Acute toxicity tests on raw leachate from a Malaysian dumping site
Fatihah Sujá, Arij Yusof and Md. Anuar Osman

ABSTRACT
Leachate samples collected from the Ampar Tenang open dumping site at Dengkil, Malaysia, were analyzed for acute toxicity. Two in vivo toxicity tests, Acute Oral Toxicity (AOT) and Primary Skin Irritation (PSI), were performed using Sprague Dawley rats and New Zealand Albino rabbits, respectively. The leachate samples were also analyzed chemically for nitrate and phosphate, ammonia-nitrogen, Kjeldahl-nitrogen and Chemical Oxygen Demand (COD). Results from both the AOT and PSI tests showed that the leachate did not contribute to acute toxicity. The AOT test yielded a negative result: no effect was observed in at least half of the rat population. The PSI test on rabbits produced effects only at a leachate concentration of 100%. However, the skin irritation was minor, and the test returned a negative result. The four chemical tests showed high levels of nutrient pollution in the leachate. The nitrate and phosphate concentrations were 2.1 mg/L and 23.6 mg/L, respectively. Further, the ammonia-nitrogen concentration was 1,000 mg NH₃-N/L the Kjeldahl-nitrogen level was 446 mg NH₃-N/L, and the Chemical Oxygen Demand was 1,300 mg/L. The in vivo toxicity and chemical analyses showed that the leachate is polluted but not acutely toxic to organisms.

Key words | acute oral toxicity, in vivo toxicity, landfill leachate, primary skin irritation test

INTRODUCTION
In Malaysia, open dumping sites are still common; only a few managed sanitary landfills are being built around the country. It is well-known that sanitary landfills manage solid waste more efficiently than open dumping sites due to technology such as liner systems, leachate-collection systems, biomass gas control systems and environmental management systems (Crawford & Smith 1985).

In an open dumping site, solid wastes are clearly exposed to the surroundings, and there is no technology in place to manage the waste and its by-products, including leachate and biomass gas. However, most open dumping sites have a leachate lagoon, where the leachate around the dumping site is collected. Due to the lack of a liner system and the existence of the leachate lagoon, the probability of leachate from open dumping sites penetrating deep into the groundwater is high, potentially contaminating the water supply.

Hence, leachate is one of the major issues handled by solid waste management facilities due to the numerous problems it poses to both the management and the community.

The composition of a raw leachate is highly variable and is dependant on several factors. One significant factor is the type of solid wastes dumped into the landfill (Christensen et al. 1992). At an open dumping site, solid wastes originate mainly from households. The Malaysian government restricts the dumping of industrial wastes to these sites to avoid the accumulation of hazardous toxic substances. However, due to the presence of chemical substances in many common household products, chemical reactions could occur in the leachate. These reactions could produce toxic materials that could then be channeled to the environment via the leachate.
Previous literature has shown that the toxicity of leachate can be determined by various tests, either *in vivo* or *in vitro* (Houk 1992). An *in vivo* test is where subjects (animals and plants) are exposed to a potentially toxic environment, while an *in vitro* test is where specific cell lines are exposed to samples (such as leachate, hospital wastewater, or effluent) at a given concentration. For example, *Salmonella* cells are used in the Ames Test (Houk 1992).

Toxic effects are divided into two categories, acute and chronic. The acute toxicity of a substance is its likelihood to cause either local damage (e.g. to the skin or eyes) or systemic damage (affecting the body as a whole) as a result of brief exposure to relatively large amounts of the substance. This type of exposure is sudden and often produces an emergency situation. Chronic toxicity is the likelihood of a substance to cause damage as a result of repeated exposure to relatively small amounts over a prolonged time period, such as the repeated consumption of contaminated food or water. Chronic toxicity usually does not produce effects until exposure has continued for some time (National Research Council 1991).

In investigating the toxicity of any sample, especially environmental samples, acute toxicity is the first thing to be studied. These results might give first-hand information as to whether the sample has the potential of being toxic upon sudden exposure or consumption. After that, proceeding with tests for chronic toxicity is often appropriate.

