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Mutagenicity Assays of Leachate from Domestic Waste Landfills in Japan: The Establishment of a Protocol for Measuring Mutagenicity Levels of Leachate

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In modern society, production of industrial and domestic waste is increasing so much that waste disposal has become a serious problem in many countries in terms of both difficulty in obtaining a location for waste disposal and concern for the hazardous effects upon the human-environmental system. There have been many reports concerning the effects of waste disposal on public health and environmental pollution (Griffith et al. 1989). Waste is composed of a great variety of substances, including both combustible and incombustible matter. In Japan, combustible waste is burned in an incinerator and the cinders are buried in landfills together with incombustible waste, although in former days both combustible and incombustible waste were buried together in their natural state. These buried substances are degraded by soil bacteria and the physico-chemical milieu existing underground. This requires a great deal of time. However, leachate from the waste landfill is unceasingly dispersed into the environment throughout the degradation period. It is known that leachate contains various kinds of chemicals both organic and inorganic, resulting from the degradation process. Leachate should therefore be examined from the point of view of both environmental pollution and hazardous effects on human health. Though some reports concerning the acute toxicity of leachate to some kinds of fish, bacteria, and algae have been published (Cameron and Koch 1980; Atwater et al. 1983; Plotkin and Ram 1984), the mutagenicity of the leachate has not yet been well examined.

In an attempt to evaluate the mutagenicity of leachate, we endeavored to establish some methods, in particular a protocol for the preconcentration of mutagens in the leachate and for the examination of the mutagenicity of leachate obtained from waste landfills in Japan using the Salmonella typhi-

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Table 1. Description of the eight areas of municipal landfill.

Area	Period	Waste component	Method of Landfilling
A	1965~1968	Combustible & Incombustible	Anaerobic
B	1973~1976	Combustible & Incombustible	Sanitary
C	1975~1977	Combustible & Incombustible	Semiaerobic
D	1977~1978	Incombustible & Incinerator ash	Semiaerobic
E	1978~1980	Incombustible & Incinerator ash	Semiaerobic
F	1980~1981	Incombustible & Incinerator ash	Semiaerobic
G	1982~1988	Incombustible & Incinerator ash	Semiaerobic
H	1988~	Incombustible & Incinerator ash	Semiaerobic

murium/mammalian-microsome system (Ames test).

MATERIALS AND METHODS

XAD-2 and XAD-8 resins were obtained from Japan Organo Co. Ltd., Tokyo, Japan. They were purified by sequential solvent extractions with methanol, acetonitrile, and diethyl ether according to Junk et al. (1974). Blue rayon was purchased from Daiwa Engineering, Tokyo, Japan. Other chemicals were of analytical grade or higher.

Leachate samples were collected from a municipal domestic waste landfill in Fukuoka Prefecture. This landfill, which has been used as a waste disposal site since 1965 was composed of eight areas. Details of each area are described in Table 1. The leachate samples were collected at the spot where the leachate from the eight areas combined. A sample collection was undertaken in autumn, 1988. Tentatively, chloroform was added to the sample at a rate of 0.1 mL per 1 L for the purposes of inhibiting bacterial proliferation. The sample was then stored at room temperature until the assay was performed. This was within 2 wk from the time of sampling.

The leachate sample was filtered with plugged glass wool to remove suspended material and the filtrate was applied using either the XAD-2/XAD-8 resin column technique or the blue rayon batch technique (Hayatsu

et al. 1983).

For the XAD resin technique, XAD-2/XAD-8 glass column (20 x 500 mm) was prepared by packing 19 mL of XAD-2 resin followed by the same amount of XAD-8 resin. Prior to loading the leachate sample, the column was washed with 1 L of distilled water. One liter of leachate filtrate whose pH was adjusted to 2 was loaded on the XAD-2/XAD-8 column, then the column was washed with 100 mL of distilled water. Nitrogen gas was introduced into the top of the column to complete the removal of the water retained in the column. Adsorbed material on the resin was eluted with 200 mL of acetone. In all the processes, the flow rate was regulated to 10 mL per min. The eluted fraction was evaporated to complete dryness under reduced pressure in a rotary evaporator at 37° C and the residue was dissolved in 0.5 mL dimethyl sulfoxide (DMSO). No mutagenicity was detected when distilled water was extracted in a similar manner. In the initial experiments to set the above conditions, the pH of the leachate sample before extraction, the type of XAD resins, the volume of XAD resins and the volume of the eluting solvent were varied (see results).

