh2006.7, which is both excision and recombination repair-deficient, was also used. All of the tester strains are isogenic with Bacillus subtilis 168wt, which has all repair capabilities intact and was used as a

biological control (Donnelly et al., 1987).

The procedures of Felkner et al. (1979) were used to conduct the Bacillus subtilis DNA repair spot test. All tester strains were kindly supplied by Dr. I.C. Felkner of Clements Assoc., Washington, D.C. Each tester strain was incubated in overnight cultures with shaking at 37°C in BHI (brain heart infusion, Difco, Detroit,MI) broth. The overnight cultures were diluted with fresh BHI broth to an approximate cell density of 5 x 10^4 organisms/mL. Inocula from the cultured and diluted repair deficient strains were streaked radially on a minimal glucose agar plate from a centered sensitivity disk, to which 0.1 mL of a 200 mg/mL sample dose had been applied. The doses applied in terms of mg/plate and L of original aqueous sample were calculated with the same general equations used in calculating doses in the Ames test. The plates were incubated at 37°C for 24 hours. After incubation, the resulting zones of inhibition (mm) for each repair deficient strain were measured using a micrometer, and compared to the zone of inhibition measured for the wild type repair proficient strain (168 wt). A response was considered positive if the zone of inhibition for one of the repair deficient strains was more than 2.5 mm greater than the zone of inhibition for the repair proficient 168 wt (Donnelly et al., 1987).

Two micrograms of Mitomycin C (Sigma) and 2 uL of methylmethanesulfonate (Aldrich) dissolved in 0.1 mL of DMSO

and applied to centered sensitivity disks were used as positive controls. DMSO (0.1 mL) applied to centered sensitivity disks was used as a solvent control. Untreated sensitivity disks were used as negative controls. Duplicate plates were run on two independent test dates at the tested dose level for each sample.

Aspergillus nidulans Assay

The diploid Aspergillus nidulans chromosome damage test was conducted on the leachate and groundwater samples by Dr. Barry R. Scott of the Phoenix Corporation's Lone Oak Laboratories in Smithville, Texas, according to the procedure of Kafer et al. (1982). Haploid conidia were combined on minimal media agar plates and allowed to spontaneously form diploid conidia. The diploid conidia were mixed in 0.1 mL of a non-selective media with 0.1 mL of the sample being tested. The mixture was incubated at 37°C in a pilot study to determine the 10% survival time of the diploid conidia. A mixture of 0.1 mL of non-selective liquid media and 0.1 mL of sample was then combined with 0.8 mL of cold phosphate buffer. It was applied to a non-selective media agar plate and incubated for the survival time determined in the pilot study (approximately 5-10 days depending on the sample). The percent of abnormal colonies (as identified by reduced growth and/or conidiation) was measured. The doses applied in terms of mg/plate and L of original aqueous sample were calculated with the same general equations used in calculating doses in the

Ames test and Bacillus subtilis spot test.

The negative control used for the test was distilled water; the solvent control was DMSO. The relative index for each sample was calculated using the following equation:

$$\text{relative index} = \frac{\%\text{ abnormal colonies in sample test}}{\%\text{ abnormal colonies in negative control test}}$$

Since DMSO was slightly positive as the solvent control, the relative index was also calculated by dividing the percent abnormal colonies in the sample test by the percent abnormal colonies in the DMSO test. Thus, the chromosome damage based only on the sample, without the influence of the solvent, was determined. Using the two-fold rule (Chu et al., 1981) a sample is considered positive in the diploid Aspergillus nidulans chromosome damage assay if it has a relative index of 2 or greater as compared to the solvent control.

Microtox Assay

The Microtox acute toxicity test was also conducted by Dr. Barry R. Scott of Lone Oak Laboratories, according to the general procedure developed by Bulich (1979). Suspensions of marine luminescent bacteria in aqueous solutions were exposed to 1.5 mL each of four serial dilutions of the original sample dose of 200 mg/mL. The doses applied in terms of mg/test and L of original aqueous sample were calculated with the same general equations used to calculate doses in the Ames test, Bacillus subtilis spot test, and the Aspergillus nidulans diploid chromosome assay.

The test was run for 30 minutes at a temperature of 15°C, with measurements of toxicity at 5, 15, and 30 minutes. Toxicity was determined by measuring light output using the gamma effect [ratio of light loss during test time (t) divided by light remaining at time (t)]. The average response of reagent blanks was used to correct for drifts in light output in the test over time and due to dilution effects.

The Microtox analyzer measures the light output of the luminescent bacteria, and decreasing light output is an indication of acute toxicity. Toxicity is quantified using the EC 50 (the effective concentration that causes a 50% decrease in light output by the bacteria). The EC 50 was reported as the percent dilution of the sample with respect to the original sample dose (200 mg/mL) for each sample at each test time.

The relative toxicity was calculated for each leachate sample using the following equation:

$$\text{relative toxicity} = \frac{\text{\% EC 50 of negative control}}{\text{\% EC 50 of leachate sample}}$$

The relative toxicity of the negative control (Groundwater 003) was similarly calculated by dividing its % EC 50 by the % EC 50 of a weak positive control (DOW G). The Chu two-fold rule (Chu et al., 1981) was used to determine a positive response using the relative toxicity. If the toxic response for the sample was at least two times greater than the response for the negative control, the sample was

considered to be acutely toxic at the corresponding % EC 50.

CHEMICAL ANALYSIS

Purgeable Chlorinated Organic Compounds

The raw leachate (Leachate 001, Leachate 002) and groundwater (Groundwater 003) samples collected without headspace in 40 mL VOA vials were analyzed for purgeable volatile and semi-volatile chlorinated organic priority pollutants. The analysis of the samples and standards was conducted using a Tekmar 4200 automated heated sampler module and a Tekmar 4000 dynamic headspace concentrator in conjunction with a Tracor 540 gas chromatograph.

The run conditions for the Tekmar 4200 sampler and Tekmar 4000 concentrator were similar to the procedure recommended by the U.S. EPA for the purge and trap analysis of purgeable chlorinated organic priority pollutants (USEPA, 1986). The Tekmar 4200 was automatically programmed for a 1.7 minute preheat stage, followed by a 5 minute prepurge stage. The Tekmar 4200 sample purging then began with a sample temperature of 60°C and a purge gas (nitrogen) flow rate of approximately 40 ml/minute. The Tekmar 4000 concentrator was automatically programmed to begin an 11 minute purge stage with commencement of the sample purging by the 4200 headspace sampler. The purged sample constituents were adsorbed to a Tenax trap during the purge stage. After completion of the purge stage, the 4000 concentrator entered a short pre-desorb stage, at which time the trap was heated to the desorb

temperature of 180°C. When the desorb temperature was reached, the 4000 concentrator entered a 4 minute desorb stage, and finished with a 7 minute bake stage to clear the trap and prepare for subsequent runs. All line and valve temperatures in the Tekmar 4200 and Tekmar 4000 were maintained at 100°C to prevent condensation of the purged sample constituents.

The Tracor 540 gas chromatograph was interconnected with the Tekmar 4000 concentrator so the run program began when it received a trigger signal from the Tekmar 4000 at the onset of the trap desorb stage. The gas chromatograph was equipped with an electron capture detector (ECD) and a polar, fused silica DB-624 megabore capillary column (J & W Scientific, Folsom, CA) for high-sensitivity detection of chlorinated organic compounds.

Nitrogen was used as the carrier gas, with a flow rate of 30 mL/minute. The temperature of the column inlet was maintained at 300°C to ensure instantaneous vaporization of the sample. The run conditions programmed for the Tracor 540 were as follows: 40°C for 5 minutes, a ramp of 5°C/minute to 140°C, a hold at 140°C for 1 minute, a ramp of 10°C/minute to a final temperature of 250°C, which was held for 15 minutes, for a total run time of 52 minutes. Analytes of interest were eluted by the midway point in the program at 140°C, but the second ramp and hold was used to ensure clearing of the column of late eluting compounds to prepare for subsequent runs.

A Spectra Physics 5240 integrator was interconnected with the Tracor 540 gas chromatograph to begin recording automatically with commencement of a sample run. The 5240 integrator was programmed to record retention time for each analyte peak, and to stop recording after completion of the 52 minute run program. The integrator was programmed to produce report printouts which included analyte retention times and peak areas.

The desorb stage of the Tekmar 4000 concentrator to the Tracor 540 gas chromatograph is analogous to direct injection into the column and detector system. Thus, standards of chlorinated organic pollutants suspected to be in the samples were injected directly to determine their respective retention times. One VOA sample of Leachate 001 was run to determine the approximate concentrations of the respective analytes, and thus to prepare standard solutions of the suspected analytes at concentration ranges that would include their sample concentrations. The standard solution dilutions were analyzed to determine absolute retention times and peak areas of the respective standard analytes. The retention time window for each standard was developed by averaging its retention times in the standard dilutions to calculate its mean absolute retention time as recommended by the U.S. EPA procedures, The standard deviation of the retention time was used to calculate a retention time window of $\pm$ 3 times the standard deviation of the mean absolute retention time (USEPA, 1986).

The external standard method was used to determine sample analyte concentrations identified by absolute retention time. The complexity of the samples prohibited the use of internal standards, due to the possibility of their interference with the sample analyte retention times and peak areas. The accuracy and precision of the external standard method depends on identical volumes being used for the analysis of all standards and samples. The volume used in the purge and trap procedure was 5 mL for all standards and samples, as recommended by the U.S. EPA (USEPA,1986). The large volume used minimized significant variation in analyte peak areas due to inherent errors in measuring sample size. The large sample size used in the purge and trap procedure also allowed for more reliable detection of the low concentrations of purgeable chlorinated organic priority pollutants in the samples.

Linear regression was used to calculate the external standard curve equations for each analyte, using the relationship between standard analyte concentration in ug/L and peak area. Analytes identified by absolute retention time in the samples were quantified by using their peak area responses in the external standard regression equations to calculate concentration in ug/L. As the samples purged were volumes of unextracted sample, no dilution or concentration factor was necessary to calculate environmental concentrations of the sample analytes.

Semi-Volatile Aromatic Compounds

The original aqueous leachate samples D001, Leachate 001, and Leachate 002, and the original aqueous groundwater sample Groundwater 003 were analyzed for semi-volatile non-chlorinated aromatic organic compounds. These compounds were not detected in the purge and trap procedure because of the nature of the EC detector and polar DB-624 column used for the detection of chlorinated organic compounds.

The raw leachate and groundwater samples were extracted with methylene chloride in an attempt to recover and concentrate benzene, toluene, ethylbenzene, and xylene for analysis by gas chromatography. The procedure recommended by the U.S. EPA for methylene chloride extraction of semi-volatile compounds in aqueous samples was followed. The procedure calls for extraction of a 1000 mL aqueous sample with 180 mL of methylene chloride, using three separate 60 mL additions followed by 2 minutes of shaking and venting and 10 minutes of separation time per addition (USEPA, 1986). Due to smaller sample volumes, smaller volumes of methylene chloride were used in the separatory funnel extractions.