Accordingly, in the present study, leachate samples were tested for acute toxicity by *in vivo* toxicity tests. The leachate was also analyzed for chemical components such as nitrogen and phosphorus. This analysis is important because the acute toxicity of landfill leachate has been shown to have a high degree of correlation with the presence of ammonia (Dave & Nilsson 2005). However, tests for the detection of heavy metals were not conducted, since the solid wastes originated mainly from households instead of industrial plants. Thus, the concentrations of heavy metals were likely negligible. The Chemical Oxygen Demand value was measured to determine the level of environmental pollution caused by the leachate.

**METHODOLOGY**

**Leachate sampling**

Leachate was collected from the Ampar Tenang open dumping site in Dengkil, in the state of Selangor, with permission from the Sepang City Council. All leachate near the site was collected in a lagoon 3 to 6 m deep, and samples were taken from the lagoon surface. The samples were collected in a 5-liter polyethylene bottle and two 100-ml glass bottles.

Figures 1 and 2 below show the lagoon where the leachate was sampled and the sampling procedure. After retrieval, the sample bottles were kept in an ice-filled polystyrene box for transport back to Universiti Kebangsaan Malaysia (UKM). A Chain of Custody form was given to the representative from Sepang City Council to be signed. Masks and gloves were worn as precautions throughout the collection process. The leachate samples were taken to two locations. The 5-litre polyethylene bottle was sent to Environmental Laboratory of the Faculty of Engineering at UKM, while the other two glass bottles were taken to the Bioserasi Laboratory at UKM. All samples were kept below 4°C, and no chemical preservatives were added.

**Laboratory analysis**

Laboratory analyses consisted of both *in vivo* toxicity and chemical tests.
In vivo toxicity test

Two in vivo toxicity tests were performed: the Acute Oral Toxicity (AOT) test and the Primary Skin Irritation (PSI) test. Both are categorized as in vivo since they involve the use of animals (Sprague Dawley rats and New Zealand White Rabbits). These tests were successfully conducted with the help of the Bioserasi Laboratory, UKM.

(i) Acute oral toxicity (AOT) test. The standard procedure used for this test was taken from the Organization for Economic Co-Operation and Development (OECD) Guidelines, number 401.

Thirty-five Sprague Dawley rats were divided into four groups as shown in Table 1 and were acclimatized for five days prior to the test. After the acclimatization period, food was withheld from the rats for 24 hours, and the AOT test was carried out on the sixth day. The rats were fed only once with leachate samples in one of three concentrations (100%, 50% or 25%), as shown in Table 1. Normal saline was used as the negative control. After exposure to the samples, observations took place at 1, 3, and 24 hours, and thereafter every day for 14 days. The rats were fed with food and water after the first three hours of observation. Clinical forms were used to record their physical characteristics, during the 14-day period. The rats were weighed on the first, seventh and fourteenth days to identify any changes. On the fifteenth day, a necropsy was performed on the Sprague Dawley rats to detect any physiological changes in their organs.

(ii) Primary skin irritation (PSI) test. The PSI test was carried out using the standard protocol A-3-PSI-ISO 10993. Six New Zealand White Rabbits were used as test subjects. The day before the test, the rabbits were shaven on their dorsal sides to expose the skin where the leachate sample would be placed. The skin was examined to ensure there was no bruising before the test was conducted. The following day, the rabbits were weighed, and their skin was marked to indicate the position of the dose sites. Whatman filter papers (1 inch × 1 inch) were dipped into the leachate and patched onto the marked dose sites.

The leachate doses were given in three concentrations: 100%, 50% and 25%. Figure 3 shows the positions of the patches on the rabbit’s skin. The skin on the right side of the patch area was abraded, and the left side was not. After patching the samples, the application sites were wrapped with gauze cloth, surgical tape and rubberized material to prevent movement of the samples. The patches were removed after 24 hours. After removal, observations were performed over the course of an hour, and repeated at 24, 48 and 72 hours. The goal of the observations was to

Table 1 | Sprague Dawley rats sample population

<table>
<thead>
<tr>
<th>Sample</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>100% leachate</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>50% leachate</td>
<td>5</td>
<td>5</td>
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<tr>
<td>25% leachate</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Normal saline</td>
<td>2</td>
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determine if there was any erythema (redness) or edema (swelling) on the rabbits’ skin. The patch areas were given a score between 1 and 4. A score of 1 was given for light erythema or edema while a score of 4 indicated severe cases. A non-detectable effect was given a score of 0. These scores were used to determine the Primary Irritation Index.

