Bull. Environ. Contam. Toxicol. (1987) 38:337–344

Bulletin of Environmental Contamination and Toxicology

Toxicological Evaluation of the Leachate from a Closed Urban Landfill

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Landfilling is one of the commonly used methods of disposal of solid wastes in small and medium size municipalities. Leachate (water that percolates through solid waste and enroute picks up dissolved and/or particulate material) produced in the landfills can contaminate underground sources of water (Schomaker 1977; Demetracopoulos et al. 1984). Though precautionary measures to reduce the risk of leachate migration into underground sources of water are included in the selection of sites for landfilling and in their design and construction, the risk of contamination is not completely eliminated (Demetracopoulos et al. 1984; Moore 1980). Such a risk depends to a great degree on the nature and concentration of contaminants present in the leachate at the place of origin.

The production, quality and movement of leachate have been studied by many investigators to observe the pollution potential of land fills (Moore 1980; Wigh 1984; Brown et al. 1982; Leekie and Parey 1979; Anderson and Dornbush 1967; Apgar and Langmuir 1971; Fungaroli 1971; USEPA 1976 and 1980). These studies are limited to monitoring the quality and movement of leachate by routine chemical analyses and in vitro mutagenicity and aquatic toxicity tests. More recently, chemistry and aquatic toxicity of raw oil shale leachates have been reported by Meyer et al. (1985). These studies reveal that leachate contains fairly high amounts of heavy metals, e.g., arsenic, barium, cadmium, chromium, copper, iron and lead, as some of its constituents (Brown et al. 1982; USEPA 1976 and 1980; Meyer et al. 1985). These metals can cause neuro-, nephro-, hepato-, or immuno-toxicity (Hammond and Beliles 1980; Gossel and Bricker 1984; Luster et al. 1982). Though chemical and in vitro toxicity tests on leachates have been conducted, not many studies are related to long-term toxicity of leachate in animal models. Therefore, toxicological evaluation of leachates is needed in order to predict possible undesirable effects of consumption of leachate-contaminated water on human populations. In this study, leachate obtained from a closed landfill, which served a city

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population of about 60,000, was evaluated for its toxicity to mice. Animals were given leachate as drinking water for 65 days, and general toxicity to selected end points was observed.

MATERIALS AND METHODS

Leachate was obtained from a landfill located on a 157-acre tract of land near Fargo, North Dakota. Details of the location, geologic description and method of operation of the landfill are given elsewhere (Berreth and Griffin 1983; North Dakota State Water Commission 1982). The site was active between 1950 to 1981. The leachate was collected in August 1983 from one of several 6'' (15.2 cm) diameter drilled holes located on the landfill using polyvinyl chloride pipes. These wells had been used to obtain samples of leachate and groundwater in the vicinity for chemical analyses (Berreth and Griffin 1983). The leachate was stored in a refrigerator until the present study started in December, 1983. Chemical analyses of the leachate sample used in the present study are given in Table 1. The analyses were performed by the North Dakota State Department of Health, Public Health Laboratory, Bismarck, ND (Berreth and Griffin 1983), utilizing the methods recommended by the U.S. Environmental Protection Agency (USEPA) (USEPA 1979, 1980 and 1982). Atomic absorption spectrophotometry was used in the determination of inorganic ions, while gas chromatographic techniques were used for the detection of various pesticides.

Sodium pentobarbital and phencyclidine (PCP) hydrochloride were supplied by Abbott Laboratories, North Chicago, IL and the National Institute on Drug Abuse, Rockville, MD, respectively. Reagent kit for the determination of serum glutamic-pyruvic transaminase (SGPT) was supplied by Sigma Chemical Co., St. Louis, MO. Before use, fresh drug solutions were prepared in saline (0.9% NaCl). Solutions for SGPT assay were prepared in deionized water.