A 50 mL volume of D001 was extracted with three 3 mL additions of methylene chloride, for a total extractant volume of 9 mL. Thirty mL volumes of Leachate 001, Leachate 002, and Groundwater 003 were each extracted with three 1.8 mL additions of methylene chloride, for a total extractant volume of 5.4 mL per sample. All sample extracts were collected and

filtered, using 0.2 micron chemical resistant filters, into clean 2 mL crimp-top glass vials. The samples were stored in a walk-in cold room at 4°C prior to analysis.

The methylene chloride semi-volatile extract samples were analyzed using a Tracor 540 gas chromatograph in conjunction with a Spectra Physics 5240 integrator, as in the purge and trap analysis of volatile and semi-volatile chlorinated organic compounds. The gas chromatograph was equipped with a flame ionization detector (FID) and a non-polar DB-5 megabore capillary column (J & W Scientific, Folsom, CA) for detection of non-chlorinated organic compounds. Helium was used as a carrier gas, with a flow rate of 30 mL/minute. The programmed run conditions for the Tracor 540 were the same as in the analysis of the chlorinated compounds. However, analytes of interest were eluted at 140°C before the midway point in the program. The run program was manually advanced to the final temperature of 250°C to shorten the length of each run, while ensuring the clearing of the column of late eluting compounds to prepare for subsequent runs.

The Spectra Physics 5240 integrator was interconnected with the Tracor 540 gas chromatograph to begin recording automatically with commencement of a sample run. The integrator was programmed to produce report printouts including analyte retention times and peak areas as in the analysis of purgeable chlorinated compounds.

The aromatic organic compounds were identified by

absolute retention times of the analyte standards. U.S. EPA procedures were used to establish absolute retention times and their respective windows of ± 3 times the standard deviation (USEPA,1986).

The internal standard method was used to calculate sample analyte concentrations identified by absolute retention times. Styrene was used as the internal standard, due to the similarity of its chemical structure to the aromatic analytes being considered. Styrene was added to each sample analyzed in volumes necessary to produce a concentration of 5 mg/L.

Standard solutions containing the analytes of interest and styrene as the internal standard were prepared by serial dilution to produce a range of concentrations that would approximate analyte concentrations in the samples. The detector responses of each analyte were compared to styrene at each concentration in order to calculate the response factor (RF) for each analyte as follows:

$$RF = \frac{(\text{peak area of styrene})(\text{mg/L of analyte standard})}{(\text{peak area of analyte standard})(\text{mg/L of styrene})}$$

The mean response factors were calculated for each analyte, and were used to calculate the analyte concentrations in the samples. According to U.S. EPA procedure, the mean response factor can be used through the working range of the method, when the standard deviation associated with the mean response factor is < ± 20% of the mean response factor (USEPA, 1986).

The response factors of each analyte were used to calculate the concentrations of the analytes in the samples as follows:

$$\text{mg/L of analyte} = \frac{\text{peak area of analyte x mg/L of styrene x RF}}{\text{peak area of styrene}}$$

As previously mentioned, the concentration of the styrene used as the internal standard was 5 mg/L in each sample. Thus, the above equation could be used to calculate sample analyte concentrations, using their respective peak areas, response factors, and the peak area of the styrene internal standard.

The calculated sample analyte concentrations were divided by 5.56, to correct for the concentration factor of extracting each 5.56 mL of sample with 1 mL of methylene chloride. This calculation provided the environmental concentrations of the analytes in the original aqueous samples.

Non-Volatile Organic Compounds

The fractions of D001, Leachate 001, Leachate 002, Groundwater 003, and Leachate 004 used for analysis in the bioassays were also chemically analyzed for non-volatile organic compounds. Due to the complexity of the bioassay fractions, the major non-volatile components of each sample were qualitatively identified by Ross Evans of Texas A&M University at Galveston. A Hewlett Packard 5971 gas chromatograph/mass spectrometer equipped with an STE5 capillary column was used to identify the major analytes in

each sample. Helium was used as the carrier gas, at a flow rate of 1 mL/minute. The run program began with a 5 minute hold at 30°C, and was ramped twice to achieve a final temperature of 270°C, which was held for 20 minutes.

The sample analytes identified by gas chromatography/mass spectrometry were identified by absolute retention time and quantified on a Tracor 540 gas chromatograph in conjunction with a Spectra Physics 5240 integrator. The gas chromatograph was equipped with an FID and DB-5 megabore capillary column for detection of non-chlorinated organic compounds as in the analysis for semi-volatile non-chlorinated aromatic compounds in the methylene chloride extracts. The gas chromatograph was also equipped with a ECD and DB-624 column for the detection of chlorinated organic compounds as in the analysis of purgeable chlorinated compounds.

The run conditions for the Tracor 540 were the same used in the analysis of volatile and semi-volatile organic compounds. The program was allowed to run to completion due to the presence of late eluting analytes of interest. The 15 minute hold time at 250°C ensured that late eluting compounds would be cleared from the column in preparation for subsequent runs.

The Spectra Physics 5240 integrator was interconnected with the Tracor 540 gas chromatograph to begin recording automatically with commencement of a sample run. The integrator was programmed to produce report printouts

including analyte retention times and peak areas as in the analysis for volatile and semi-volatile organic compounds.

The major analytes in each sample identified by gas chromatography/mass spectrometry were identified on the Tracor 540 gas chromatograph by the absolute retention times of the analytes in the standard dilutions. The major non-volatile analytes were identified by absolute retention time and their respective windows of $\pm$ 3 times the standard deviation (USEPA, 1986).

The internal standard method was used to calculate sample analyte concentrations identified by absolute retention times. Three internal standards were used: 2,4-dimethyl phenol for the earlier eluting phenols, cresols, organic acids, camphor, and a-terpineol; dimethyl phthalate for the later eluting phthalates; and 2-chlorophenol for the chlorinated phenols. The 2,4-dimethyl phenol was added to each sample analyzed in the necessary volumes to produce a concentration of 100 mg/L. Dimethyl phthalate was added to each sample analyzed in the necessary volumes to produce a concentration of 50 mg/L. The 2-chlorophenol was added to each sample analyzed in the necessary volumes to produce a concentration of 500 mg/L.

Standard solutions containing the analytes of interest and internal standards were prepared by serial dilution to produce a range of concentrations that would approximate analyte concentrations in the samples. The detector response of each analyte was compared to the respective internal

standard to calculate their response factors.

The mean response factors were calculated, and used through the working range of the method with the associated standard deviations $<\pm$ 20% of the mean response factor (USEPA, 1986). The calculated mean response factors and the concentrations of the internal standards, along with the peak areas of the analytes and internal standards, were used to calculate sample concentrations of the analytes.

The calculated sample analyte concentrations were divided by a concentration factor specific for each sample, to correct for the concentration of the original aqueous volume for each sample to the final volume extracted and concentrated in the XAD resin and acetone extraction procedure.

ESTIMATED CUMULATIVE CANCER RISK ASSESSMENT

In contrast to the bioassays described above which utilize living organisms to directly estimate the toxicity of a sample, Crouch et al. (1983) developed a mathematical procedure to estimate the risk of chemicals in drinking water. While the authors are not proposing that individuals would drink landfill leachate directly, the procedure can be used as a tool to compare the relative toxicity of various leachates. This provides a basis for being able to decide which leachate constitutes the greatest threat to groundwater reserves.

The estimated cumulative cancer risk of the leachate and groundwater samples was calculated using the method of comparing the risk of chemicals in drinking water developed by

Crouch et al. (1983). Brown and Donnelly (1988) utilized this method to estimate the cancer risks associated with the organic compounds for several municipal and hazardous waste landfills. The equation utilized by Brown and Donnelly (1988) to calculate the median annual cancer risk, R_{md} for a lifetime intake (70 years) of 2 L/day of water contaminated with a mixture of chemicals is as follows:

$$R_{md} = 4 \times 10^{-7}[\text{sum}(B_i d_i)]$$

where:

B_i is the carcinogenic potency of each individual chemical in kg day/mg; and d_i is the concentration of each individual chemical in ug/L.

Crouch et. al. (1983) recommend calculating the mean and 98th percentile risk in order to determine the probability distribution for the risk equation. The equations utilized by Brown and Donnelly (1988) to calculate the mean (R_m) and 98th percentile (R_{98}) cancer risk for a mixture of chemicals in landfill leachates are as follows:

$$R_m = \exp(s^2/2) \times R_{md}$$

$$R_{98} = \exp(2s) \times R_{md}$$

The variable s is the total uncertainty in measuring carcinogenic potency.

The carcinogenic potency for each compound in the leachate and groundwater samples was acquired directly from the literature as rodent carcinogenicity data, or calculated using the oral rat LD_{50} acute toxicity data. The carcinogenic

potency (B) for a chemical is derived from its oral rat LD_{50} data using the following equation from Crouch et al. (1983):

$$B = 6.5(LD_{50})^{-1.1}$$

The use of oral rat LD_{50} acute toxicity data in estimating the carcinogenic potency increases the uncertainty of the estimation of cumulative cancer risk. The procedure can be used to compare the cumulative cancer risks of complex mixtures, such as landfill leachates, containing chemicals for which limited carcinogenic data is available (Brown and Donnelly, 1988). The equations used in calculating estimated cumulative cancer risk were developed and kindly supplied as a computer software program by Dr. E.A.C. Crouch of Harvard University.

RESULTS AND DISCUSSION

BIOASSAYS

Salmonella/Microsome Assay

None of the leachate or groundwater samples tested were mutagenic in the Ames test with or without metabolic activation at any dose level. However, three of the four leachate samples tested were acutely toxic with at least one Salmonella typhimurium tester strain and at least one dose level. The groundwater sample (Groundwater 003) was not toxic with any of the tester strains at the various levels of metabolic activation and dose. Table 1 shows the volumes of original aqueous samples required for the organic residue dose levels applied in the Salmonella/microsome test. Table 2 with

Table 1. Volumes of original aqueous samples required for dose levels applied in the Salmonella/microsome mutagenicity test.

Dose (mg/mL)	Dose (mg/plate)	Sample Volume (L) x 10^4 Leachate D001	Leachate 001	Leachate 002	Grdwater 003	Leachate 004
1	.05	40	12	19	657	25
2	.10	81	24	37	1,315	50
5	.25	202	60	93	3,288	126
10	.50	403	120	187	6,576	252
20	1.0	807	240	374	13,152	504
50	2.5	2,018	600	935	32,863	1,260
100	5.0	4,035	1,200	1,870	65,762	2,520
200	10.0	8,071	2,400	3,740	131,524	5,041

Table 2. Mutagenicity, in strain TA 98 of Salmonella typhimurium, of sample extracts at various dose and metabolic activation levels.

