Induction of Hepatic Cytochrome P450 Activities by Toxaphene in the Rat

Henk-Jan Drenth, Chris J. C. Van Oevelen, Christian J. K. Buitenhuis and Martin Van Den Berg

Research Institute Toxicology, Utrecht University, PO Box 80176, NL-3508 TD, Utrecht, the Netherlands.

Introduction

Until its ban in the U.S. in the early 1980s, toxaphene has been used as a pesticide on a huge scale (1). Toxaphene is a complex mixture consisting of more than 670 polychlorinated constituents (2).

Exposure to exogenous compounds can generally lead to the induction of certain cytochrome P450 enzymes (CYPs). Only a few studies have reported effects of toxaphene on hepatic CYPs in the rat (1,3). The possibilities of determining effects of compounds on the hepatic CYPs include measurements of mRNA levels, protein levels, or enzyme activities. Among the CYP activity measurements, the O-dealkylation of alkoxyresorufins by the hepatic microsomal fraction has been used extensively. The O-dealkylation of methoxy- (MROD), ethoxy- (EROD), and pentoxyresorufin (PROD) by rat hepatic microsomes, is indicative for the presence of CYP1A2, CYP1A1, and CYP2B1, respectively (4). Another frequently used method is the metabolism of steroids. Several microsomal CYPs can hydroxylate testosterone or 17β-estradiol (E_2) at specific carbon atoms. Although there always remains an overlap in the formed metabolites, the overall metabolite profile formed by the microsomes can be indicative for the CYPs present in the microsomal fraction (5-7).

Haake *et al.* (3) reported the induction of testosterone hydroxylase activities in rat hepatic microsomes after exposure to toxaphene. In this study, male rats were injected i.p. with a high dose (400 µmol/kg) of technical toxaphene in corn oil. No dose-response relationship was studied in this experiment.

The goal of our present study is to investigate a dose-response relationship between oral exposure of rats to technical toxaphene, and some selected hepatic microsomal CYP activities.

Experimental Animals, Material and Methods

Thirty two mature female SPF rats (Uw:U(CPB), weighing approximately 350 g) were randomly divided into 8 groups. The animals were allowed to acclimatize for 5 days. One day before dosing, technical toxaphene (Riedel de Haën, Seelze, Germany) was dissolved in 5% tween-80. The different doses were derived, by diluting the stock emulsion with 5%-tween. The control group received vehicle only. Rats in the other groups received 0.012; 0.069; 0.42;

2.5; 15; or 40 mg/kg bw of toxaphene. The solutions were administered orally using a 2 ml syringe with a metal canule. To assess the effect of tween-80, one group received no treatment at all. Rats were killed after 24 h by placing them in an atmosphere of 100% carbondioxide. Livers were removed and microsomes were prepared as described by Bouwman *et al.* (8). Protein content of the microsomal fraction was determined using the Bradford method with crystalline BSA as a standard.

MROD, EROD and PROD activities were measured (fluorimetric: excitation wavelength of 530 nm, emission wavelength 590 nm) according to Burke *et al.* (9) with some minor adjustments as described by Rutten *et al.* (10).

Testosterone hydroxylase activities of hepatic microsomes were determined according to Sonderfan *et al.* (11) and adapted by Wortelboer *et al.* (12). Briefly, microsomes were incubated in phosphate buffer (pH=7.4, 37°C, 15 min) with 250 µM testosterone. The incubation mixture contained 1 mM NADP, 5 mM glucose-6-phosphate, 3 mM $MgCl_2$, and 1 unit/ml glucose-6-phosphate dehydrogenase. The formed androstenedione and hydroxy metabolites (OHT) were determined using a HPLC method (13), as described by Wortelboer *et al.* (12). As an internal standard 11β-OHT was added.

17β-Estradiol hydroxylation was determined as described by Suchar *et al.* (6,7), with some modifications. Microsomes were incubated in phosphate buffer (pH=7.4) for 60 min at 37°C with 250 µM E_2. The incubation mixture contained 1 mM NADP, 5 mM glucose-6-phosphate, 3 mM $MgCl_2$, and 1 unit/ml glucose-6-phosphate dehydrogenase. 16α-Hydroxyestrone (16α-OHE_1) (Sigma, St Louis, MO, USA) was used as an internal standard. Metabolites were analyzed using a HPLC method described by Suchar *et al.* (6). Detection was performed with UV absorption at 280 nm. Metabolites were identified and quantified using standards. 16α-OHE_2 (estriol, E_3), 2-OH E_2, 4-OH E_2, and 6α-OHE_2, E_2, and E_1 were purchased from Sigma (St. Louis, MO, USA).

The Levine-test was used to test whether the variances between the dose-groups were different ($p<0.05$). If so, then the Kruskal-Wallis test was used ($p<0.05$), otherwise the Bonferroni Anova test was performed ($p<0.05$). Statistical differences between the control and the highest dose groups were determined using contrast groups.

Results and Discussion

No overt toxicity was observed in any of the dose groups. Administration of the vehicle only (tween-80) did not induce any of the CYP activities (data not shown).

In hepatic microsomes from the highest dose group, an increase of PROD activity was observed (Figure 1A). The activity increased from 1.9 ± 0.41 to 7.5 ± 3.4 pmol/mg prot·min. No effects of toxaphene exposure on MROD (14 ± 2.3 pmol/mg prot·min) and EROD (12 ± 5.1 pmol/mg prot·min) activities were observed (data not shown).