Male ICR mice (21 to 24 g), obtained from Harlan Sprague Dawley Inc., Indianapolis, IN, were housed in plastic cages (29.2 x 19 x 12.7 cm), in groups of 5, in a centralized animal care facility in the College of Pharmacy of North Dakota State University. The facility maintained at 24 to 25^{o} C with the relative humidity of 40-60% was kept in a light cycle for 12 h darkness (7 PM to 7 AM) and 12 h light (7AM to 7 PM) from fluorescent lights. Animals were provided tap water (or leachate) and mouse chow (Ralston Purina Co., St. Louis, MO) ad libitum.

Twenty mice were exposed to the leachate, while 20 control mice were provided tap water in 250 ml plastic bottles (Evenflo Products, Ravenna, OH), daily for 65 days. The leachate was neither filtered nor analyzed for microbes before administering to animals. One mouse in the experimental group died on Day 7. Postmortem examination could not establish the cause of death. The tap water was from the city water supply for drinking, and its chemical analyses obtained from North Dakota State Department

of Health, Public Health Laboratory, Bismarck, ND is given in Table 1. The chemical analyses of the tap water were performed in accordance with USEPA-recommended methods (USEPA 1980).

Body weight and feed and water (or leachate) consumption were recorded between 8 AM to 1 PM on designated days. The average body weight per mouse was calculated by weighing all animals of each cage to obtain the mean $\pm$ SE from 4 cages. The feed and water consumption data were obtained as cage group means (n=4).

On Day 65, 11 randomly selected mice from each group, control and experimental, were used for the measurement of basal locomotor activity by administering saline (10 ml/kg, i.p.). Further, 6 or 7 additional animals from each group were challenged with PCP to evaluate the effect of leachate exposure on the PCP-induced locomotion. Six mice, pretreated with the saline, were utilized for the pentobarbital-induced sleep experiment. Seven animals, not treated with pentobarbital or PCP, were first weighed and then killed using diethyl ether for blood and tissue collection. Blood samples, taken from the heart by opening the chest cavity, were pooled to obtain sufficient amount of serum for the SGPT assay (Reitman and Frankel 1957). Organs, e.g., brain, liver, spleen, and kidneys, were removed, blotted dry and weighed (n=7). Tissues were prepared for histologic evaluation by fixation in 10% neutral buffered formalin and embedded in paraffin. Histologic sections were cut at 6 µm and stained with hemotoxylin and eosin (Preece 1972). Changes in these tissues at ultrastructural level were also evaluated according to the method of Dawes (1971).

The influence of leachate on pentobarbital (60 mg/kg, i.p., in saline)-induced sleep was determined by the duration of the loss of righting reflex in mice after 65 days of treatment (Chaturvedi et al. 1981). The experiment was performed between 1 to 4 PM.

The effect of 65 days-intake of leachate on the PCP (16.4 µmol/kg, i.p., in saline)-induced locomotion in mice was measured using 11 animal activity cages (Chaturvedi 1984). To the interior of each cage, six photocell beams were crossed, interruption of which were additively recorded by counters. The change in the activity as measured by the counts registered by the counters is considered as a criterion for the effect of leachate on the brain function, since the altered pharmacological response reflects the altered neural substrates, e.g., receptors, neurotransmitters etc. (Zenick 1983; MacPhail et al. 1983). Administering pharmacological challenges to organisms exposed to toxic substances is an important strategy in behavioral toxicology (Zenick 1983). The basal and PCP-induced locomotion of the control and experimental groups were measured (Table 2). It has been established that 16.4 µmol/kg, i.p., dose of PCP, a central nervous system stimulant, produces a submaximal stimulation in mice (Chaturvedi 1984; Chen et al. 1959; Hu et al. 1984), with the effect being over in 60 min. The activity (counts/mouse/60 min.) was measured between 1 to 5 PM.

Values of various parameters of toxicity are presented as mean ± SE of 4–11 determinations. The difference between 2 means was tested at 0.05 significance level. Significance of difference between means of all observations including body weight, feed intake, water consumption, organ weight, locomotor activity, etc., was checked by Student's t test (Goldstein 1964).

