Changes in the microsomal mixed function oxidase system with long-term trichloroethylene administration

Key words: Trichloroethylene—Cytochrome P-450—Cytochrome b5—NADPH-cytochrome c reductase—Mixed function oxidase

Trichloroethylene (Tri) continues to be extensively used as an industrial solvent for fats and oils.1) As a xenobiotic metabolized by the microsomal mixed function oxidase system (MFOS) Tri is able to destroy, inhibit and induce microsomal enzymes. Costa et al.2) reported that incubation of hepatic microsomes with NADPH and trichloroethylene resulted in decreased levels of cytochrome P-450, but no alteration in the levels of NADPH-cytochrome c reductase or cytochrome b5. In vivo study, a single inhalation of Tri (10,000 ppm) for a few hours3) or a single intraperitoneal injection of Tri (1.0 ml/kg body weight)4) did not change the levels of cytochrome P-450 or NADPH-cytochrome c reductase in rats. However, Savolainen et al.5) reported that repeated inhalation of Tri (200 ppm) for several days increased hepatic cytochrome P-450. Pessayre et al.4) reported that repeated administration of Tri (1.0 ml/kg, i.p.) for 5 days decreased microsomal P-450 but increased NADPH-cytochrome c reductase activity. These studies suggest that MFOS is destroyed or induced by the dose and duration of Tri administration. These studies of the effect of Tri on microsomal enzymes, however, have been done over less than 5–6 days’ duration. It is not clear how microsomal enzymes are altered when Tri is administered or inhaled for longer durations. In this communication, the authors report the effect of Tri administration for 15 weeks on cytochrome P-450, cytochrome b5 levels and NADPH-cytochrome c reductase activity. We also investigated changes in body weight and main organ weights.

Adult male Wistar rats weighing 200 g served as subjects. They were divided into two groups. Group 1 (8 rats) was given trichloroethylene (2.0 g/kg body weight) in olive oil subcutaneously twice a week for 15 weeks. Group 2 (7 rats) was injected with olive oil in the same manner and served as a control. The animals were fed with a standard laboratory diet and water ad libitum. The room temperature was kept at 22±5°C.

In statistical analysis, differences between mean values were assessed using Student's unpaired t-test.

Animals were sacrificed one week after the final injection of Tri. Livers were perfused in situ with ice-cold 0.9% saline, rapidly excised, blotted dry, weighed, mixed and homogenized in ice-cold Tris-acetate buffer (pH 7.4) containing 0.1 M KCl and 1.0 mM EDTA. Hearts, lungs, spleens, thymus, kidneys and brains were also blotted dry and weighed. Liver microsomes were isolated by the method
Liver protein was estimated by the method of Lowry et al. using bovine serum albumin as the standard. The levels of cytochrome P-450 and cytochrome b5 were determined with a Hitachi double-beam recording spectrometer (model 557) by the method of Omura and Sato. NADPH-cytochrome c reductase activity was determined by the method of Masters et al.

Figure 1 shows the effect of chronic trichloroethylene treatment on body weight gain. The mean body weights of the Tri-treated group were slightly less than those of the control group for each week. However, there were no significant differences in body weight between the two groups. Table 1 shows the organ weights at the end of the experiment. The mean liver weight of the Tri-treated rats was 1.15 times larger than that of control rats, but there was no significant difference. Relative
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Liver weights (i.e., liver weight as a percentage of body weight) of the Tri-treated group were significantly larger (about 20%) than those of the control group (p<0.01). The weights of heart, lung, spleen, thymus, kidney and brain were not changed by long-term Tri treatment. Kjellstrand et al.10) studied the influence of continuous inhalation of 150 ppm Tri for 30 days on body, liver, spleen and kidney weights in rats. An age-dependent decrease in body weight gain was observed in female rats exposed to Tri, but this decrease was not observed in male rats. Liver enlargement (20–30% in relative liver weight) was caused by Tri exposure. These data are in good agreement with our findings. They also reported that the spleen weight appeared unaffected or somewhat smaller in Tri-exposed animals. In our experiment, the weight of spleen did not change with Tri treatment.

Table 2 shows the effects of chronic trichloroethylene treatment on microsomal protein, cytochrome P-450, cytochrome b5 and cytochrome c reductase. Tri treatment caused a significant increase (about 20%) in microsomal protein (p<0.01). Microsomal cytochrome P-450 content in microsomal protein did not show a significant change with Tri treatment, but the content in liver showed a significant increase (p<0.05). Trichloroethylene treatment also caused a significant increase in cytochrome b5 level (about 15%) and NADPH-cytochrome c reductase activity (about 23%).

Long-term administration of Tri increased the liver weight/body weight ratio, microsomal proteins, cytochrome b5 content and NADPH-cytochrome c reductase activity. These results likely indicate the induction of drug-metabolizing enzymes by Tri. It may be said that long-term Tri treatment did not specifically induce cytochrome P-450, because the cytochrome P-450 content in microsomal protein did not increase in spite of the increase of microsomal protein in liver. Are there any enzymes which were induced by long-term Tri treatment besides cytochrome b5 and NADPH-cytochrome c reductase? Pessayre et al.4) demonstrated that repeated Tri administration for five days decreased microsomal mixed function oxidase activity with some substrates but increased it with others. They suspected that cytochrome P-450 can be simultaneously destroyed and induced

<table>
<thead>
<tr>
<th></th>
<th>Microsomal protein (mg/g liver)</th>
<th>Cytochrome P-450</th>
<th>Cytochrome b5</th>
<th>NADPH-cytochrome c reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/mg protein)</td>
<td>(nmol/mg protein)</td>
<td>(nmol/g liver)</td>
<td>(nmol/mg protein)</td>
</tr>
<tr>
<td>Control</td>
<td>10.64 ± 0.876</td>
<td>1.342 ± 0.205</td>
<td>14.28 ± 2.41</td>
<td>0.714 ± 0.056</td>
</tr>
<tr>
<td>trichloroethylene</td>
<td>12.58 ± 0.712**</td>
<td>1.456 ± 0.159</td>
<td>18.34 ± 2.31*</td>
<td>0.822 ± 0.092*</td>
</tr>
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(*) p<0.05, **: p<0.01
(Each figure represents mean and S.D.)
with chronic administration of Tri. As Pessayre et al. mentioned, is the fact that cytochrome P-450 was not changed with long-term Tri administration the net result of destruction and induction? These questions arose from our experiments, and we hope to do further investigation to solve them.

REFERENCES


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