(ANTI)ESTROGENIC EFFECT OF FOUR TOXAPHENE CONGENERS AND THE TECHNICAL MIXTURE IN STABLY TRANSFECTED HUMAN T47D.LUC BREAST CANCER CELLS

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Abstract

(Anti)estrogenic effects of four persistent toxaphene congeners and the technical mixture have been examined in T47D human breast cancer cells. None of the four congeners (CHB 26, 32, 50 and 62) showed any estrogenic effect in the same nominal concentration range (up to 50 μM) as the technical mixture. The technical toxaphene mixture exerted an antiestrogenic effect as determined by measuring the inhibition of luciferase activity induced by 10 pM 17β-estradiol. A maximal inhibition of 45 %, and an EC_{50} of 0.58 μM were calculated. None of the four individuals congeners showed any antiestrogenic effect.

Introduction

The technical toxaphene mixture consists of of mainly polychlorinated bornanes (CHBs). Despite its ban in North America and Europe toxaphene is still used on a small scale in African and Latin American countries [1, 2]. At present toxaphene can be detected in the most remote areas, such as the Arctic region. Due to the relative persistence of some congeners towards biotransformation, concentrations in biota, especially those in species at higher trophic levels, can even exceed those of DDTs and PCBs [1]. Bioaccumulation of toxaphene is congener specific and only a limited number of congeners are present in e.g. tissues from aquatic biota [1]. The congeners CHB26, CHB50, and to a lesser extend CHB62 (Parlar nomenclature) [3, 4] are persistent and bioaccumulate in marine mammals, fish and human tissue and milk [1, 5, 6]. CHB32 is more easily degraded and only occasionally detected in biota [1]. Recently, several *in vitro* tests have been developed to assess estrogenic and anti-estrogenic effects of chemicals.

Toxaphene has been added to the growing list of xenobiotic compounds possessing estrogenic activity [7]. This was shown by increased proliferation of MCF-7 human breast cancer cells [8-10], induction of progesterone receptor and pS2 levels [8]. In addition, toxaphene induces the ER-mediated production of vitellogenin in *Xenopus laevis* [11]. In contrast a number of other *in vitro and in vivo* studies could not demonstrate ER-mediated effects of toxaphene [9, 12-15]. The ER-CALUX assay uses T47D breast cancer cells stably transfected with an ER-regulated luciferase reporter gene and is highly specific, sensitive, and responsive to (anti)estrogenic substances [16]. Essentially all toxaphene studies reported to date assessed estrogenic effects of the technical mixture. In the present study the ER-CALUX assay was used to investigate possible (anti)estrogenic effects of four individual toxaphene congeners (CHB26, CHB32, CHB50, and CHB62), in comparison to the technical mixture.

Material and Methods

Chemicals. The four toxaphene congeners CHB26 (2-endo, 3-exo, 5-endo, 6-exo, 8,8,10,10-octachlorobornane), CHB32 (2,2,5-endo,6-exo,8,9,10-heptachlorobornane), CHB50 (2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-nonachlorobornane), and CHB62 (2,2,5,5,8,9,9,10,10-nonachlorobornane) were obtained via Promochem (Wesel, Germany).

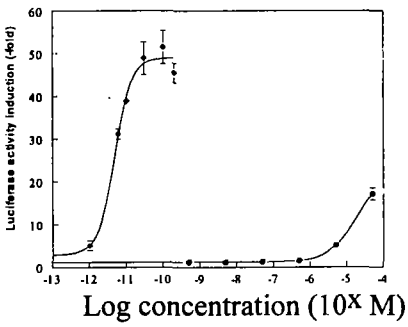

Figure 1. Effect of 17β-estradiol (◆) and technical toxaphene (●) on luciferase activity in T47D.Luc cells.