(b) Chemical testing

Four chemical assays were chosen to characterize the leachate. Ion chromatography was used to measure nitrate and phosphate; the titrimetric method was used to determine ammonia-nitrogen levels; Kjeldahl-nitrogen was quantified with the Macro-Kjeldahl method; and Chemical Oxygen Demand was determined using the open reflux method. These tests were carried out using the standard procedures of the American Public Health Association (Cleresci et al. 1998).

RESULTS

In vivo toxicity tests

(a) Acute oral toxicity (AOT) test

The clinical reviews indicated that most of the Sprague Dawley rats showed physical changes in terms of motor activity; specifically, slow reactions to stimulation. For example, the rats would initially run when grabbed suddenly, but after exposure to the leachate they moved more slowly and exhibited malaise. The average weight of the rats increased steadily throughout the 14 days of observation, with no sign of weight loss (see Figures 4 and 5).

In addition, the fur on some of the rats also showed changes. Fur differences were noted on one male (Male No.5_Leachate 50%) and two females (Female No.1 and 2_Leachate 50%). The same male rat also showed respiratory changes; his breathing became rapid and shallow starting on the eleventh day and continued until the last day of observation.

After the necropsies were performed, eight organs (the brain, spleen, liver, heart, pancreas, lungs, kidneys and stomach) were examined for differences in size, color and appearance between the leachate-fed rats and the negative controls. Among the treated rats, only one (Male No.5_Leachate 50%) showed a change: one lung was enlarged and lobulated compared to the negative control rats (Figure 6).

After examination, the organs were weighed, and the average weights were calculated. The lungs showed the most significant results out of all eight organs. As shown in Figure 7, the average weight of the lung per body weight (g/kg) for the male rats fed with 50% leachate was 12.97 g/kg, which is almost 80% higher than the negative control male population. This could be explained by the lobulated lung shown in Figure 6, which came from one of the male rats in the 50% leachate sample group. This

![Figure 4](https://iwaponline.com/wst/article-pdf/61/2/389/447835/389.pdf)

**Figure 4** | Male Sprague Dawley average weight.
lobulated lung, which was bigger and thus heavier, could have skewed the average lung weight of this group.

The AOT test is categorized as LD_{50}, which means that the test only yields a positive result if the sample severely affects 50\% of the test subjects. Just a few of Sprague Dawley rats were biologically affected, which is clearly less than 50\%. Even though most of the rats showed slow reactions to stimulation, the outcome was not severe.

**Primary skin irritation test**

The average scores for primary skin irritation were determined using the rating system shown in Table 2. A score of 1 indicated light erythema or edema while a score of 4 indicated severe cases. Undetectable skin irritation was given a score of 0. The average skin irritation (min) scores were used to determine the Primary Skin Irritation Index, as shown in Table 3. This index value was used as the overall outcome of the PSI test. The index values for the three leachate concentrations were compared with values from the Classification of Skin Irritation, as shown in Table 4. In this study, doses of 100\% leachate caused only slight irritation to the skin of New Zealand White rabbits. The other two leachate concentrations had no effect on the test subjects.

**Chemical testing**

The results of the four chemical assays are shown in Table 5. The concentrations of nitrate and phosphate ions were 2.1 mg/L and 23.6 mg/L, respectively. The ammonia–nitrogen test returned a value of 1,000 mg NH_3-N/L, and the Kjeldahl-nitrogen concentration was 446 mg NH_3-N/L. Lastly, the Chemical Oxygen Demand was 1,300 mg O_2/L.

**DISCUSSION**

In Malaysia, open dumping sites have been the final destination of household and domestic wastes for years. The locations selected for these dumping sites are far away from residential areas. The Ampar Tenang open dumping site is situated at an old and abandoned palm tree field. However, due to the rapid development in Selangor, Malaysia, land use demand is high. Currently, there is a housing project being built not more than 2 kilometers from the Ampar Tenang open dumping site.

![Figure 6](image_url)
Due to this situation, which is occurring elsewhere in Selangor as well, the authorities have decided to convert all the open dumping sites to sanitary landfills starting in 2008. However, this conversion process might take years, and the housing project might finish and become inhabited long before then. There are many potential problems with people living near the dumping sites due to their associated pollution. In particular, the water supply can become contaminated by leachate penetrating into the groundwater table or being carried by surface water into the river during rain events.