Sample	Dose (mg/ml)	Total his+ Revertants[1] -S-9	+low S-9	+ med S-9	+ high S-9
Leachate D001	0	21 ± 6	31 ± 9	24 ± 6	27 ± 6
	1	14 ± 6	--	--	25 ± 0
	2	24 ± 1	--	--	25 ± 3
	5	25 ± 0	45 ± 0	--	28 ± 10
	10	20 ± 4	35 ± 12	33 ± 4	34 ± 14
	20	21 ± 6	46 ± 14	46 ± 8	29 ± 9
	50	--	33 ± 4	38 ± 5	30 ± 7
	100	--	35 ± 19	27 ± 8	20 ± 6
	200	--	25 ± 8	20 ± 9	20 ± 3
Leachate 001	0	17 ± 1	31 ± 9	24 ± 6	27 ± 6
	1	20 ± 7	--	--	27 ± 0
	2	16 ± 1	--	--	22 ± 7
	5	24 ± 1	46 ± 1	--	24 ± 6
	10	25 ± 3	41 ± 12	41 ± 6	24 ± 11
	20	24 ± 1	49 ± 12	36 ± 9	29 ± 11
	50	--	30 ± 6	29 ± 6	24 ± 1
	100	--	33 ± 10	24 ± 6	32 ± 17
	200	--	26 ± 10	22 ± 5	28 ± 9
Leachate 002	0	--	31 ± 9	24 ± 6	25 ± 9
	1	--	--	--	--
	2	--	--	--	--
	5	--	41 ± 6	--	--
	10	--	38 ± 10	32 ± 5	16 ± 1
	20	--	36 ± 9	32 ± 8	21 ± 8
	50	--	24 ± 4	23 ± 5	15 ± 0
	100	--	35 ± 13	21 ± 6	0 ± 0
	200	--	26 ± 7	15 ± 7	0 ± 0
Grdwater 003	0	21 ± 6	31 ± 9	24 ± 6	27 ± 6
	1	28 ± 7	39 ± 5	38 ± 4	26 ± 7
	2	22 ± 7	43 ± 11	39 ± 6	23 ± 11
	5	27 ± 0	30 ± 8	34 ± 6	19 ± 12
	10	35 ± 0	38 ± 9	38 ± 3	22 ± 13
	20	41 ± 8	38 ± 11	29 ± 5	21 ± 10
	50	--	--	--	--
	100	--	--	--	--
	200	--	--	--	--

Table 2. (continued).

Sample	Dose (mg/ml)	Total his+ Revertants[1] -S-9	+low S-9	+ med S-9	+ high S-9
Leachate 004	0	17 ± 1	27 ± 6	24 ± 6	27 ± 5
	1	16 ± 1	--	--	21 ± 8
	2	25 ± 3	--	--	25 ± 0
	5	25 ± 3	--	--	27 ± 8
	10	28 ± 7	38 ± 5	55 ± 7	29 ± 4
	20	22 ± 7	41 ± 6	37 ± 11	28 ± 6
	50	--	33 ± 10	38 ± 14	--
	100	--	21 ± 6	33 ± 11	32 ± 7
	200	--	32 ± 30	15 ± 7	30 ± 7

[1] Total his+ revertants are reported as the mean and standard deviation calculated from at least four tests (two duplicate plates on at least two independent test dates).

shows the results of the mutagenicity testing on all samples tester strain TA 98 and various levels of metabolic activation. Table 3 depicts the results of the mutagenicity testing of Leachate D001 with tester strains TA 97 and TA 1538, and Leachate 001 with tester strains TA 97, TA 100, TA 102, and TA 1538.

Leachate 002 was toxic using a high level of metabolic activation at the three highest dose levels (15 $\pm$ 0 revertants at 50 mg/mL, and 0 revertants at 100 mg/mL and 200 mg/mL). A toxic response in the Salmonella/microsome assay when using tester strain TA 98 is a response of $\leq$ 15 revertants, or approximately half the historical background level of revertants.

Two leachate samples not toxic with Salmonella typhimurium tester strain TA 98 were toxic when using tester strains TA 97 and TA 1538. Leachate D001 was toxic using tester strain TA 97 with high levels of metabolic activation at the three highest dose levels (39 $\pm$ 8 revertants at 50 mg/mL and 100 mg/mL, and 0 revertants at 200 mg/mL). Leachate D001 was also toxic with tester strain TA 1538 at the highest dose level (0 revertants at 200 mg/mL) with a high level of metabolic activation. A toxic response in the Salmonella/ microsome assay in tester strain TA 97 is $\leq$ 50 revertants and in tester strain TA 1538 a toxic response is $\leq$ 15 revertants, or approximately half the historical background levels of 100 and 30 revertants, respectively, for each strain.

Table 3. Mutagenicity, in various tester strains of Salmonella typhimurium with high metabolic activation, of leachate extracts at various dose levels.

Sample	Strain	Dose (mg/ml)	Total his+ Revertants[1] +high S-9
Leachate D001	TA 97	0	100 ± 14
		10	70 ± 18
		20	75 ± 3
		50	39 ± 8
		100	39 ± 8
		200	0 ± 0
	TA 1538	0	24 ± 11
		5	24 ± 5
		10	27 ± 10
		20	22 ± 9
		100	16 ± 21
		200	0 ± 0
Leachate 001	TA 97	0	100 ± 14
		10	56 ± 16
		20	69 ± 6
		50	40 ± 10
		100	36 ± 13
		200	0 ± 0
Leachate 001	TA 100	0	89 ± 10
		10	66 ± 16
		20	65 ± 14
		50	60 ± 4
		100	62 ± 21
		200	39 ± 8
	TA 102	0	221 ± 54
		10	208 ± 26
		20	111 ± 8
		50	56 ± 1
		100	0 ± 0
		200	4 ± 5
	TA 1538	0	24 ± 11
		10	26 ± 8
		20	13 ± 5
		100	34 ± 20
		200	0 ± 0

[1] Total his+ revertants are reported as the mean and standard deviation calculated from at least four tests (two duplicate plates on at least two independent test dates).

Leachate 001 was also toxic in the Salmonella/microsome assay when using tester strains TA 97 and TA 1538. Leachate 001 was toxic at the three highest dose levels (40 ± 10 revertants at 50 mg/mL, 36 ± 13 revertants at 100 mg/mL, and 0 revertants at 200 mg/mL) with a high level of metabolic activation using TA 97. In addition, Leachate 001 was toxic at the highest dose level (0 revertants at 200 mg/mL) with a high level of metabolic activation using TA 1538.

Leachate 001 was also toxic in the Salmonella/microsome assay when using tester strains TA 100 and TA 102 with high levels of metabolic activation. Leachate 001 was toxic with tester strain TA 100 at the highest dose level (39 ± 8 revertants at 200 mg/mL). Leachate 001 was toxic with tester strain TA 102 at the three highest dose levels (56 ± 1 revertants at 50 mg/mL, 0 revertants at 100 mg/mL, and 4 ± 5 revertants at 200 mg/mL). A toxic response in tester strain TA 100 is $\leq$ 50 revertants and in tester strain TA 102 a toxic response is $\leq$ 100 revertants or approximately half the background levels of 100 and 200 revertants, respectively, for each strain.

It should be noted that although Leachate D001 and Leachate 001 resulted in approximately the same amount of toxicity using tester strains TA 97 and TA 1538, Sample D001 required more than three times the volume of original aqueous leachate as Leachate 001 to supply the organic residue doses applied. With equal environmental volumes, Leachate 001 could

be more toxic than Leachate D001.

It must be emphasized that the Salmonella/microsome assay or Ames test is designed as a test for mutagenicity. The Ames test is not designed for accurately assessing acute toxicity, due to the mutant characteristics of the various tester strains. Thus, toxicity as generally observed in the Salmonella/microsome assay should be further identified and quantified in a bioassay specifically designed for acute toxicity, such as the Microtox test.

In addition, the acutely toxic effects in the Ames test cause a lower level of background revertants available for reverse mutations, which may mask possible mutagenic effects normally resulting in increased revertants. Thus, other bioassays that are more resistant to acutely toxic samples should be used to further assess mutagenicity and chronic toxicity. The insensitivity of the Ames test in detecting most chlorinated organic compounds (Rinkus and Legator, 1979) is another limitation of using the Salmonella/microsome assay to assess complex mixtures such as landfill leachates.

As previously discussed, the Bacillus subtilis assay to detect genetic mutagenicity indirectly as DNA damage can be useful for assessing wastewater samples that are acutely toxic to the Salmonella typhimurium tester strains (Fort et al., 1981). The Bacillus subtilis system has another advantage in comparison to the Ames test; it is sensitive to chlorinated chemicals, while the Salmonella/microsome assay is not (Matsui

et al., 1989).

The Aspergillus nidulans fungal bioassay is also more resistant to toxicity than the Ames test. The Aspergillus nidulans assay measures chronic toxicity in terms of chromosome damage, and also as point mutations as in the Ames test (Donnelly et al., 1987).

Bacillus Subtilis Assay

One leachate sample tested was positive in the Bacillus subtilis DNA repair spot test. Leachate 004 was positive in the Bacillus subtilis assay using tester strain mc-1. Table 4 shows the volumes of original aqueous leachates required for the organic residue doses applied in the Bacillus subtilis assay. Table 5 shows the activities of the various leachate samples as tested with the various Bacillus subtilis tester strains. Table 6 presents the activity in the Bacillus subtilis assay expressed as zone of inhibition mm/L of original aqueous leachate sample.

Tester strain mc-1 is deficient in recombination (Rec^-) DNA repair capabilities. The zone of inhibition produced in mc-1 with Leachate 004 was 8.0 $\pm$ 1.6 mm, greater than the 2.5 mm difference above the wild type strain, 168wt (5.0 $\pm$ 1.1 mm) necessary for a response to be considered positive.

Leachate 004 was also borderline positive in the Rec^- and Exc^- tester strain fh2006.7. The zone of inhibition (7.4 $\pm$ .48 mm) was 2.4 mm greater than the corresponding zone of

Table 4. Volumes of original aqueous leachate samples required for dose levels applied in the Bacillus subtilis DNA repair spot test.

Sample	Dose (mg/mL)	Dose (mg/plate)	Sample Volume (L)
Leachate D001	200	20	1.61
Leachate 001	200	20	0.48
Leachate 002	200	20	0.75
Leachate 004	200	20	1.01

Table 5. Lethal effects, in the Bacillus subtilis spot test, of leachate extracts on various DNA repair proficient and deficient strains.

	Zone of inhibition (mm) for each Bacillus subtilis tester strain.[1]				
		repair deficient			
	168wt	recA8	mc-1	fh2006.7	hcr9
Sample					
Leachate D001	2.0 ± 0.76	3.5 ± 1.0	3.5 ± 0.58	3.3 ± 0.50	2.8 ± 0.96
Leachate 001	2.9 ± 0.78	3.2 ± 1.3	3.8 ± 0.96	3.3 ± 1.5	3.4 ± 1.2
Leachate 002	2.2 ± 1.0	3.9 ± 0.63	3.3 ± 1.5	3.9 ± 1.0	2.3 ± 0.96
Leachate 004	5.0 ± 1.1	7.3 ± 1.3	8.0 ± 1.6	7.4 ± 0.48	6.5 ± 1.7
Control					
Negative	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Solvent (DMSO)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Positive (MMC)	7.1 ± 2.3	25.0 ± 4.8	18.0 ±14.0	11.0 ± 3.0	12.0 ± 2.4
Positive (Mit. C)	15.0 ± 1.9	22.0 ± 3.0	25.0 ± 1.7	30.0 ± 3.4	22.0 ± 3.7

[1] Zone of inhibition reported as mean and standard deviation calculated from four independent tests (two duplicate plates on two independent test dates).