RESULTS AND DISCUSSION

The daily consumption of leachate for 65 days affected spleen weight and neurobehavior of mice. Relative to the controls, there was no significant ($p > 0.05$) difference in feed intake, water (or leachate) consumption, or body weight gain. The ratio of liver or kidney weight to body weight also did not change (Table 2). No significant difference was evident in the pentobarbital-induced sleep time or SGPT level of both groups. No significant tissue changes at light microscopic or ultrastructural levels were observed in brain, liver, kidneys, or spleen of the leachate mice. However, the weight of the spleen of the experimental mice was 74% less than the controls (Table 2). The basal locomotor activity in both groups was the same, but the PCP-induced locomotion in the leachate group increased by 43% ($p < 0.05$) relative to controls.

Leachates are composed of several inorganic ions and organic matter (Brown et al. 1982; USEPA 1976 and 1980; Meyer et al. 1985). The concentration of these constituents varies with the source of leachate. Effects of the exposure to leachate will depend on the amount and nature of these constituents and their interactions. Relative to the control tap water, the leachate investigated in the present study contains fairly high amounts of metals, e.g., magnesium, arsenic, barium, chromium, lead, iron, cadmium, etc. In the leachate organic pesticides, e.g., endrin, lindane, methoxychlor, toxaphene, 2,4-D etc., were not detected (Berreth and Griffin 1983; Table 1). Approval limits of heavy metals and agricultural chemicals for health prescribed by the USEPA's Interim Primary Drinking Water Standards are also given in Table 1 (USEPA 1976). Levels of these metals are several times higher in the leachate than the safe level for drinking water established by the USEPA (1976) and U.S. Public Health Service (1970). Some of these metals have the potential to produce undesirable effects on the nervous system, kidney, liver, and immune system (Hammond and Beliles 1980; Gossel and Bricker 1984; Luster et al. 1982). In the present study, the exposure to the leachate for 65 days affected neurobehavior, measured as change in PCP-induced locomotion, and spleen weight of mice. The observed enhancement in the locomotion may be due to the interaction among the constituents of the leachate, particularly metals, resulting in altered neural substrates (Zenick 1983; MacPhail et al. 1983). The possibility of such treatment on the metabolism of PCP, thereby on its influence, exists. However, it is less likely as leachate treatment did not alter the pathology of liver and the pentobarbital-induced sleep. Hypoplastic spleens have been reported to occur following exposure to

Table 1. Chemical quality of the leachate and control water used in the study

Parameter	Concentration[a]	
	Leachate	Tap water
Conductivity	10130/8444	213
Total dissolved solids	7030	114
pH	7.8	8.8
Total alkalinity (as $CaCO_3$)	5930	59
Total hardness (as $CaCO_3$)	1890	80
Flouride	0.1	1.0
Chloride	1050	14
Nitrate (as N)	0.029	0.0
Total Kjeldahl nitrogen	156	–[b]
Sulphate	39	24
Total and ortho phosphate	1.18, 1.0	–[b]
HCO_3	7240	54.0
CO_3	0.0	9.0
Calcium	69.5	17.0
Magnesium	418.0	9.0
Manganese	0.132	0.004
Potassium	301.0	3.95
Sodium	1580	10.0
Sodium adsorption ratio	15.8	0.49
Iron	15.5	0.04
Arsenic	16.3	0.0 (0.05)[c]
Barium	820.0	0.0 (1.00)
Cadmium	2.38	0.001 (0.01)
Chromium	23.4	0.0 (0.05)
Copper	16.0	0.011
Lead	45.8	0.0 (0.05)
Selenium	0.03	0.0 (0.01)
Zinc	326	0.004
Chemical oxygen demand	1030	–[b]
Endrin	None detected	–[b] (0.0002)
Lindane	None detected	–[b] (0.004)
Methoxychlor	None detected	–[b] (0.100)
Toxaphene	None detected	–[b] (0.005)
2,4 - Dichlorophenoxyacetic acid (2,4-D)	None detected	–[b] (0.100)
2 - (2,4,5 - Trichlorophenoxy)-propionic acid (2,4,5-TP)	None detected	–[b] (0.010)