Rat hepatic microsomes formed E_3, 2-OHE_2, and 4-OHE_2, and E_1 from E_2. The formation rates were 23 ± 3.1, 141 ± 68.0, 38 ± 22 and 307 ± 90.6 pmol/mg prot·min respectively. Only the formation rate of 2-OHE_2 was induced significantly ($p<0.05$) by a factor of 2.5 in the highest dose group compared to the control group (Figure 1B). All other formation rates of E2 metabolites remained unchanged (data not shown).

The control hepatic microsomes hydroxylated testosterone at several positions. The formed metabolites included 6β-, 7α-, 15α-, 15β-, 16β-OHT, and androstenedione.

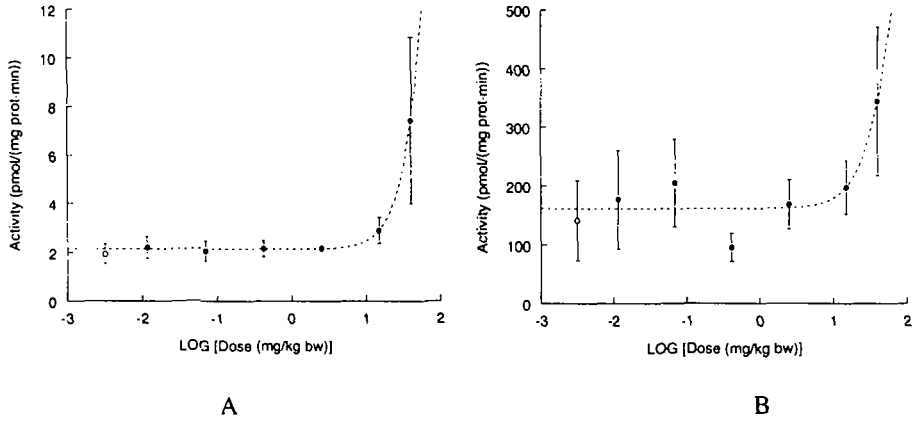

Figure 1. PROD activity (A) and Estradiol 2-hydroxylase activity (B) of female rat hepatic microsomes. Rats were exposed orally to graded doses of technical toxaphene in 5% tween-80. Control rats received vehicle only. The dose is expressed as the logarhythm of the dose in mg toxaphene per kg of bodyweight. The vertical lines through the data points represent the standard deviation. The dashed lines represent the fitted sigmoidal curves using SlideWrite Plus for Windows 4.0 (Advanced Graphics Software, Carlsbad, CA, USA).

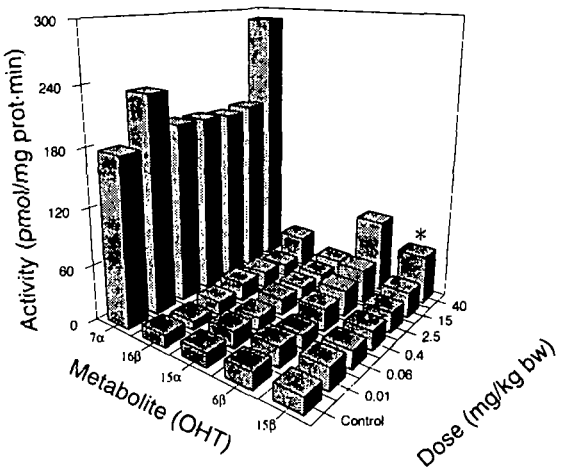

Figure 2. Testosterone hydroxylation. The formation rates of 15β-, 6β-, 15α-, 16β-, and 7α-hydroxytestosterone from testosterone (250 µM) by rat hepatic microsomes (300-500 µg protein) in 15 min at 37 °C and pH 7.4. An asterix is used to indicate a significant difference ($p<0.05$) between the dose group and the control group.

In our experiment we have observed a significant induction of the 15β-OHT formation rate by a factor of 3.1.The formation rates of 15α-OHT and androstenedione were not induced.

Though not significant at $p<0.05$, but significant at $p<0.1$, the formation rates of 6β-, 7α-, and 16β-OHT were induced by a factor of 4.4, 1.6, and 2.5, respectively (Figure 2). Two metabolites, 16α- and 2α-OHT, were formed at a low rate (<20 pmol/mg prot·min) by only a few of the microsomal fractions.

Haake *et al.* have reported induction of 6β-, 16α-, and 16β-OHT, while the formation rates of 6α-, 15β-, 7α-OHT, and androstenedione remained unchanged (3). In this experiment four weeks old male Long Evans rats were injected i.p. with 400 µmol/kg toxaphene in corn oil as a vehicle. Assuming an average molecular weight of 414 g/mol this dose is equivalent to 165 mg/kg. Animals were sacrificed at day 5 after dosing, and were fasted 24 h. prior to sacrifice. The differences in CYP activity induction by toxaphene in both studies can be explained by the differences in experimental design, strain, age and gender of the rats. It has been observed that the presence and regulation of some CYPs depends on the gender (14).

The induction of any of the CYP activities by toxaphene, is only observed in the highest dose group. These rats received a relative high dose, namely half the LD_{50} (80 mg/kg bw for female rats (15)). For many organochlorine compounds, like PCBs and dioxins, specific induction of CYP activities occurs at much lower dose levels compared to their LD_{50} values. Therefore, we conclude that induction of hepatic CYP activities by toxaphene in rats is not a very susceptible effect. The use of CYP activity induction as a biomarker for exposure to environmental relevant dose levels of toxaphene, does not seem to be feasible.

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