IN VITRO AND IN VIVO TUMOR PROMOTING POTENCY OF TECHNICAL TOXAPHENE, UV-IRRADIATED TOXAPHENE, AND BIOTRANSFORMED TOXAPHENE.

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INTRODUCTION

Toxaphene, a complex mixture of polychlorinated camphenes, was first introduced in 1945 by Hercules Co. as Hercules 3965. Until the mid 1980s, it was mass produced and widely used as an insecticide, particularly in the cotton growing industry. It was also used as a piscicide to control rough fish in various water systems[1,2]. The lipophilic, persistent, and volatile nature of toxaphene, has contributed to its global dispersion throughout the fresh water and marine environment. It has even been found in remote areas such as the Arctic where the pesticide was never used[3]. In addition to bioaccumulation in biota inhabiting these regions, it is also been detected in humans[4,5].

Data on the toxicological risk for humans associated with toxaphene exposure is scanty. Reports on the mutagenic and carcinogenic properties of toxaphene in mammalian test systems, have led to the assumption that toxaphene poses a serious thread to humans[1,2]. Human exposure mainly occurs through the consumption of toxaphene contaminated fish. Due to weathering and biotransformation, the bornane composition of technical toxaphene (TT) differs from the bornane composition in consumption fish. Therefore information on the carcinogenicity and general toxicology of weathered and biotransformed TT would be of major interest. To mimic the weathered toxaphene found in fish, we developed a so-called 'realistic exposure' procedure for toxaphene. This procedure makes use of cod that were exposed to TT. Toxaphene residues that were extracted from cod liver (CLE), were then used in in vitro and in vivo studies to obtain information on its tumor promoting potency. Besides CLE, we also studied the tumor promoting properties of UV-irradiated toxaphene (UVT) and TT.

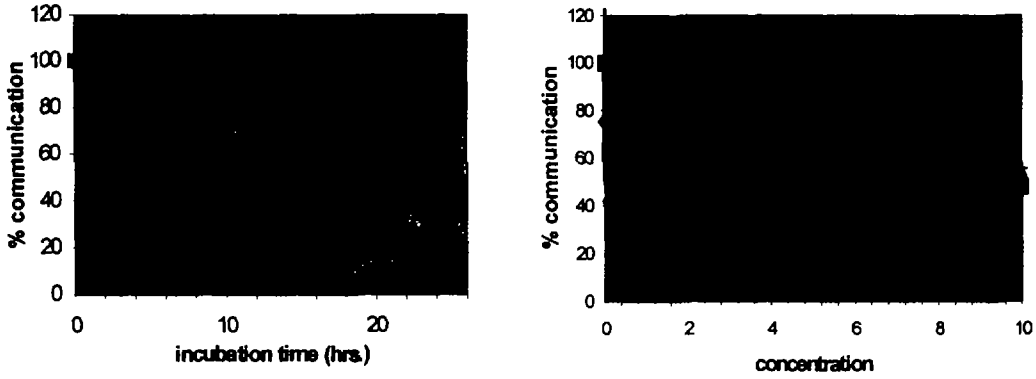
Material and methods

Chemicals: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD; 99% pure) was obtained from Schmidt (Amsterdam, Netherlands). Technical toxaphene (TT) was purchased from Promogem (Germany). Uv-irradiated toxaphene (UVT) was a kind gift from Dr. H. Parlar. Biotransformed toxaphene (Cod Liver Extract, CLE) was obtained by feeding cod (2 years of age) a toxaphene enriched diet (30 ppm technical toxaphene/pellet) for 4 months. After the dosing period, toxaphene residues were extracted from cod liver.

In vitro studies: The in vitro tumor promoting potency of TT, UVT, and CLE was evaluated by studying inhibition of gap junctional intercellular communication (GJIC). Hepa 1c1c7 cells were allowed to grow in small disks for 48 hrs after which a small amount of lucifer yellow was injected into single cells and the number of communicating cells counted. 0-24 hrs prior to

injection of lucifer yellow, growing medium was replaced by exposure medium spiked with CLE (1-10 mg/ml), UVT or TT (0.5-5, and 1-10 mg/ml respectively).