[a] Concentrations in mg/L except for conductivity (micromhos/cm)
[b] Not determined
[c] Values in parentheses are available EPA Approved Limits (USEPA, 1976)

Table 2. Effects of daily leachate consumption for 65 days on various parameters of toxicity in mice

Parameter	Control (tap water) Mean ± SE	Experimental (leachate) Mean ± SE
Body weight (g;n = 7)	35.3 ± 1.5	33.5 ± 0.9
Liver weight (g)	1.79 ± 0.14	1.67 ± 0.07
Liver/body weight ratio (x 100)	5.1	5.0
Kidney weight (g)	0.554 ± 0.02	0.524 ± 0.01
Kidney/body weight ratio (x 100)	1.57	1.56
Spleen weight (g)	0.181 ± 0.029	0.104* ± 0.001
Spleen/body weight ratio (x 100)	0.51	0.31
SGPT[a] (units/ml)	67.3 ± 0.6	53.8 ± 1.7
Pentobarbital-induced sleep (min) (n=6)	83.9 ± 10.9	82.5 ± 5.7
Locomotor activity (counts/mouse/h)		
Basal-saline (n = 11)	2789 ± 193	2975 ± 126
PCP-induced	4767 ± 667 (n = 7)	6799* ± 526 (n = 6)

[a] 1 unit = 4.82×10^{-4} µmol glutamate/min at pH 7.5 and 25°C.

* Blood samples from 7 animals were pooled to obtain sufficient amount of serum sample. The assay was run in triplicate. $p < 0.05$

environmental chemicals (Luster et al. 1982). Metals like lead, cadmium and arsenic produce immunosuppressive effects (Luster et al. 1982). Therefore, the decrease in the spleen weight in the absence of detectable pathology might be associated with the metal-caused immunotoxicity. Thymic organ weight determination might have been further helpful in relating the spleen weight decrease to probable immunotoxicity.

This study is significant because of the real possibility of pollution of groundwater bodies by leachates. However, the contaminants of leachate may not reach the ground water bodies in the same concentration as at the place of origin due to filtration, dispersion and attenuation within the soil matrix. The attenuation or magnification of the potential of leachate to produce toxicity as it moves through the soil medium and contaminates ground water is not well understood at present. Nevertheless, such studies on toxicity of leachates from landfills using animal models will be necessary to predict the

toxicological consequences of contamination of groundwater by leachates. Although leachability of many individual organic chemicals are fairly well understood and predictable, the combined presence of these chemicals makes it difficult to predict toxicity of leachates at locations along the path of migration. Definite causal relationship between toxic effects and the constituents of leachate can be established only through further investigation using synthetic mixtures. Further studies using animal models are also needed on leachates at the source as well as other locations along the path of migration to improve the understanding of the variation of toxicity as leachates migrate toward water bodies.

Acknowledgements. The authors thank the administration of the Departments of Pharmaceutical Sciences and Toxicology of the College of Pharmacy for providing the animal research facilities.

REFERENCES

Anderson JR, Dornbush JN (1967) Influence of sanitary landfill on groundwater quality. Amer Water Assoc J 59:457-470

Apgar MA, Langmuir D (1971) Groundwater pollution potential of a landfill above a water table. Groundwater 9:76-94

Berreth GW, Griffin DM Jr (1983) Geochemical investigation of the old Fargo landfill. Contract No: DACW 37-82-M-1817. Final Report to U.S. Army Corps of Engineers

Brown DK, Francis CW, Maskarinee MP, Larimer FW (1982) Toxicity of leachates: Comparison of extraction procedure extracts and landfill leachates. Project-Summary. EPA-600/52-82-049 Municipal Environmental Research Laboratory. USEPA. Cincinnati, OH.