Table 6. Lethal effects of leachate extracts on various DNA repair proficient and deficient strains, expressed as zone of inhibition (mm/L) of original aqueous leachate sample.

	Zone of inhibition (mm/L) for each Bacillus subtilis tester strain.[1]				
	repair deficient				
	168wt	recA8	mc-1	fh2006.7	hcr9
Sample					
Leachate D001	1.2 ± 0.47	2.2 ± 0.62	2.2 ± 0.36	2.0 ± 0.31	1.7 ± 0.59
Leachate 001	6.1 ± 1.6	6.7 ± 2.7	7.9 ± 2.0	6.9 ± 3.1	7.1 ± 2.5
Leachate 002	2.9 ± 1.3	5.2 ± 0.84	4.4 ± 2.0	5.2 ± 1.3	3.1 ± 1.3
Leachate 004	5.0 ± 1.1	7.2 ± 1.3	7.9 ± 1.6	7.3 ± 0.48	6.4 ± 1.7

[1] Zone of inhibition (mm/L) of original aqueous leachate sample is reported as the mean and standard deviation of the zone of inhibition (mm) divided by the original aqueous leachate sample volume required for the applied plate dose as reported in Table 4.

inhibition in 168wt (5.0 ± 1.1 mm). The excision repair-deficient tester strain (Exc$^-$) hcr-9 showed a much smaller response to Leachate 004 than the tester strains deficient in recombination repair (recA8, mc-1, and fh2006.6). Exc$^-$ tester strain hcr-9 produced only a 1.5 mm greater zone of inhibition than the wild-type strain 168 wt. These results agree with the observations of Felkner et al. (1981) who determined that Bacillus subtilis tester strains that are recombination repair-deficient are more sensitive to sample induced DNA damage than strains that are only excision repair-deficient.

None of the other leachate extracts were positive in any of the Bacillus subtilis repair-deficient strains. However, by calculating the zone of inhibition (mm)/L of original aqueous leachate sample (Table 6), it should be noted that Leachate 001 would produce approximately the same level of lethal effects as Leachate 004 at equal environmental volumes. With equal environmental volumes, Leachate D001 and Leachate 002 both would produce significantly fewer lethal effects than Leachate 004 and Leachate 001.

It should be emphasized that the Bacillus subtilis assay is an indirect measure of mutagenicity through its sensitivity to DNA damage in repair deficient tester strains. However, as previously discussed, Kada et al. (1972) confirmed a correlation between DNA damage and mutagenicity as measured in the Ames test. In addition, Ames and McCann (1981) estimated that 83% of known carcinogens are mutagenic in the

Salmonella/microsome assay.

It should be noted that Leachate 004 was collected from a currently operating municipal solid waste landfill located in an industrialized area, but subject to regulations preventing the disposal of hazardous waste within it. Leachate 004 was positive in the Bacillus subtilis DNA repair assay, which can be correlated with mutagenicity and carcinogenicity. Leachate D001, which was collected from a closed municipal landfill subject to co-disposal of hazardous waste, was negative in the Bacillus subtilis assay. These data provide evidence that currently operating municipal solid waste landfills, particularly those receiving industrial as well as residential waste, are producing leachate which is as carcinogenic as that from older landfills that were subject to co-disposal of hazardous waste. It appears that either the current regulations banning hazardous materials are not effective in keeping hazardous materials out of the MSW landfills, or the toxins are coming from household hazardous wastes and small quantity generators. In addition, changes in the types and quantities of materials used by society may also contribute to changes in the toxicity of leachates produced.

Aspergillus Nidulans Assay

Two of the four leachate samples tested were positive in the diploid Aspergillus nidulans chromosome damage assay. Table 7 shows the volumes of original aqueous samples required for the organic residue dose levels applied. Table 8 shows

Table 7. Volumes of original aqueous samples required for dose levels applied in the Aspergillus nidulans diploid chromosome damage test.

Sample	Dose (mg/mL)	Dose (mg/plate)	Sample Volume (L)
Leachate D001	20	2	0.16
Leachate 001	20	2	0.05
Leachate 002	20	2	0.07
Grdwater 003	20	2	2.63
Leachate 004	20	2	0.10

Table 8. Abnormal colonies formed and relative indexes for leachate and groundwater extracts as determined with the Aspergillus nidulans diploid chromosome damage test.

Sample	% Abnormal Colonies	Relative Index to: Distilled Water	DMSO
Leachate D001	25	8.8	4.3
Leachate 001	19	6.7	3.2
Leachate 002	--	--	--
Grdwater 003	7.2	2.4	1.2
Leachate 004	9.9	3.4	1.6
Control			
Negative (distilled water)	2.9	1.0	--
Solvent (DMSO)	5.9	2.1	1.0

the abnormal colonies formed and relative indexes for each sample in the Aspergillus nidulans assay. Table 9 presents the activity in the Aspergillus nidulans assay expressed as relative index/L of original aqueous sample.

Using the two-fold rule (Chu et al., 1981) Leachate D001 and Leachate 001 were positive as compared to the solvent control. Leachate D001 produced 25% abnormal colonies in the diploid chromosome assay, resulting in a relative index of 4.3 as compared to the DMSO solvent control. Leachate 001 also tested positive, with 19% abnormal colonies formed, resulting in a relative index to DMSO of 3.2. The relative indexes compared to a negative control, distilled water, were also calculated. However, because the solvent, DMSO, has a positive relative index as compared to distilled water, it must be used to assess the chromosome damage in the samples caused only by the organic constituents in the sample. Groundwater 003 and Leachate 004, which were determined to be genetically toxic in the Bacillus subtilis assay, were both negative in the Aspergillus nidulans chromosome damage assay. Leachate 002 was toxic to the Aspergillus nidulans system, thus its genetic toxicity in the form of chromosome damage was not assessed.

It should be noted that Leachate D001 has a relative index approximately one third greater than Leachate 001. However, Leachate D001 required nearly four times the volume of original aqueous leachate than Leachate 001 to supply the

Table 9. Relative indexes for leachate and groundwater extracts in the Aspergillus nidulans diploid chromosome damage test, expressed as relative index/L of original aqueous sample.

Sample	Relative Index/L of original aqueous sample[1]: Distilled water	DMSO
Leachate D001	55	27
Leachate 001	140	67
Leachate 002	--	--
Grdwater 003	0.95	0.46
Leachate 004	34	17

[1] Relative Index/L of original aqueous sample is reported as the relative index divided by the original aqueous sample volume required for the applied plate dose as reported in Table 7.

organic residue dose levels applied. When relative index/L of original aqueous leachate sample is calculated (Table 9), it should be noted that Leachate 001 would be more genetically toxic than Leachate D001 at equal environmental volumes. These data provide evidence that the leachates produced from recently closed municipal solid waste landfills receiving primarily residential waste (Leachate 001) are likely to be more genetically toxic as measured by Aspergillus nidulans than leachates produced from older closed landfills which were subject to co-disposal of hazardous waste (Leachate D001).

Microtox Assay

All four leachate samples were confirmed to be acutely toxic in the Microtox luminescent bacteria acute toxicity test. Table 10 shows the volumes of original aqueous samples required for the organic residue dose levels applied in the Microtox test. Table 11 depicts the relative acute toxicities of the leachate samples in the Microtox test at the various test times. Table 12 presents the activity in the Microtox test expressed as relative toxicity/L of original aqueous sample volume.

Using the two-fold rule (Chu et al., 1981) all leachate samples were acutely toxic at all test times. Leachate 001 (relative index of 3.8 at 5 minutes to 4.4 at 30 minutes) and Leachate 002 (relative index of 2.3 at 5 minutes to 2.7 at 30 minutes) both increased in toxicity relative to the negative control (Groundwater 003) with increasing test times.

Table 10. Volumes of original aqueous samples required for dose levels applied in the Microtox acute toxicity test.

Sample	Dose (mg/mL)	Dose (mg/test)	Sample Volume (L)
Leachate D001	200	300	24.2
Leachate 001	200	300	7.2
Leachate 002	200	300	11.2
Leachate 004	200	300	15.1
Grdwater 003	200	300	394.6

Table 11. Acute toxicities, in the Microtox test, of sample extracts at various test times.

Sample	Test time	% EC 50	Relative Toxicity	Average Relative Toxicity[1]
Leachate D001	5	2.5	7.0	6.9 ± 1.2
	15	2.7	5.7	
	30	2.0	8.0	
Leachate 001	5	4.6	3.8	4.1 ± 0.31
	15	3.8	4.2	
	30	3.6	4.4	
Leachate 002	5	7.5	2.3	2.5 ± 0.20
	15	6.5	2.5	
	30	6.0	2.7	
Leachate 004	5	4.0	4.4	4.2 ± 0.15
	15	3.8	4.2	
	30	3.9	4.1	
Grdwater 003 (Negative control)[2]	5	18.0	1.0	1.0 ± 0.0
	15	16.0	1.0	
	30	16.0	1.0	

[1] Average relative toxicity is reported as the mean and standard deviation of the relative toxicities at three test times.

[2] Relative toxicities of .70, .64, .64 (.66±.03) at 5, 15, and 30 minutes, respectively, when tested against a standard positive control (DOW G).

Table 12. Acute toxicities of sample extracts in the Microtox test, expressed as relative toxicity/L of original aqueous leachate sample.

Sample	Average Relative Toxicity/ L of original aqueous leachate sample[1]
Leachate D001	0.29 ± 0.05
Leachate 001	0.57 ± 0.04
Leachate 002	0.22 ± 0.02
Leachate 004	0.28 ± 0.01

[1] Average relative toxicity/L of original aqueous leachate sample is reported as the mean and standard deviation of relative toxicity divided by the original aqueous leachate sample volume required for the applied test dose as reported in Table 10.

Leachate D001 decreased in relative toxicity from 7.0 at 5 minutes to 5.7 at 15 minutes. However, it did increase in relative toxicity at 30 minutes (relative index of 8.0) as compared to 5 and 15 minutes. Leachate 004 decreased slightly in relative toxicity with increasing test time, from 4.3 at 5 minutes to 4.1 at 30 minutes.

At the applied dose levels, Leachate D001 (6.9 ± 1.2) was nearly twice as acutely toxic as either Leachate 001 (4.1 ± .31) or Leachate 004 (4.2 ± 1.5) at all test times. Leachate 001 and Leachate 004 were in turn nearly twice as toxic as Leachate 002 (2.5 ± .20) at all test times. When relative toxicity for each leachate is calculated as relative toxicity/L of original aqueous sample (Table 12) it should be noted that Leachate 001 (.57 ± .04) would be approximately twice as acutely toxic as Leachate D001 (.29 ± .05), Leachate 002 (.22 ± .02), and Leachate 004 (.28 ± .01) at equal environmental volumes. Thus, leachates from municipal solid waste landfills (Leachate 001, Leachate 002, Leachate 004) were at least as acutely toxic as leachates produced from landfills that were subject to co-disposal of hazardous waste (Leachate D001).