In the blue rayon technique, 2 g of blue rayon was added to 1 L of the filtrate in a 2 L beaker. The mixture was slowly stirred with a magnetic stirrer for 30 min at room temperature. This adsorption procedure was repeated two more times with fresh batches of 2 g and 1 g of blue rayon. The three portions of blue rayon were combined and washed 3 times with 500 mL of water, and the water was wrung out of the blue rayon following each treatment. The adsorbed components on blue rayon were eluted by stirring the blue rayon slowly in 200 mL of ammoniacal-methanol (methanol:concentrated ammonium hydroxide [ca. 28-30%] = 50:1, v/v) for 30 min. This elution procedure was repeated three times. These three eluates were combined and taken to dryness under reduced pressure in a rotary evaporator. The residue was dissolved in 0.5 mL DMSO.

Mutagenicity tests were carried out by the standard plate incorporation test as described by Maron and Ames (1983), with the Salmonella typhimurium strain TA98 (supplied by Dr. B. N. Ames, University of California, Berkeley, U.S.A.). A 0.0125 to 0.2-mL sample in DMSO (equivalent to 25 to 400-mL leachate) was plated onto Vogel-Bonner's basal agar plate with 2 mL of soft agar, 0.1 mL of an overnight culture of TA98, and 0.5 mL of S9 mixture. The S9 mixture contained 50 uL of hepatic S9 (prepared from male Wistar-King rats (250 - 300 g) pretreated with polychlorinated biphenyls, KC-500 (Kanegafuchi Kagaku, Osaka, Japan)).

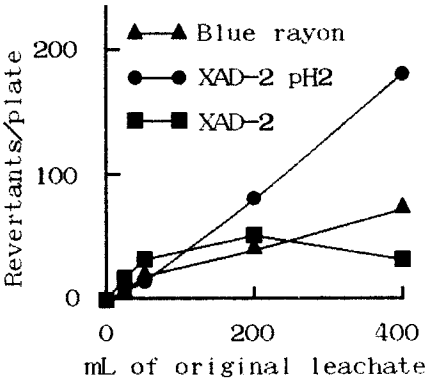

Figure 1. Comparison of the XAD resin column technique and the blue rayon batch technique for efficiency in extracting mutagens from the leachate sample. All were tested with 100 μL of S9 fraction per plate with tester strain TA 98.

Each sample was plated in duplicate and His+ revertants were scored after 48 hr incubation of the plate at 37° C. All data for mutagenicity were presented as averages of revertant numbers in duplicate after subtracting the spontaneous revertant numbers (39 revertants/plate). As a positive control for this assay, we used benzo(a)pyrene (B(a)P). The revertants level of 5 ug B(a)P was 799.

RESULTS AND DISCUSSION

In a preliminary experiment, the mutagenicity of the leachate concentrate which was prepared by XAD-2 treatment was examined, using tester strains TA98 and TA100 with or without S9 mixture. A strain TA98 in the presence of S9 mixture gave only positive results, more than twice the number of revertants as in the concurrent negative control (data not shown). This indicates that mutagens in the leachate are pro-mutagens of the frame-shift type(Maron and Ames 1983). Thus, we decided to use TA98 as a tester strain and S9 mixture for the metabolic activation in later experiments.

In mutagenicity assays of the leachate, it is an important step to prepare a leachate concentrate for use in the Ames test. The XAD resin column technique and the blue rayon batch technique, which have been widely used for extracting mutagens from aqueous samples, were compared for their efficiency in extracting mutagens from leachate. Weak mutagenicity was detected in the samples prepared by treatment with both XAD-2 and blue rayon. However, when the leachate was acidified before XAD-2 treatment, much stronger

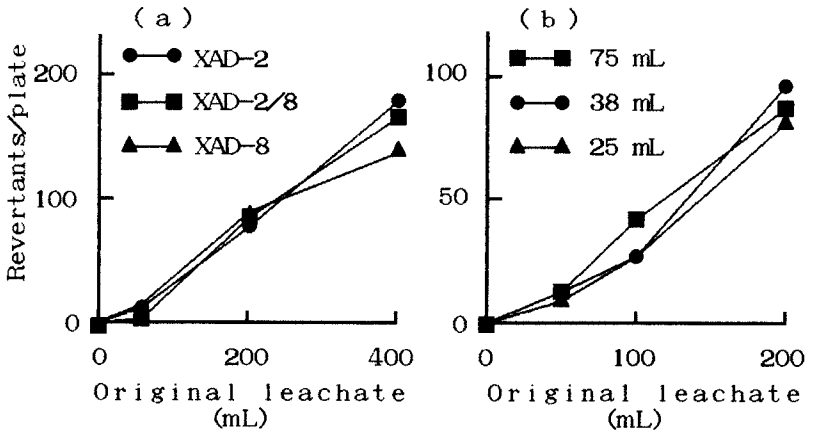

Figure 2. The effect of different types of XAD resins (a), and different volumes of XAD-2/XAD-8 resins in series packed in a glass column (b) on the extraction of mutagens from 1 L of leachate. All were tested with 100 μL of S9 fraction per plate with tester strain TA 98.

