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LITERATURE CITED


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Genotoxic Effects of Linear Alkyl Benzene Sulfonate, Sodium Pentachlorophenate and Dichromate on Tetrahymena pyriformis

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ABSTRACT. DNA in macro- and micronuclei of Tetrahymena pyriformis treated with linear alkyl benzene sulfonate (LAS) and sodium pentachlorophenate (PCP-Na) were determined by microspectrophotometry. The effects on rate of formation of macronuclear DNA extrusion bodies were also studied. We found DNA content of micronuclei in 0.14 ppm LAS and 0.9 ppb PCP-Na was lower than that of the control, and LAS was able to increase the formation rate of macronuclear DNA extrusion bodies (the formation rate was 54% in 11.3 ppm LAS and 25.6% in 16.7 ppm dichromate). We concluded that 0.14 ppm LAS (below the maximum acceptable toxicant concentration) was genotoxic, whereas 0.014 ppm LAS was not. Dichromate 0.05 ppm and 0.9 ppb PCP-Na equally to and below the maximum acceptable toxicant concentration, respectively, were potentially genotoxic.

Key words. DNA, macronuclear DNA extrusion bodies, micronucleus, microspectrophotometry.

O VER 1,000 chemicals are introduced in the world each year. These chemicals may not only be acutely toxic but may also have long-term harmful effects. Among these chemicals, insecticides, detergents and heavy metals deserve particular attention. In addition to safe levels and mechanisms of toxicities, potential genetic effects are important.

The detergents used in China are primarily linear alkyl benzene sulfonates (LAS). Judging from global trends, LAS will remain the major detergent, and hence will continue to accumulate in the environment. In China sodium pentachlorophenate (PCP-Na), which is widely used as a preservative, insecticide, herbicide, and antibiotic, often leads to significant local pollution. Chromium, an important element in industry, is causing increasing concern since its discovery as a possible carcinogen. Chromium can cause skin ulcers, nose inflammations, and genetic malformations. Dichromate is a frequently used laboratory material. Therefore, the toxic effects of LAS, PCP-Na and dichromate to organisms merit more study. Reports on the toxic effects of LAS, PCP-Na and dichromate on Tetrahymena pyriformis have been limited to their effects on cell growth, motility, and other physiological processes [4, 6, 9-12]; hence, we studied the mechanisms of toxicity and the genotoxicity. There are at least five kinds of genotoxicity: gene mutation, chromosome aberration, primary damage of DNA, interference with DNA topoisomerases and carcinogenic transformation.

1 To whom correspondence should be addressed.
Table 1. Effects of LAS and PCP-Na on DNA content in the macro- and micronuclei of *T. pyriformis* S1 (mean ± SD).

<table>
<thead>
<tr>
<th>Nuclei group</th>
<th>LAS concentration (ppm)</th>
<th>PCP-Na concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.14 ppm</td>
<td>0.15 ppm</td>
</tr>
<tr>
<td>Micronuclei</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment</td>
<td>2,861.90 ± 345.57</td>
<td>1,391.72 ± 223.06</td>
</tr>
<tr>
<td>Control</td>
<td>3,494.82 ± 516.63</td>
<td>1,789.78 ± 282.74</td>
</tr>
<tr>
<td>Significance (t-test)</td>
<td><em>P &lt; 0.001</em></td>
<td><em>P &lt; 0.001</em></td>
</tr>
<tr>
<td>Macronuclei</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment</td>
<td>11,434 ± 1,872</td>
<td>13,020 ± 1,886</td>
</tr>
<tr>
<td>Control</td>
<td>13,534 ± 1,731</td>
<td>14,440 ± 2,416</td>
</tr>
<tr>
<td>Significance (t-test)</td>
<td><em>P &lt; 0.002</em></td>
<td><em>P &gt; 0.05</em></td>
</tr>
</tbody>
</table>

*The number of measured micro- and micronuclei is 50 and 35, respectively.*

**MATERIALS AND METHODS**

**Organisms and growth medium.** *Tetrahymena pyriformis* strains S1 and BJ4 were grown axenically at 25–28°C in 250–
ml flasks in a medium consisting of 2% (w/v) polypeptone and 0.1% (w/v) glucose plus 0.1% (w/v) yeast extract.