CHEMICAL ANALYSIS

Volatile and Semi-volatile Chlorinated Organic Compounds

Both hermetically collected raw leachate samples, as well as the groundwater sample, were determined to contain several

U.S. EPA Priority Pollutants, Target List Compounds, Appendix IX Compounds, and compounds on the National Drinking Water List. Table 13 shows the average concentrations and ranges of the volatile and semi-volatile pollutants in VOA samples of Leachate 001, Leachate 002, and Groundwater 003. Table 14 shows the average concentrations of the analytes in municipal solid waste landfill leachates, as well as their respective promulgated regulatory standards.

Leachate 001 contains 12 U.S. EPA regulated compounds, including two priority pollutants that exceed the promulgated standards. The analyte 1,1-dichloroethane is present in an average concentration (6 ug/L) more than 10 times the promulgated standard of .58 ug/L. Dichloromethane (methylene chloride) is present in an average concentration (188 ug/L) nearly 40 times the EPA standard of 4.8 ug/L.

Leachate 002 contains 8 U.S. EPA regulated compounds, including three priority pollutants that exceed the promulgated regulatory standards. Dichloromethane is present in an average concentration (276 ug/L) nearly 60 times the promulgated standard of 4.8 ug/L. The analyte 1,1,2-trichloroethane is present in an average concentration (16 ug/L) approximately 2.5 times the promulgated standard of 6.3 ug/L. Trichloroethylene is present in an average concentration (20 ug/L) four times the promulgated standard of 5 ug/L.

Groundwater 003 contains 9 U.S. EPA regulated compounds,

Table 13. Average concentrations (ug/L with ranges in parentheses) of volatile and semi-volatile chlorinated organic compounds in hermetically collected samples as determined with purge and trap concentration and gas chromatography.

Compound	Sample		
	Leachate 001[1]	Leachate 002[2]	Grdwater 003[2]
Carbon tetrachloride[3]	trace	0	0
1,3-dichlorobenzene3	19 (11.5-27)	5.4 (4.6-6.4)	2.3 (0-3.5)
1,1-dichloroethane[3]	6 (0-12)	0	trace
1,2-dichloroethane[3]	trace	0	0
dichloromethane[3]	188 (0-376)	276 (0-827)	133 (0-226)
1,1,2,2-tetrachloroethane[3]	trace	0	0
trans-1,2-dichloroethylene[3]	trace	4,616 (0-6,582)	trace
1,2,3-trichlorobenzene[4]	trace	trace	trace
1,2,4-trichlorobenzene[3]	3.8 (0-4.3)	trace	trace
1,1,1-trichloroethane[3]	trace	123 (33-169)	trace
1,1,2-trichloroethane[3]	2.5 (0-5)	16 (0-24)	trace
trichloroethylene[3]	trace	20 (0-60)	trace

[1] average of 2 individual VOA vial samples
[2] average of 3 individual VOA vial samples
[3] compound on :

Priority Pollutant List. Promulgated by the U.S. EPA under authority of the Clean Water Act of 1977. Federal Register, 44, 233, December 3, 1979.

Target Compound List. Promulgated by the U.S. EPA under authority of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) of 1980 and the Superfund Amendments and Reauthorization Act (SARA) of 1986.

Appendix IX List. Promulgated by the U.S. EPA under authority of the Solid Waste Disposal Act of 1965 and the Resource Conservation and Recovery Act (RCRA) of 1976. Federal Register, 52, 131, July 9, 1987.

National Drinking Water List. Promulgated by the U.S. EPA under authority of the Safe Drinking Water Act, as amended in 1986. Federal Register, 52, 130, July 8, 1987.

[4] compound on: National Drinking Water List

Table 14. Average concentrations in municipal solid waste landfill leachates and promulgated regulatory standards for volatile and semi-volatile chlorinated organic compounds identified in samples Leachate 001, Leachate 002, and Groundwater 003.

Compound	Average Concentration (ug/L) (USEPA,1988b)	Promulgated Standards (ug/L) (USEPA,1988c)
carbon tetrachloride	202	5[1]
1,3-dichlorobenzene	--	--
1,1-dichloroethane	1,715	.58[2]
1,2-dichloroethane	1,841	5[1]
dichloromethane	5,352	4.8[2]
1,1,2,2-tetrachloroethane	210	1.7[2,3]
trans-1,2-dichloroethylene	568	--
1,2,3-trichlorobenzene	--	--
1,2,4-trichlorobenzene	--	--
1,1,1-trichloroethane	887	200[1]
1,1,2-trichloroethane	378	6.3[2,3]
trichloroethylene	187	5[1]

[1] Maximum Contaminant Level, EPA drinking water standard

[2] Constituent is considered carcinogenic when ingested orally; concentration is based on a unit risk of 10^{-6} except as noted.

[3] Concentration based on 10^{-5} risk (Class C carcinogen)

including one priority pollutant that exceeds the promulgated standard. Dichloromethane is present in an average concentration (133 ug/L) nearly 30 times the promulgated standard of 4.8 ug/L.

Most of the compounds in Leachate 001 and Leachate 002 were also present in much lower concentrations, in Groundwater 003. The presence of the U.S. EPA regulated pollutants in Groundwater 003 can most likely be attributed to the close proximity of the monitoring well samples and the landfill cells from which Leachate 001 and Leachate 002 were collected. These data show that potential contamination risks exist from recently closed and currently operating municipal solid waste landfills that are equipped with liners and leachate collection systems. This points to the need for more stringent regulations on monitoring requirements for MSW landfills and the need to develop environmentally safe alternatives to landfill disposal.

Semi-Volatile Aromatic Organic compounds

All three methylene chloride extracted leachate samples contain U.S. EPA Priority Pollutants, Target List Compounds, Appendix IX Compounds, and compounds on the National Drinking Water List. Table 15 shows the concentrations of the semi-volatile aromatic compounds in the samples. Table 16 lists the average concentrations of the sample analytes in municipal solid waste landfill leachates, as well as their promulgated

Table 15. Concentrations (ug/L) of semi-volatile aromatic compounds in original aqueous samples as determined with gas chromatography.

Compound	Sample			
	Leachate D001	Leachate 001	Leachate 002	Grdwater 003
ethylbenzene[1]	trace	2,329	529	0
m-xylene[2]	0	165	0	0
toluene[1]	18	10,200	597	0

[1] compound on :

Priority Pollutant List. Promulgated by the U.S. EPA under authority of the Clean Water Act of 1977. Federal Register, 44, 233, December 3, 1979.

Target Compound List. Promulgated by the U.S. EPA under authority of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) of 1980 and the Superfund Amendments and Reauthorization Act (SARA) of 1986.

Appendix IX List. Promulgated by the U.S. EPA under authority of the Solid Waste Disposal Act of 1965 and the Resource Conservation and Recovery Act (RCRA) of 1976. Federal Register, 52, 131, July 9, 1987.

National Drinking Water List. Promulgated by the U.S. EPA under authority of the Safe Drinking Water Act, as amended in 1986. Federal Register, 52, 130, July 8, 1987.

[2] compound on: Target Compound List, Appendix IX List, National Drinking Water List.

Table 16. Average concentrations in municipal solid waste landfill leachates and promulgated regulatory standards for semi-volatile aromatic compounds identified in samples Leachate D001, Leachate 001, Leachate 002, and Leachate 004.

Compound	Average Concentration (ug/L) (USEPA, 1988b)	Promulgated Standard (ug/L) (USEPA, 1988c)
ethylbenzene	274	4,000[1]
m-xylene	222[2]	70,000[1,2]
toluene	1,016	10,000[1]

[1] Concentration based on EPA critical dose for systemic toxicants and the assumption of a 70 kg adult consuming 2 liters of water per day.

[2] Includes all xylene isomers

standards.

Leachate D001 contains two U.S. EPA regulated aromatic compounds. Ethylbenzene is present at trace concentrations. Toluene is present at low (18 ug/L) but quantifiable concentrations.

Leachate 001 contains significant concentrations of three U.S. EPA regulated aromatic compounds. Toluene is present in a concentration (10,200 ug/L) that exceeds the U.S. EPA standard of 10,000 ug/L. Ethylbenzene is present in a concentration (2,329 ug/L) more than half of the promulgated regulatory standard of 4000 ug/L.

Leachate 002 contains significant concentrations of ethylbenzene (529 ug/L) and toluene (597 ug/L). Groundwater 003 did not contain any of the analyzed semi-volatile aromatics.

A surrogate sample containing concentrations of toluene, ethylbenzene, and m-xylene approximating the sample concentrations was extracted using the same methylene chloride procedure (USEPA, 1986) as previously described for the samples. The extraction and analysis of the surrogate sample showed full recovery of toluene, ethylbenzene, and m-xylene. Benzene added to the surrogate sample was not recovered. This suggests that methylene chloride extraction may be of limited use in recovering benzene from aqueous leachate samples. However, small volumes of the original aqueous samples were analyzed by gas chromatography and showed no benzene present

in any of the leachate samples.

It should be noted that the concentrations of ethylbenzene and toluene are much higher in Leachate 001 (from the recently closed cell) than in Leachate 002 (from the recently opened and operating cell). This difference could be due to the disposal of more potentially hazardous wastes in the landfill cell closed earlier. The difference could also reflect the stage of each cell. Leachate 002 was gray-orange, indicating it was exposed to a primarily aerobic, oxidizing environment that was moving into a reducing anaerobic stage. Leachate 001 was oily-black, indicating it was exposed to an anaerobic, reducing environment. Thus, the differences in analyte concentrations found in various landfills is likely a result of biotransformations within the landfill, as well as the type of waste disposed.

It should again be emphasized that much higher concentrations of semi-volatile aromatic priority pollutants were present in Leachate 001 and Leachate 002, as compared to Leachate D001. This provides evidence that municipal solid waste landfills opened after development of regulations prohibiting co-disposal of hazardous waste (Leachate 001 and Leachate 002) may produce leachates with much greater concentrations of semi-volatile aromatic priority pollutants than leachates from landfills where hazardous wastes were co-disposed (Leachate D001).

Non-Volatile Organic Compounds

All of the leachate samples contain quantifiable concentrations of acutely or chronically toxic non-volatile organic compounds. Table 17 shows the concentrations of the non-volatile organic compounds in the bioassay fraction of the leachate and groundwater samples. Table 18 shows the average concentrations of the sample analytes in municipal solid waste landfill leachates, as well as their promulgated regulatory standards.

A surrogate sample containing the non-volatile analytes (excluding phthalates) in concentrations approximating their sample concentrations was extracted and concentrated using the XAD resin column and acetone procedure (Brown and Donnelly, 1984) as previously described for the sample extractions. The extraction and analysis of the surrogate sample showed trace levels of diethyl phthalate and bis-(2-ethylhexyl) phthalate. Based on the surrogate sample extraction, and a comparison of the phthalate concentrations in the leachate and groundwater samples, the concentrations of phthalates were quantified but not included in the calculation of estimated cumulative cancer risk.