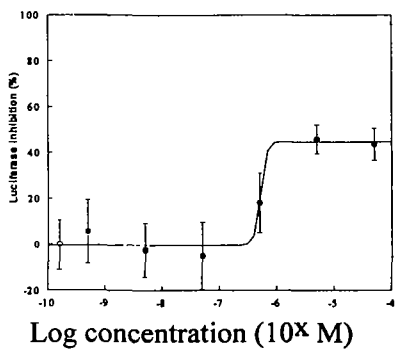

Figure 2. Antiestrogenic effect of technical toxaphene (●) on luciferase activity in T47D.Luc cells. Cells were incubated with 10 pM 17β-estradiol and several concentrations of toxaphene or solvent control (O).

Cell culture and luciferase activity. The cell culturing conditions were similar to those described by Legler et al. [16]. The cells were maintained in medium without phenol red, supplemented with 5% dextran-coated charcoal stripped fetal calf serum. The ER-CALUX assay was performed as described previously by Legler et al. [16].

(Anti) estrogenic effects. The nominal concentration of the toxicants in the incubation medium were: 0.5, 5.0, 50 nM, 0.5, 5.0, and 50 µM. an average molar weight of 414 g/mol for the technical toxaphene mixture was used [6]. An E_2 standard curve (1 to 200 pM) was included in each assay. A dose of 10 pM E_2 was included as a positive control, as well as DMSO solvent as a negative control. Every concentration was tested in triplicate. Antiestrogenic effects were tested at the same nominal concentrations. Cells were co-incubated with E_2, at a nominal concentration of 10 pM. This is the approximate concentration with a half maximal inductive effect (EC50) on luciferase activity [16]. An antiestrogenic effect was defined as the capability of a chemical or mixture to inhibit the luciferase activity induced by 10 pM E_2. Cytotoxicity of the toxicants was assayed in the T47D.luc cells by determination of the MTT activity [17]. The highest nominal concentration (50 µM) of the four congeners and the technical mixture were assayed. Technical toxaphene or the individual CHB congeners were not cytotoxic to the T47D.luc cells.

Results and Discussion

Estrogenic effects. Technical toxaphene and E_2 induced luciferase activity dose-dependently in the T47D.Luc cells (Fig. 1). The nominal EC_{50} concentration for E_2 was 4.81 pM and the lowest observed effect concentration (LOEC) was calculated as 0.14 pM. The EC_{50} and LOEC of technical toxaphene were calculated as 18.7 and 0.40 µM, respectively. This indicates a potency of approximately six orders of a magnitude less than E_2. CHB26, CHB32, CHB50, or CHB62, even at the highest dose, did not induce any dose-dependent increase in luciferase activity in the same concentration range as the technical mixture.

Anti-estrogenic effects. Technical toxaphene inhibited E_2 induced luciferase activity (Fig. 2). A maximal inhibition of 45% was observed at concentrations of 5 µM or greater. The calculated EC_{50} for inhibition is 0.58 µM. The inhibition of technical toxaphene was reversed upon incubation with higher E_2 concentrations. Incubation of 30 or 100 pM E_2, and 5 µM technical toxaphene resulted in an induction of luciferase activity comparable to the induction exerted by E_2 alone. None of the individual CHB congeners showed a dose-dependent inhibitory effect on E_2 induced luciferase activity in T47D.Luc cells.

In our study technical toxaphene causes estrogenic and antiestrogenic effects and these results might be explained by a role of toxaphene (congeners) as (a) partial agonist(s). Another explanation could be the presence of both (an) estrogenic and (an) antiestrogenic

compound(s) in the technical mixture. The antiestrogenic effect of technical toxaphene was shown to be reversible by E_2, which suggests a competitive mode of action. None of the four individual CHB congeners exerted an estrogenic or an antiestrogenic effect at the same concentrations as the technical mixture.

Therefore, it can be suggested that other constituents of technical toxaphene are responsible for the observed effects. CHB26, CHB50, and CHB62 are major contributors to the total toxaphene concentration in several foodstuffs, including fish, and in human milk samples [1, 18-20]. Our results suggest that the estrogenic risk of toxaphene exposure may be overestimated when studies are done solely with the technical mixture. Thus, risk assessment based on results from *in vitro* studies with technical toxaphene mixtures only should be interpreted with caution.

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