Furthermore, although the wastes deposited in open dumping sites originate mainly from households, most modern daily-use products contain chemical substances. Therefore, chemical reactions in the leachate could produce toxic materials, which would then be released into the environment. Hence, to determine whether the leachate is toxic to living organisms, performing acute toxicity tests is necessary. These tests provide initial and immediate information regarding the toxicity of the leachate.

The Acute Oral Toxicity (AOT) test and the Primary Skin Irritation (PSI) test are the main in vivo assays used by researchers to determine acute toxicity. The AOT test is also known as LD$_{50}$, where LD stands for lethal dose, and 50 refers to the dose of the sample used. The AOT test is considered positive if half or more of the test subjects were severely affected by the substance. The PSI test is performed to determine the effect of the samples on the skin of the test subjects.

In the present study, the AOT test showed that the leachate was not acutely toxic towards Sprague Dawley rats, as only a few of the rats were affected. Even though most of the rats showed slow responses to stimulation after ingesting the leachate, the effects was not considered severe. Two female rats fed with the 50% leachate solution showed fur changes. Their white, soft fur became a bit yellowish and coarse. One male rat from the same treatment group had similar fur changes. The same male rat showed rapid and shallow breathing from the eleventh day until the last day of observation, and the necropsy revealed a lobulated lung.

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<th>Scoring system for skin reactions</th>
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<td>Reaction</td>
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<td>Erythema (E)</td>
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Source: Standard protocol A-3-PSI-ISO 10993.
Only one rat showed severe effects from exposure to the leachate. A possible reason for this could be that the rat was already in poor health at the time of exposure. Its pre-existing condition might have worsened upon consuming the contaminated samples. These situations are often unavoidable when performing the \textit{in vivo} toxicity tests using animals.

In the PSI test conducted on the New Zealand White rabbits, only 100\% leachate concentrations produced effects, yielding a Primary Skin Irritation Index value of 0.5. The PSI Index values for the 50\% and 25\% leachate concentrations were zero. According to the Classification of Skin Irritation shown in Table 4, 100\% leachate caused slight irritation to the skin of the rabbits, while the other two concentrations had no effect.

Based on the ammonia–nitrogen assay, the ammonia concentration (1,000 mg NH$_3$-N/L) indicates high levels of toxicity. However, even though this concentration appears high, it is still below the level producing toxic effects (1,100 mg/L) given by the Agency for Toxic Substances and Disease Registry of the U.S. Public Health Service.

Furthermore, Olivero-Verbo et al. (2008) stated that sample toxicity decreases with increasing ion concentrations and increases with organic component concentrations.

The COD test provides a rough estimation of the concentration of organic compounds, because COD measures the quantity of oxygen needed to convert the organic matter into carbon dioxide and water. Higher COD values correspond to a higher quantity of organic compounds in the sample.

The high Chemical Oxygen Demand (COD) level (1,300 mg O$_2$/L) indicates that the leachate samples are toxic. Yet, given the negative results of the \textit{in vivo} toxicity tests, we could only determine while that the leachate showed high levels of pollution, it did not cause acute toxicity in the test subjects.

**CONCLUSIONS**

The results of the \textit{in vivo} toxicity and chemical tests performed on raw leachate samples from the Ampar Tenang open dumping site in Dengkil, Selangor showed no acute toxicity to living organisms. Although chemical measurements of COD and ammonia are high, results of the \textit{in vivo} toxicity tests were considered negative. However, the toxicity of the leachate should be explored further. Since this study showed that the leachate did not produce acute toxicity, more research should be conducted to determine the possible chronic toxicity of the leachate. It is known that one of the major toxicants in landfills is ammonia (McBride et al. 1979; Cameron & Koch 1980; Clemment & Merlin 1995), and given the high ammonia concentrations measured in this study, it is important to determine the potential for chronic toxicity.

Chronic toxicity is relatively more dangerous than acute toxicity. Chronic symptoms often go undetected for longer periods after exposure has occurred, when it is too late to prevent damage. Cancer is an excellent example of chronic toxicity, which is caused by toxic substances called carcinogens.
A thorough understanding of the toxicity of raw landfill leachate is vital, and research using *in vivo* and *in vitro* tests for both acute and chronic toxicity is necessary. More information is needed to increase our understanding of leachate’s toxic effects, not only for our benefit but also for the sake of future generations.

REFERENCES