It is assumed that the phthalates, which are common plasticizers (Agarwal et. al., 1985), were dissolved in significant concentrations by the samples from the plastic barrels in which the samples were stored. Both diethyl phthalate and bis-(2-ethylhexyl) phthalate are present in many

Table 17. Concentrations (ug/L) of non-volatile organic compounds in bioassay sample fractions as determined with gas chromatography/ mass spectrometry and gas chromatography.

Compound	Sample				
	Lea D001	Lea 001	Lea 002	Grd 003	Lea 004
bis-(2-ethylhexyl) phthalate[1]	30	58	47	29	49
camphor	--	--	--	--	79
4-chlorophenol	4,616	--	--	--	--
decanoic acid	--	777	6,897	--	--
diethyl phthalate[1]	30	119	36	trace	45
nonanoic acid	0	614	2,917	--	--
o-cresol	trace	101	185	--	--
p- and m-cresol[1]	trace	3,187	513	--	--
phenol[1]	trace	254	902	--	--
a-terpineol	14	1,970	--	--	--
3-tert-butylphenol	--	--	--	--	60

[1] compound on :

Priority Pollutant List. Promulgated by the U.S. EPA under authority of the Clean Water Act of 1977. Federal Register, 44, 233, December 3, 1979.

Target Compound List. Promulgated by the U.S. EPA under authority of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) of 1980 and the Superfund Amendments and Reauthorization Act (SARA) of 1986.

Appendix IX List. Promulgated by the U.S. EPA under authority of the Solid Waste Disposal Act of 1965 and the Resource Conservation and Recovery Act (RCRA) of 1976. Federal Register, 52, 131, July 9, 1987.

Table 18. Average concentrations in municipal solid waste landfill leachates and promulagated regulatory standards for non-volatile organic compounds identified in samples Leachate D001, Leachate 001, Leachate 002, Groundwater 003, and Leachate 004.

Compound	Average Concentration (ug/L) (USEPA,1988b)	Promulgated Standard (ug/L) (USEPA,1988c)
bis(2-ethylhexyl)phthalate	184	70[1]
camphor	--	--
4-chlorophenol	--	--
decanoic acid	--	--
diethyl phthalate	118	460,000[1]
nonanoic acid	--	--
o-cresol	--	--
p- and m-cresol	2,394[2]	2,000[1,2]
phenol	2,456	1,000[1]
a-terpineol	--	--
3-tert butyl phenol	--	--

[1] Concentration based on EPA verified reference dose for systemic toxicants and the assumption of a 70 kg adult consuming 2 liters of water per day.

[2] Concentration includes only p-cresol

municipal solid waste landfill leachates (Table 18). However, it is likely that the majority of their concentrations in the samples are due to contamination from the sample collection and extraction. Thus, phthalates were not included in the calculation of estimated cumulative cancer risk, or considered in the discussion of the non-volatile toxic analytes present in the samples.

Leachate D001 contained five toxic non-volatile organic compounds. U.S. EPA regulated compounds include p- and m-cresol and phenol. Although not regulated, 4-chlorophenol was present in significant concentrations, and is a known mutagenic compound (Strobel and Grummt, 1987).

Leachate 001 and Leachate 002 both contain appreciable concentrations of several toxic and U.S. EPA regulated compounds. Leachate 001 contains concentrations of the U.S. EPA Priority Pollutants p- and m-cresol (3,187 ug/L) in excess of the promulgated standard of 2000 ug/L. It should be noted that because of the co-elution of p- and m-cresol, their concentrations in samples are based on standards including 50% of each analyte. The actual sample concentrations of each isomer were not measurable, and thus were reported as the concentration of a 50%/50% mixture of the isomers.

Leachate 001 also contains phenol (254 ug/L), another U.S. EPA Priority Pollutant. Also present in Leachate 001 are significant concentrations of the known toxic compounds nonanoic acid, decanoic acid, and o-cresol (RTECS, 1989) and

a-terpineol (Opdyke, 1974).

Leachate 002 contains all of the toxic compounds found in Leachate 001 with the exception of a-terpineol. Phenol is present in a concentration (902 ug/L) very near the promulgated standard of 1000 ug/L. The analytes p- and m-cresol are present in lower concentrations than in Leachate 001, however, o-cresol is present in a higher concentration than in Leachate 001. The organic acids, nonanoic and decanoic acids, are present in much higher concentrations in Leachate 002 than Leachate 001. This contradicts what would be expected regarding organic acid concentrations, as Leachate 002 is from a recently opened cell that appeared to be in an aerobic, oxidizing state. Larger concentrations of acids would be expected in the anaerobic, reducing state present in the closed cell Leachate 001 was collected from.

Groundwater 003 contained no non-volatile organic compounds, and Leachate 004 contained only two non-volatile organic compounds. The known toxic compounds identified in Leachate 004 included camphor (Brown and Donnelly, 1988) and 3-tert butylphenol (RTECS, 1989).

Leachate D001, Leachate 001, and Leachate 002 all contain at least one compound that has tested positive in the Salmonella/microsome mutagenicity assay or the Aspergillus nidulans chromosome damage assay. Leachate D001 contains phenol, which has been determined to be mutagenic in the Ames test with metabolic activation (Gocke et al, 1981) and

positive in the Aspergillus nidulans chromosome damage assay (RTECS, 1989). It also contains 4-chlorophenol, which has been determined to be mutagenic in the Ames test with and without metabolic activation (Strobel and Grummt, 1987). Leachate D001 was not positive in the Ames mutagenicity test, thus the phenol and 4-chlorophenol did not induce mutagenic effects due to either low concentrations or the acutely toxic effects of the sample matrix masking the mutagenic response.

Leachate 001 and Leachate 002 both contain appreciable levels of phenol, which has been determined to be mutagenic in the Ames test as discussed previously (Gocke et al, 1981). However, the phenol did not induce mutagenic effects due to either low concentrations or acute toxicity of the sample matrix, as in Leachate 001. Phenol has also tested positive in the Aspergillus nidulans chromosome damage assay (RTECS, 1989), as previously mentioned. Phenol is present in trace concentrations in Leachate D001, and in significant concentrations in Leachate 001 (254 ug/L) and Leachate 002 (902 ug/L).

As discussed previously, Leachate D001 and Leachate 001 were both positive in the Aspergillus nidulans chromosome assay. It is likely that some of the abnormal colonies formed by Leachate D001 and Leachate 001 in the chromosome damage assay were caused by phenol. It is also likely that the high levels of 4-chlorophenol (4,616 ug/L) in Leachate D001 contributed to the positive response in the Aspergillus

nidulans assay.

The acute toxicity of Leachate 002 should be noted, and is likely due to the high concentrations of nonanoic (2,917 ug/L) and decanoic (6,897 ug/L) acids, both known to be acutely toxic (RTECS, 1989). A response for chromosome damage that would have resulted from the much greater level of phenol (902 ug/L) than was present in Leachate 001 may have been masked by the toxicity from the high concentrations of acutely toxic organic acids.

Considering only the analytical data of Leachate D001, Leachate 001, Leachate 002, and Leachate 004, it should be noted that leachates produced from municipal solid waste landfills receiving primarily residential waste (Leachate 001 and Leachate 002) contain higher concentrations of more toxic and U.S. EPA regulated compounds than leachates from municipal solid waste landfills receiving both industrial and residential municipal waste (Leachate 004) and older co-disposal landfill leachates (Leachate D001).

Estimated Cumulative Cancer Risk Assessment

Three of the four leachate samples were at least within one order of magnitude of the estimated cumulative carcinogenic risk of the Love Canal leachate. Table 19 shows the estimated carcinogenic potencies of the organic compounds identified in the leachate and groundwater samples. Table 20 shows the estimated cumulative cancer risks for the leachate

Table 19. Estimated Carcinogenic Potency of organic compounds identified in leachate or groundwater samples.

Compound	Potency (B) (kg day/mg)	Total s Assumed	Method[1]	Reference
Purgeable Chlorinated Organics:				
carbon tetrachloride	4.0×10^{-3}	1.52	FS	1
1,3-dichlorobenzene	$<8.8 \times 10^{-2}$	2.50	LD	2
1,1-dichloroethane	4.6×10^{-3}	1.53[2]	LD	4
1,2-dichloroethane	9.4×10^{-3}	1.53	LD	2
dichloromethane	2.2×10^{-3}	1.80	LD	1
1,1,2,2,-tetra chloroethane	4.2×10^{-3}	1.60[3]	FS	4
trans-1,2-dichloro ethylene	2.6×10^{-5}	1.60	LD	1
1,2,3-trichlorobenzene	2.3×10^{-3}	2.10[4]	LD	4
1,2,4-trichlorobenzene	4.4×10^{-3}	2.10	LD	2
1,1,1-trichloroethane	1.7×10^{-5}	1.68	FS	2
1,1,2-trichloroethane	5.9×10^{-3}	1.60	FS	1
trichloroethylene	7.3×10^{-4}	1.52	FS	1
Semi-volatile Aromatics:				
ethylbenzene	3.4×10^{-4}	1.62	FS	1
m-xylene	5.6×10^{-4}	1.60	LD	4
toluene	9.0×10^{-4}	1.62	FS	1

Table 19. (continued).

Compound	Potency (B) (kg day/mg)	Total s Assumed	Method[1]	Reference
Non-volatile Organics:				
bis-(2-ethylhexyl) phthalate	1.4×10^{-3}	1.87	LD	1
camphor	8.9×10^{-3}	1.92	LD	1
4-chlorophenol	7.0×10^{-3}	2.01[5]	LD	4
decanoic acid	7.7×10^{-4}	1.71[6]	LD	4
diethyl phthalate	5.5×10^{-4}	2.41	LD	1
nonanoic acid	1.7×10^{-4}	1.71[6]	LD	4
o-cresol	3.3×10^{-2}	2.78[7]	LD	4
p- and m-cresol	1.2×10^{-2}	2.78	LD	1
phenol	1.7×10^{-2}	1.97	FS	1
a-terpineol	6.6×10^{-4}	1.71[6]	LD	3
3-tert butyl phenol	9.9×10^{-4}	1.71[6]	LD	4

[1] Method:
FS = rodent feeding study to determine carcinogenic potency (B)
LD = carcinogenic potency (B) calculated from oral rat LD_{50} acute toxicity data

Total s Assumed (uncertainty of measuring potency, B):
[2] s for 1,2 dichloroethane
[3] s for tetrachloroethane
[4] s for 1,2,4 trichlorobenzene
[5] s for chlorophenol
[6] average s
[7] s for p-cresol

References:
1-Brown and Donnelly (1988)
2-Crouch et al. (1983)
3-Opdyke (1974)
4-Registry of Toxic Effects of Chemical Substances (1989)

Table 20. Estimated Cumulative Cancer Risk for organic compounds in leachate and groundwater samples.

Sample	Estimated Cancer Risk x 10^9		
	Median	Mean	98th
Leachate D001[1]	14,000	100,000	824,000
Leachate 001	72,800	877,000	5,160,000
Leachate 002	54,000	284,000	1,920,000
Grdwater 003	428	2,470	16,400
Leachate 004[2]	378	1,900	15,000
Love Canal[3]	117,000	234,000	1,280,000

[1] Does not include volatile and semi-volatile chlorinated organic compounds.