**Determination of DNA content in micro- and macronuclei.** Cells were grown in stationary vessels. Cells were obtained by
gentle centrifugation (700 g) and the pellet was washed three times in isotonic Osterhout solution. The cells were cen-
trifuged and suspended. The chemicals were added at a final concentration of 0.14 ppm LAS, 11.31 ppm LAS, 9 × 10 ppm PCP-
Na, and 0.15 ppm PCP-Na. Cells cultured in an isotonic medium served as controls. Culture medium was centrifuged off after 5 h and cells were fixed in Carnoy’s solution for 0.5 h. Slides were
Feulgen stained. DNA was measured spectrophotometrically.
The conditions of microspectrophotometry in this test were as
follows: 1) Measurement of micronuclei DNA—X-field (steps)
8, Y-field (steps) 8, X-stepsize (μm) 0.5, Y-stepsize (μm) 0.5,
the wave of light 560 nm; 2) Measurement of macronuclei DNA—
X-field (steps) 10, Y-field (steps) 10, X-stepsize (μm) 2, Y-step-
size (μm) 2, the wave of light 560 nm.

**Measurements of macronuclear DNA extrusion bodies.** Cultures of cells (amicronucleate strain BJ4) in the log phase were
used and treated as described above. Final concentrations were
0.014 ppm LAS, 0.14 ppm LAS, 1.4 ppm LAS, 11.30 ppm LAS,
0.05 ppm dichromate, 5 ppm dichromate, and 16.7 ppm dichro-
atate. The chemicals were removed after 4 h. The cells were
recultured for 4 h, then fixed with Carnoy’s solution, stained
with Feulgen, and counterstained with Fast Green. The mac-
ronuclear DNA extrusion bodies (MaEB) were identified under
an oil lens using over 2,000 cells for each sample. Because the
*Tetrahymena pyriformis* strain BJ4 is amicronucleate, the mi-
cronuclei discovered under an oil lens should be toxicological
MaEB.

**RESULTS**

**Determination of DNA content in macro- and micronuclei of**
*Tetrahymena pyriformis* S1. We already know that 12h-LC50
of LAS *Tetrahymena* is 17.21 ppm [5], 12h-LC50 of K2CrO3 is
14.098 ppm and that of PCP-Na is 0.117 ppm (Wu, unpubl.
data). We chose two doses for the former two chemicals; the
high dose was near 12h-LC50 and the low dose was near or lower
than the maximum acceptable toxicant concentration (MATC).
The MATC of LAS is 0.17 ppm [5], and that of K2CrO3 is 0.05
ppm according to the National Drinking Water Quality Stan-
ard (TJ20-76, a serial number of the legal standard in China).
The MATC of PCP-Na is < 0.01 ppm according to the National
Fishery Water Quality Standard (TJ35-39). The effects of LAS
and PCP-Na at the two doses are given in Table 1.

After the cells are treated with 11.31 ppm LAS or 0.15 ppm
PCP-Na, the DNA contents of macro- and micronuclei are lower
than that of the control. Their difference is significant by
Student’s t-test (*P < 0.05*). Additionally, after the cells are treated
with 0.14 ppm LAS and 0.9 ppm PCP-Na (these doses are below
the MATC), the DNA content of the micronuclei is lower than
that of the control (*P < 0.001*), whereas that of the macronuclei
was not significantly different from that of the control (*P >
0.05*). The micronuclei of *T. pyriformis* S1 were more sensitive
to the chemicals than were the macronuclei.

**MaEB test.** The effects of LAS and dichromate on the for-
mation rate of MaEB of *T. pyriformis* BJ4 are shown in Tables
2 and 3. From these results we can conclude the following:
1) LAS and K2CrO3; can induce the formation of MaEB of *T.
pyriformis* BJ4; 2) the formation rate of MaEB increases with
increasing doses of LAS and K2CrO3; 3) LAS can greatly in-
crease the formation rate of MaEB (the formation rate was 54% in
11.31 ppm LAS and 25.6% in 16.7 ppm K2CrO3), and 4) from
the results above, the safe concentration of LAS from a
 genetic standpoint was < 0.014 ppm, and that of K2CrO3 was
> 0.05 ppm. Yet, according to the National Drinking Water
Quality Standard, the safe concentration for LAS is 0.3 ppm
and for K2CrO3, 0.05 ppm. This means that the concentra-
tions as set cannot be regarded as genetically harmless to the
protozoan cell.

**DISCUSSION**

**Measurement of micro- and macronuclei DNA content of**
*Tetrahymena.* From the effect of LAS and PCP-Na on the content
of nuclear DNA alone, we still cannot say that these chemicals
are directly responsible for the damage to the structure and

Table 2. Effects of LAS on formation rate of MaEB for *T. pyriformis* BJ4 (mean ± SD).