Chaturvedi AK, Rao NGS, Berg IE (1981) The role of hepatic microsomal enzymes in the modulation of phencyclidine-induced toxicity. Toxicology 22:245-254

Chaturvedi AK (1984) Effects of mecamylamine, nicotine, atropine, and physostigmine on the phencyclidine-induced behavioral toxicity. Pharmac Biochem Behav 20:559-566

Chen G, Ensor CR, Russell D, Bohner B (1959) The pharmacology of 1-(1-phenycyclohexyl)-piperidine HCl. J Pharmacol Exp Ther 127:241-250

Dawes CJ (1971) Chemical Fixatives. In: Biological Techniques in Electron Microscopy. Barnes and Noble, New York, pp. 17-47

Demetracopoulos AC, Korfiatis GP, Bonrodimos EL, Nawy EG, (1984) Modeling for design of landfill bottom liners. ASCE J Environ Eng 110:1084-1097

Fungaroli AA (1971) Pollution of subsurface water by sanitary landfills. Report SW/12rg Vol I USEPA Washington D.C.

Goldstein A (1964) Biostatistics. Macmillan, New York.

Gossel TA, Bricker JD (1984) Principles of clinical toxicology. Raven Press, New York, pp. 153-187

Hammond PB, Beliles RP (1980) Metals. In: Doull J, Klaassen CD, Amdur MO (eds.) Toxicology: The Basic Science of Poisons, 2nd ed Macmillan Publishing Co, Inc, New York, pp. 409-467

Hu CY, Choudhuri MSK, Berg IE, Rao NGS, Chaturvedi AK (1984) Toxicity of 1-phenylcyclohexene and its interaction with phencyclidine. Toxicol Appl Pharmacol 76:403-413
Leekie JO, Parey JG (1979) Landfill management with moisture control. ASCE J Enviro Eng Div 105:337-355
Luster MI, Dean JH, More JA (1982) Evaluation of Immune Functions in Toxicology. In: Hayes AW (ed) Principles and Methods of Toxicology. Raven Press, New York, pp. 561-586
MacPhail RC, Crofton KM, Reiter LW (1983) Use of environmental challenges in behavioral toxicology. Federation Proc 42: 3196-3200
Meyer JS, Sanchez DA, Brookman JA, McWhorter DB, Bergman HL (1985) Chemistry and aquatic toxicity of raw oil shale leachates from Piceance Basin, Colorado. Envior Toxicol Chem 4:559-572
Moore CA (1980) Landfill and surface impoundments evaluation. EPA/530/SW-869C. Municipal Environmental Research Laboratory. USEPA. Cincinnati, OH
North Dakota State Water Commission (1982) Pumping test records.
Preece A (1972) Paraffin Tissue Processing Method. In: Preece A (ed.) A Manual for Histologic Technicians, 3rd ed. Little Brown and Co, Boston, MA pp. 57-73
Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Amer J Clin Pathol 28:56-63
Schomaker NB (1977) Management of gas and leachate in landfills. Proc of the Third Annual Municipal Solid Waste Research Symposium at St. Louis MO. EPA-600/9-77-026:1-12 Municipal Environment Research Laboratory. USEPA Cincinnati, OH
USEPA (1976) Office of Water Supply, National interim primary drinking water regulations, EPA-570/9-76-003
USEPA (1979) Method for chemical analysis of water and wastes, 600/4-79-020
USEPA (1980) Toxicology of Leachates, Office of Water and Waste Management, Washington, D.C.
USEPA (1980) Standard methods for the examination of water and waste water, 15th Edition
USEPA (1982) Methods for organic chemical analysis of municipal and industrial waste water, EPA-600/4-82-057
USEPA (1982) Test methods for evaluating solid waste, physical/ chemical methods, EPA-SW-846, 2nd Edition
U.S. Public Health Service (1970) Community water supply study: Analysis of national survey findings. U.S. Department of Health Education and Welfare, Washington, D.C.
Wigh RJ (1984) Landfill research at the Boone county field site. Project-Summary. EPA-600/2-84-050. Municipal Environmental Research Laboratory. USEPA. Cincinnati, OH
Zenick H (1983) Use of pharmacological challenges to disclose neurobehavioral deficits. Federation Proc 42:3191-3195

Received June 2, 1986; accepted August 23, 1986