[2] Includes only non-volatile organic compounds

[3] Estimated Cumulative Cancer Risk for suspected carcinogenic chemicals, as calculated by Brown and Donnelly (1988)

and groundwater samples.

Although Leachate 004 is several orders of magnitude lower in estimated cumulative cancer risk than the other leachate samples, it should be noted that its estimated risk is based only on the non-volatile organic compounds. VOA samples were not collected for analysis of volatile and semi-volatile U.S. EPA regulated compounds. Leachate D001, which tested positive for chronic toxicity in the Aspergillus nidulans assay is the same order of magnitude in median and mean estimated cancer risk as Leachate 001 and Leachate 002, but one order below in 98th percentile risk. This is likely due to the risk estimate of Leachate D001 not including volatile and semi-volatile chlorinated priority pollutants, as hermetically collected samples were not obtained.

The Groundwater 003 estimated cumulative cancer risk could be less than calculated. Most of the carcinogenic constituents were present in trace concentrations, which were entered as 1 ug/L in the risk assessment model previously discussed. This is also the case with Leachate 002 and particularly Leachate 001, both of which contain several volatile, semi-volatile, or non-volatile organic compounds at trace concentrations. However, the error in the estimation of the cumulative cancer risk due to assigning a 1 ug/L concentration to compounds found in trace concentrations should be negligible. Most of the organic analytes, particularly the non-volatile organics and semi-volatile

aromatics, were not detectable at concentrations of 1 ug/L in the standard dilutions. Thus, trace but unquantifiable concentrations of analytes in the samples could be higher than the assigned value of 1 ug/L for trace concentrations.

The nature of the risk assessment model and the assumptions inherent to it must be considered when analyzing the estimated cumulative cancer risks for the organic compounds in the leachate and groundwater samples. While no one would deliberately go out and drink landfill leachate, the fact remains that it will likely find its way into surface and/or groundwaters some of which may be used as a source of drinking water. Thus, the model can be used as a basis for comparing the cumulative cancer risk posed by various landfill leachates (Brown and Donnelly, 1988) and thus provide a means for deciding which leachates constitute the greatest potential threat to our groundwater reserves.

As previously mentioned, Leachate 001, which tested positive for chronic toxicity in the Aspergillus nidulans assay, and Leachate 002 are both on the same order of mean estimated cancer risk (10^{-4}) and 98th percentile estimated cancer risk (10^{-3}) as the Love Canal leachate. Leachate 001 has an estimated cumulative cancer risk approximately four times, or nearly half an order of magnitude, greater than the Love Canal leachate at the mean and 98th percentile levels. Leachate 002 also has an estimated cumulative cancer risk greater than the Love Canal landfill leachate at the mean and

98th percentile levels.

The cancer risk assessment provides evidence that leachates produced from recently closed (Leachate 001) or currently operating (Leachate 002) municipal solid waste landfills receiving primarily residential waste are at least as chronically toxic as leachates from co-disposal landfills (Leachate D001) and hazardous waste landfills such as Love Canal.

CONCLUSIONS

None of the leachates or groundwater samples were found to be mutagenic using the Salmonella/microsome bioassay (Table 21). However, three of the four leachate samples were acutely toxic in the Salmonella/microsome bioassay. Leachate D001, Leachate 001, and Leachate 002 all contain appreciable concentrations of compounds determined to be mutagenic in the Ames test: Leachate D001 contains 4-chlorophenol (Strobel and Grummt, 1987) and Leachate D001, Leachate 001, and Leachate 002 all contain phenol (Gocke et al., 1981). The possibility that acutely toxic effects masked a positive response for chronic toxicity as measured by mutagenicity was further investigated.

The Bacillus subtilis DNA repair spot test was utilized as another indication of genetic toxicity in the leachate samples. The Bacillus subtilis assay is less susceptible to acute toxicity than the Salmonella/microsome assay (Fort et

Table 21. Summary of genetic and acute toxicity responses and the mean cumulative cancer risk for all samples tested.

Sample	Genetic Toxicity			Acute Toxicity		
	Salmonella Microsome Assay	*Bacillus subtilis* Assay	*Aspergillus nidulans* Assay	*Salmonella typhimurium* Assay	Microtox Assay	Mean Cumulative Cancer Risk x 10^6
Leachate D001	negative	negative	positive	positive	positive	100
Leachate 001	negative	negative	positive	positive	positive	877
Leachate 002	negative	negative	negative	positive	positive	284
Grdwater 003	negative	positive	negative	negative		2.5
Leachate 004	negative	positive	negative	negative	positive	1.9
Love Canal						234

al., 1981). The Bacillus subtilis assay is also more sensitive to chlorinated organic compounds than the Ames test (Matsui et al., 1989). The Bacillus subtilis has been correlated with mutagenicity in the Ames test (Kada et al, 1972) and carcinogenicity (Ames and McCann, 1981).

Leachate 004, a sample collected from a currently operating municipal solid waste landfill receiving residential and industrial waste, tested positive for DNA damage using the recombination repair-deficient (Rec^-) tester strain mc-1. This shows that leachates from currently operating municipal solid waste landfills (Leachate 004) receiving residential and industrial waste, could be more genetically toxic as measured by DNA damage than leachates from landfills subject to co-disposal of hazardous waste (Leachate D001).

Leachate 004 also tested borderline positive using tester strains recA8 (Rec^-) and fh2006.7 (Rec^- and Exc^-), but was negative using excision repair-deficient (Exc^-) strain hcr-9. These results agree with the observations of Felkner et al. (1981), and emphasize the utility of the more sensitive Rec^- tester strains for the detection of mutagens in municipal landfill leachate samples.

Two leachate samples, Leachate D001 and Leachate 001, were found to be genetically toxic as measured by the Aspergillus nidulans diploid chromosome damage assay. With equal environmental volumes of the original aqueous leachates, Leachate 001 would be more chronically toxic than Leachate

D001. These data provide evidence that leachates from municipal solid waste landfills receiving primarily residential waste (Leachate 001) could be more chronically toxic as measured by chromosome damage than leachates from landfills subject to co-disposal of hazardous waste (Leachate D001).

The positive responses in the Aspergillus nidulans systems are likely due in part to phenols, which have tested positive in the Aspergillus nidulans test (RTECS, 1989); phenols are present in appreciable concentrations in both Leachate D001 and Leachate 001. A third leachate which contains significant concentrations of phenol, Leachate 002, may have had a positive response in the Aspergillus nidulans assay masked by high concentrations of acutely toxic organic acids. Although the Aspergillus nidulans assay is more resistant than the Ames test to interference from acutely toxic compounds, the high concentrations of organic acids present in many municipal solid waste landfill leachates may prevent an accurate assessment for chronic toxicity using mutagenicity and chromosome damage assays.

It should also be noted that the samples (Leachate D001 and Leachate 001) positive in the Aspergillus nidulans chromosome damage assay (due likely in part to the phenols present) were not positive in the Bacillus subtilis DNA repair assay. Leachate 004 does not contain phenols, and was negative in Aspergillus nidulans assay and positive in the Bacillus subtilis assay.

All of the leachate samples were determined to be acutely toxic in the Microtox luminescent bacteria acute toxicity test. When considering equal environmental volumes of the original aqueous leachates, Leachate 001 would be approximately twice as acutely toxic as Leachate D001, Leachate 002, and Leachate 004. This suggests that leachates produced by municipal solid waste landfills receiving primarily residential waste (Leachate 001 and Leachate 002) are at least as acutely toxic as leachates from municipal solid waste landfills receiving industrial and residential waste (Leachate 004) and landfills that were subject to co-disposal of hazardous waste (Leachate D001).

The acute toxicity of the municipal solid waste landfill leachate samples as determined with the Microtox test emphasizes the possibility that mutagenic effects of chlorophenols (Strobel and Grummt, 1987) and phenols (Gocke et al., 1981) in three of the leachate samples (Leachate D001, Leachate 001, Leachate 002) were being masked by the acute toxicity of the sample matrix to the Salmonella/typhimurium tester strains. Any further characterization of landfill leachates with the Ames test to determine genetic toxicity will likely require fractionation of the sample to separate the mutagenic compounds from the acutely toxic compounds.

The results of the genetic and acute toxicity bioassays re-emphasize the utility of using a battery of bioassays (Plotkin and Ram, 1984) to more fully characterize the acute

and genetic toxicity of municipal solid waste landfill leachates.

Acutely or genetically toxic volatile, semi-volatile, or non-volatile organic compounds were found in all of the leachate and groundwater samples. Leachate 001 contains concentrations of the U.S. EPA Priority Pollutants 1,1-dichloroethane, dichloromethane, toluene, and p- and m-cresol in excess of the promulgated regulatory standards. Leachate 002 contains concentrations of the U.S. EPA Priority Pollutants dichloromethane, 1,1,2-trichloroethane, and trichlorethylene in excess of the EPA regulatory standards. Groundwater 003 contained several volatile chlorinated organic U.S. EPA Priority Pollutants, including dichloromethane in concentrations in excess of the EPA regulatory standards. These data combined with that of Laine and Miklas (1989) suggest that recently closed and currently operating landfills equipped with liners and leachate collection systems (as in the landfill where Leachate 001 and 002 were collected) may be leaking and contaminating adjacent groundwater supplies with potentially hazardous concentrations of U.S. EPA Priority Pollutants.

It thus appears that more stringent regulations are needed on the construction, operation and monitoring of municipal solid waste landfills. In addition, it points to the need for developing alternate technologies for disposing of our municipal wastes in an environmentally safe manner and

thus minimizing the amount of MSW which ultimately goes to a landfill. Innovative technologies such as source separation of wastes, recycling the maximum amount of re-usable material in the waste stream, and composting the organic fraction of the waste in conjunction with sewage sludge, have the potential to greatly reduce the amount of wastes going to landfills and will likely reduce the toxicity of the leachates generated thus protecting our groundwater resources.

Two of the three municipal solid waste landfill leachates (Leachate 001 and Leachate 002) from landfills which were opened since the regulations prohibiting co-disposal of hazardous waste were determined to have mean and 98th percentile estimated cumulative cancer risks greater than a Superfund landfill leachate (Leachate D001) and the Love Canal landfill leachate. Leachate 001 and Leachate 002 both have 98th percentile estimated cumulative cancer risks on the order of 10^{-3}; the Superfund co-disposal landfill leachate (Leachate D001) has a 98th percentile estimated cumulative cancer risk on the order of 10^{-4}. All of these risks are at least as great as the current regulated acceptable cumulative cancer risk of 10^{-4}.

The use of a battery of toxicity bioassays in conjunction with chemical analysis by gas chromatography has produced data that support the possibility that municipal solid waste landfill leachates pose a hazard to drinking water supplies. Further analysis of leachates from various municipal solid

waste landfills with bioassays and chemical analysis will aid in developing improved methodology and techniques for monitoring this hazard.

LITERATURE CITED

1. Agarwal, D.K., W.H. Lawrence, L.J. Nunce, and J. Autian. 1985. Mutagenicity evaluation of phthalic acid esters and metabolites in Salmonella typhimurium cultures. J. Toxicol. Environ. Health. 16:61-99.