<table>
<thead>
<tr>
<th>LAS concentration (ppm)</th>
<th>Formation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>0.014</td>
<td>7 ± 0.33</td>
</tr>
<tr>
<td>0.14</td>
<td>9 ± 0.41</td>
</tr>
<tr>
<td>1.40</td>
<td>16 ± 0.72</td>
</tr>
<tr>
<td>11.31</td>
<td>38 ± 1.21</td>
</tr>
<tr>
<td></td>
<td>54 ± 2.10</td>
</tr>
</tbody>
</table>
Table 3. Effects of K$_2$Cr$_2$O$_7$ on formation rate of MaEB for T. pyriformis BJ4 (mean ± SD).

<table>
<thead>
<tr>
<th>K$_2$Cr$_2$O$_7$ concentration (ppm)</th>
<th>0</th>
<th>0.05</th>
<th>5</th>
<th>16.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formation rate (%)</td>
<td>7 ± 0.29</td>
<td>12.9 ± 0.42</td>
<td>14.8 ± 0.65</td>
<td>25.6 ± 1.12</td>
</tr>
</tbody>
</table>

function of DNA. They may only damage the physiological metabolism of the cell, so that they affect replication enzymes and inhibit synthesis of DNA. As for genotoxicity, the repair process is said to play an important part in exposure to chemicals of low doses. Yet, for concentrations below the MATC, say 0.14 ppm LAS and 0.9 ppb PCP-Na, when used to test Tetrahymena for 5 h, micronuclear DNA content was obviously lower than that of the control, because the genetic activity of micronuclear DNA is rather limited when compared with that of macronuclear DNA for the following reasons: 1) Its role in damage repair is not as effective as for macronuclear DNA. Cells repair DNA damage by using several different enzymes. Errors in gene replication are directly proportional to the extent of gene damage, storage ability of the genome, and efficiency of the repair process [7]. 2) After reaction with the chemicals, essential conditions for DNA replication of micronuclear DNA may not exist, making the DNA content of the test group lower than that of the control. At a low dose of the chemical, the DNA content of the macronuclei was not obviously different from that of the control. Two possible explanations exist: 1) macronuclear DNA is not as sensitive as the micronuclear DNA is to a low dose of the chemicals; or 2) macronuclear DNA may repair DNA damage in time to protect themselves. Our experiments show that the DNA content in micronuclei was already affected at the MATC level. As the MATC is established by environmental researchers on the basis that given concentrations of chemicals will affect neither growth nor reproduction of the organisms in the ecosystem, potential toxic effects of chemicals not affecting growth and reproduction of the cell are largely ignored.

For chemicals at a high dose as used in the positive control, e.g. 11.31 ppm LAS and 0.15 ppm PCP-Na (values close to their 12h-LC$_{50}$, respectively), organisms sensitive to the chemical died whereas resistant ones survived. In organisms that survived high-dose treatment, the essential demands for DNA replication cannot be satisfied. Therefore, the content of the DNA in macronuclei and micronuclei of the test group was obviously lower than that of the control group (P < 0.05).

**MaEB test.** The MaEB we identified in T. pyriformis BJ4 may be DNA extrusion bodies [8]. The formation rate of MaEB is proportional to the chemical dose. Two explanations for this exist: 1) during the S phase, these chemicals can block duplication of macronuclear DNA, inducing formation of the chromosomal extrusion in T. pyriformis [1–3]. The DNA that had not been well duplicated is extruded into the cell matrix; 2) when the cell is treated with chemicals, DNA duplication is blocked. As soon as the chemicals are removed, the metabolism of the cell will compensate, leading to excessive DNA synthesis, with an increase in the formation rate of the MaEB.

The formation rate of MaEB in the test group was higher than that in the control group when test doses of LAS and K$_2$Cr$_2$O$_7$ were below the MATC. We conclude that the MaEB test is a sensitive and useful method.

From the positive results of the MaEB test and other harmful effects on nuclear DNA, it appears that LAS can damage the genetic material of the cell when the test animal is treated with a dose of 0.14 ppm (below the MATC); however, 0.014 ppm LAS caused no identifiable change in the genetic material. K$_2$Cr$_2$O$_7$ 0.05 ppm (equal to the MATC) and 0.9 ppb PCP-Na (below the MATC) can also affect the DNA.

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