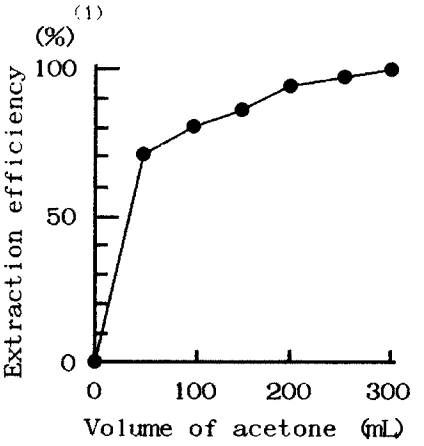

Figure 3. The effect of acetone volume on the elution of mutagens from XAD-2/XAD-8 resins column loaded with 1 L of acidified leachate.

*(1) The mutagenicity of the leachate concentrate prepared from the elution with 300 mL of acetone was taken to be 100%

mutagenicity was detected and a linear dose-response curve was obtained within a range of 0 to 400 mL equivalent of the leachate (Fig.1).

We next compared XAD-2, XAD-8 and XAD-2/XAD-8 in series for the efficiency of extracting mutagens from acidified leachate samples. Each column was prepared by packing resins with a total volume of 75 mL in a glass column (20 x 500 mm). As shown in Figure 2a, there was no difference in the extraction efficiency between the types of XAD resins. Considering that landfill leachate generally contains a variety of substances, we chose the column packed with XAD-2 and XAD-8 resins in series. This was because a combination of XAD-2 and XAD-8 resins can be expected to adsorb not only non-polar mutagens but also weakly polar mutagens.

It is also of practical interest to determine the appropriate amount of XAD resins needed to extract potential mutagens in 1 L of leachate. In the range of 25 to 75 mL of resins tested, no differences were observed in extraction efficiency (Fig.2b). Although as little as 25 mL of resins were regarded as sufficient for extracting mutagens from 1 L of leachate, we decided to use 38 mL of resins in order to have a great capacity of adsorption in reserve. The optimum amount of acetone needed for eluting mutagens from a 38 mL-XAD resins column composed of XAD-2 and XAD-8 in series was examined. As shown in Figure 3, 200 mL of acetone proved to be enough to elute mutagens from the column, since the elution of mutagens was attained almost to a maximum with 200 ml of acetone.

Finally, using the leachate concentrate equivalent to 200 mL of original leachate, an optimum amount of S9 was examined in the range of 10 to 200 uL per plate. The highest mutagenicity was obtained by the addition of 50 uL of S9 (data not shown).

We thus established a protocol for the accurate detection of the mutagenicity of leachate (See MATERIALS AND METHODS).

This study showed that the mutagenicity of leachate obtained from a municipal domestic waste landfill can be effectively detected with XAD resin technique as a preconcentration method. XAD resins have previously been used effectively for extracting mutagens from various water samples: drinking water (Meier et al. 1987), raw water (Maruoka and Yamanaka 1983), and waste water (Rappaport et al. 1979). In this study, we proved that XAD resins are helpful for preparing the leachate concentrate for mutagenicity tests using Salmonella typhimurium/mammalian-microsomes. Moreover, this study showed that the acidification of the leachate before XAD treatment is effective to disclose high mutagenicity from the leachate sample. Mutagens

in drinking water are also effectively adsorbed on XAD resins at pH 2 (Meier et al. 1987). Mutagens in leachate appear to be different from those in drinking water since mutagens in leachate are sensitive to TA98 in the presence of S9. On the other hand, mutagens in drinking water are sensitive to TA100 without S9. When we assayed the mutagenicity of the leachate sample used in this study according to our established protocol, the mutagenicity level was regarded as 426 net revertants per liter. This level is not high. However, the characteristics of mutagens in leachate should be pursued with the intention of preventing hazardous environmental pollution.

As indicated by Fuller et al. (1979), leachate widely varies in chemical composition according to the location. This is due to the quantity of the waste, the geographical conditions, and the environmental conditions of the landfills. Even in the same landfill, the quality of the leachate changes gradually because some waste components decompose quickly, while others decompose slowly (Chen and Bowerman 1974). In the study on the mutagenicity of leachate, we should take these matters into account. At present, we are studying the mutagenicities of leachate and treated water from other municipal domestic landfills, and we are examining the differences in mutagenicity according to the sampling time.

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