2. Ames, B.N., J. McCann, and E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutat. Res. 31:347-364.

3. Ames, B.N., and J. McCann. 1981. Validation of the Salmonella test: a reply to Rinkus and Legator. Cancer Res. 41:4192-4203.

4. Baxter, K.M. 1985. The effects of a hazardous and a domestic waste landfill on the trace organic quality of chalk groundwater at a site in East Anglia. Sci. Total Environ. 47:93-98.

5. Bomberger, D.C., R. Lewis, and A. Valdez. 1987. Waste characterization study: assessment of recyclable and hazardous components. Second Semi Annual Progress Report, SRI Project PHY-2530. SRI International, Menlo Park, CA.

6. Booth, C.J., and P.J. Vagt. 1986. Characterization of a landfill-derived contaminant plume in glacial and bedrock aquifers, NE Illinois. UIUC-WRC-86-202. University of Illinois at Urbana-Champaign, Water Resources Center, Urbana-Champaign, IL.

7. Brown, K.W., and K.C. Donnelly. 1982. Mutagenic potential of water concentrates from the effluent of a waste oil storage pond. Bull. Environm. Contam. Toxicol. 28:424-429.

8. Brown, K.W., and K.C. Donnelly. 1984. Mutagenic activity of runoff and leachate water from hazardous waste land treatment. Environ. Pollut. Ser. A. 35:229-246.

9. Brown, K.W., and K.C. Donnelly. 1988. An estimation of the risk associated with the organic constituents of hazardous and municipal waste landfill leachates. Hazard. Waste Mater. 5:1-30.

10. Cameron, J.D., and M.W. Stewart. 1982. Leachate disposal alternatives in a sole source aquifer environment. p.272-293. Fifth Annual Madison Conference of Applied Research and Practice on Municipal and Industrial Waste, Madison, WI, 22 Sept.-24 Sept. 1982.

11. Chu, K.C., K.M. Patel, A.H. Lin, R.E. Tarone, M.S. Linhart, and V.C. Dunkel. 1981. Evaluating statistical analyses and reproducibility of microbial mutagenicity assays. Mutat. Res. 85:119-132.

12. Crouch, E.A.C., R. Wilson, and L. Zeise. 1983. The risks of drinking water. Water Res. Res. 19:1359-1375.

13. DeWalle, F.B., and E.S.K. Chian. 1981. Detection of trace organics in well water near a solid waste landfill. JAWWA. 73(4):206-211.

14. Donnelly, K.C, K.W. Brown, and D. Kampbell. 1987. Chemical and biological characterization of hazardous industrial waste, I. Prokaryotic bioassays and chemical analysis of a wood-preserving bottom-sediment waste. Mutat. Res. 180:31-42.

15. Dressler, M. 1979. Extraction of trace amounts of organic compounds from water with porous organic polymers. J. Chromatogr. 165:167-206.

16. Dunlap, W.J., D.C. Shew, J.M. Robertson, and C.R. Toussaint. 1975. Organic pollutants contributed to groundwater by a landfill. p. 96-110. Proceedings Research Symposium on Gas and Leachate from Landfills, Rutgers University-Cooks College, New Brunswick, NJ. 24 Mar.-26 Mar. 1975.

17. Dunlap, W.J., D.C. Shew, M.R. Scalf, R.L. Cosby, and J.M. Robertson. 1979. Isolation and identification of organic contaminants in groundwater. p. 453-477. In H. Keith (ed.) Identification and analysis of organic pollutants in water. Ann Arbor Science Publishers, Inc. Ann Arbor, MI.

18. Felkner, I.C., K.M. Hoffman, and B.C. Wells. 1979. DNA-damaging and mutagenic effects of 1,2-dimethylhydrazine on Bacillus subtilis repair-deficient mutants. Mutat. Res. 68:31-40.

19. Felkner, I.C., A.D. Laumbach, and M.L. Harter. 1981. Development of a B. subtilis system to screen carcinogens/mutagens: DNA-damaging and mutation assays. p. 93-119. In I.C. Felkner (ed.) Microbial testers, probing carcinogenesis. Marcel Dekker, Inc. New York, NY.

20. Fort, C.L., M.K. Koczwara, E.J. Kirsch, and C.P.L. Grady, Jr. 1981. Evaluation of the quality of wastewater treatment effluent following chlorination or ozonation. p. 1261-1278. In R.L. Jolley et. al. (ed.) Water

Chlorination, Vol. 4. Ann Arbor Science, Ann Arbor, MI.

21. Gapinski, D.P. 1988. The impact of household hazardous wastes on landfill leachates. M.S. Thes. Rensselaer Polytechnic Institute. Troy, NY (Thes. Abstr. 88-516067).

22. Gocke, E., M.-T. King, K. Eckhardt, and D. Wild. 1981. Mutagenicity of cosmetics ingredients licensed by the European Communities. Mutat. Res. 90:91-109.

23. Grabow, W.O.K., J.S. Burger, and C.A. Hilner. 1981. Comparison of liquid-liquid extraction and resin adsorption for concentrating mutagens in Ames Salmonella/microsome assays on water. Bull. Environm. Contam. Toxicol. 27:442-449.

24. Haxo, H.E., and T.P. Lahey. 1988. Transport of dissolved organics from dilute aqueous solutions through flexible membrane liners. Hazard. Waste Hazard. Mater. 5:275-294.

25. Hooper, K., C. Gold, and B.N. Ames. 1978. Developments of methods for mutagenicity testing of wastewater and drinking water samples by the Ames Salmonella test. Report to Water Resources Control Board, Sacramento, CA.

26. Johnson, R.L., J.A. Cherry, and J.E. Pankow. 1989. Diffusive contaminant transport in natural clay: a field example and implications for clay-lined waste disposal sites. Environ. Sci. Technol. 23:340-349.

27. Kada, T., K. Tutikawa, and Y. Sadaie. 1972. In vitro and host-mediated "rec-assay" procedures for screening chemical mutagens; and phloxine, a mutagenic red dye detected. Mutat. Res. 16:165-174.

28. Kafer, E., B.R. Scott, G.L. Dorn, and R. Stafford. 1982. Asperigillus nidulans: systems and results of tests for chemical induction of mitotic segregation and mutation, I. Diploid and duplication assay systems, A report of the USEPA Gene-Tox Program. Mutat. Res. 98:1-48.

29. Kmet, P., and P.M. McGinley. 1982. Chemical characteristics of leachate from municipal solid waste landfills in Wisconsin. p. 225-254. Fifth Annual Madison Conference of Applied Research and Practice on Municipal and Industrial Waste, Madison, WI, 22 Sept.-24 Sept. 1982.

30. Laine, D.L., and M.P. Miklas, Jr. 1989. Detection and location of leaks in geomembrane liners using an electrical method: case histories. p.35-40. In

Proceedings of the 10th National Conference on Superfund, Washington, D.C. Nov. 27-29, 1989.

31. Maron, D.M., and B.N. Ames. 1983. Revised methods for the Salmonella mutagenicity test. Mutat. Res. 113:173-215.

32. Matsui, S., R. Yamamoto, and H. Yamada. 1989. The Bacillus subtilis/ microsome rec-assay for the detection of DNA damaging substances which may occur in chlorinated and ozonated waters. Wat. Sci. Tech. 21:875-887.

33. NUS Corporation. 1987. Characterization of MWC and leachates from MSW landfills, monofills, and co-disposal sites, summary volume I of VII. USEPA Rep. 530-SW-87-028A. Office of Solid Waste and Emergency Response, Washington, D.C.

34. NUS Corporation. 1988. Criteria for municipal waste landfills (40 CFR Part 258), Subtitle D of Resource Conservation and Recovery Act (RCRA) design criteria (subpart D). USEPA Rep. 530-SW-88-042. Office of Solid Waste and Emergency Response, Washington, D.C.

35. Opdyke, D.L.J. 1974. Terpineol. Food Cosmet. Toxicol. 12:997-998.

36. Plotkin, S., and N.M. Ram. 1984. Multiple bioassays to assess the toxicity of a sanitary landfill leachate. Arch. Environ. Contam. Toxicol. 13:197-206.

37. Pohland, F.G., and S.R. Harper. 1986. Critical review and summary of leachate and gas production from landfills. USEPA Rep. 600/2-86/073. Hazardous Waste Engineering and Research Laboratory, Office of Research and Development, Cincinnati, OH.

38. Rappaport, S.M., M.G. Richard, M.C. Hollstein, and R.E. Talcott. 1979. Mutagenic activity in organic wastewater concentrations. Environ. Sci. and Technol. 13:957-961.

39. Reinhard, M., and N.L. Goodman. 1984. Occurence and distribution of organic chemicals in two landfill leachate plumes. Environ. Sci. Technol. 18:953-961.

40. Registry of Toxic Effect of Chemical Substances. 1989. Quarterly report. U.S. Dept. of Health, Education and Welfare; Public Health Service; Center for Disease Control; National Institute for Occupational Safety and Health; Washington, D.C.

41. Rinkus, S.J., and M.S. Legator. 1979. Chemical characterization of 465 known or suspected carcinogens

and their correlation with mutagenic activity in the Salmonella typhimurium system. Cancer Res. 39:3289-3318.

42. Sabel, G.V., and T.P. Clark. 1984. Volatile organic compounds as indicators of municipal solid waste leachate contamination. Waste Mgmt. Res. 2:119-130.

43. Sawhney, B.L., and R.P. Kozloski. 1984. Organic pollutants in leachates from landfill sites. J. Environ. Qual. 13:349-352.

44. Shuckrow, A.J., A.P. Pajah, and C.J. Touhill. 1982. Hazardous waste leachate management manual, "Appendix A". Noyes Data Corporation, Park Ridge, NJ.

45. Strobel, K., and T. Grummt. 1987. Aliphatic and aromatic halocarbons as potential mutagens in drinking water. Part II. Chlorinated phenols. Toxicol. Environ. Chemistry. 14:143-156.

46. Stuart, J.D., H.D. Luce, S.R. Smith, and R.F. Black. 1984. Detection and effects of a periodic leachate discharges from landfills. University of Connecticut, Institute of Water Resources, Storrs, CT.

47. U.S. Environmental Protection Agency. 1985. Mutagenicity sample preparation protocols. Environmental Monitoring Systems Laboratory, E.P.A., Las Vegas, NV.

48. U.S. Environmental Protection Agency. 1986. Test methods for evaluating solid waste, Volume IB: Laboratory manual, physical and chemical methods. Office of Solid Waste and Emergency Response, Washington, D.C.

49. U.S. Environmental Protection Agency. 1988a. Operating criteria: "criteria for municipal solid waste landfills". USEPA Rep. 530-SW-88-037. Office of Solid Waste, Washington, D.C.

50. Westat, Inc. 1988. National survey of solid waste (municipal) landfill facilities. USEPA Rep. 530-SW88-034. Office of Solid Waste and Emergency Response, Washington, D.C.

51. Wood, J.A., and M.L. Porter. 1987. Hazardous pollutants in class II landfills. Hazard. Waste Mgmt. 37:609-615.