Figure 1 **(A)** Time dependent inhibition of intercellular communication in Hepa 1c1c7 cells by cornoil



(—), 2,3,7,8-TCDD (-♦-; 2 nM),technical toxaphene (-□-; 10 μg/ml), uv-irradiated toxaphene (-●-; 5 μg/ml), and CLE (-▲-; 10 μg/ml). Mean data ± sem are given as percentage of maximum communication. **(B)** Concentration dependent inhibition of intercellular communication in Hepa 1c1c7 cells by 2,3,7,8-TCDD (-♦-; nM),technical toxaphene (-□-; μg/ml), uv-irradiated toxaphene (-●-; μg/ml), and CLE (-▲-; μg/ml). Mean data ± sem are given as percentage of maximum communication. Exposure time was 24 h.

In vivo studies: The in vivo tumor potency of CLE, TT and UVT was studied using a medium-term two stage promotion assay, measuring the development of altered hepatic glutathion S-transferase (GST-p) positive foci. In short, 2/3 hepatectomy was performed on female Sprague Dawley rats, ± 6 weeks of age, followed by a single i.p. NDEA injection. Starting 5 weeks after partial hepatectomy, rats were dosed weekly for 20 weeks with CLE, TT, or UVT (0.46-12.5, 0.67-18, and 0.33-9 mg/kg/week respectively) by s.c. injection, using corn oil as a carrier. Control animals received corn oil only whereas TCDD (1 mg/kg/week) was administered as positive control. One week after the last dosing, the animals were killed and the liver was taken out. Altered hepatic foci were analysed by staining sections (4 μm) of acetone fixed tissue for gluthatione-S-transferase-P (GST-P positive foci) using GST-P specific antibodies and DAB[6]. Foci were digitised and analysed using the PC_Image Analysis and Measurement software package (Foster Findley Associates lltd). The smallest group of GST-P positive cells scored had an area of 2600 μm^2 (cut-off limit).

Table 1 Maximal inhibition (ΔA_{max}) and EC_{50} values of inhibition of gap-junctional intercellular communication in Hepa1c1c7 cells by 2,3,7,8-TCDD, technical toxaphene, uv-irradiated toxaphene, and CLE. ΔA_{max} and EC_{50} values of the compounds tested were calculated by Scatchard analysis.

	2,3,7,8-TCDD	TT	uvT	CLE
Amax (%)	75.20	63.00	79.90	52.60
EC50 (ug/ml)	$6.4*10^{-6}$	2.70	5.56	1.03
R.P.[a]	1	$2.4*10^{-6}$	$1.2*10^{-6}$	$6.2*10^{-6}$

Results

In vitro studies: Inhibition of GJIC is already observed after 0.5 h when Hepa 1c1c7 cells are incubated with either TCDD, TT, uvT, or CLE (Fig.1A). A continuous decrease of GJIC is observed in cells exposed to TCDD (up to 75% inhibition after 24 h). In contrast, a partial recovery of GJIC was found in TT, uvT, and CLE exposed cells between 1 and 6 h of incubation, followed by a further decrease of GJIC up to 54, 38, and 47% respectively after 24 h.. TCDD, TT, uvT, and CLE inhibited GJIC in a dose-dependent manner (Fig. 1B). EC_{50} values for inhibition of GJIC, maximal response (ΔA_{max}) and K_f values for the TCDD, TT, uvT and CLE exposed cells were calculated by Scatchard analysis using converted 24 h dose-response data (Table 1). EC_{50} values for the tested toxaphene mixtures varied form 1.03 ug/ml for CLE to 5.56 ug/ml for uvT. Scatchard analysis showed that maximal inhibition of intercellular communication was highest for uvT (79.9%) followed by TT (63.0%) and CLE (52.6%). The response constant K_f inhibition of intercellular communication was highest for CLE and lowest for uvT.

In vivo studies: Mean foci area was increased, but not significantly, in the TCDD treated rats as compared to control rats (fig. 2a). Both the area fraction of GST-p positive foci as well as the number of altered hepatic foci (AHF) larger than 75,000 μm^2 per cm^2 liver tissue were significantly increased in TCDD treated rats as compared to cornoil treated rats (4-fold and 5.8-fold respectively) (fig. 2b + c). Although no significant differences were found betweeneither TT, uvT, or CLE exposed rats and cornoil treated rats, a slight dose dependent increase in the area fraction of AHF and the number of large AHF could be observed.

Conclusions

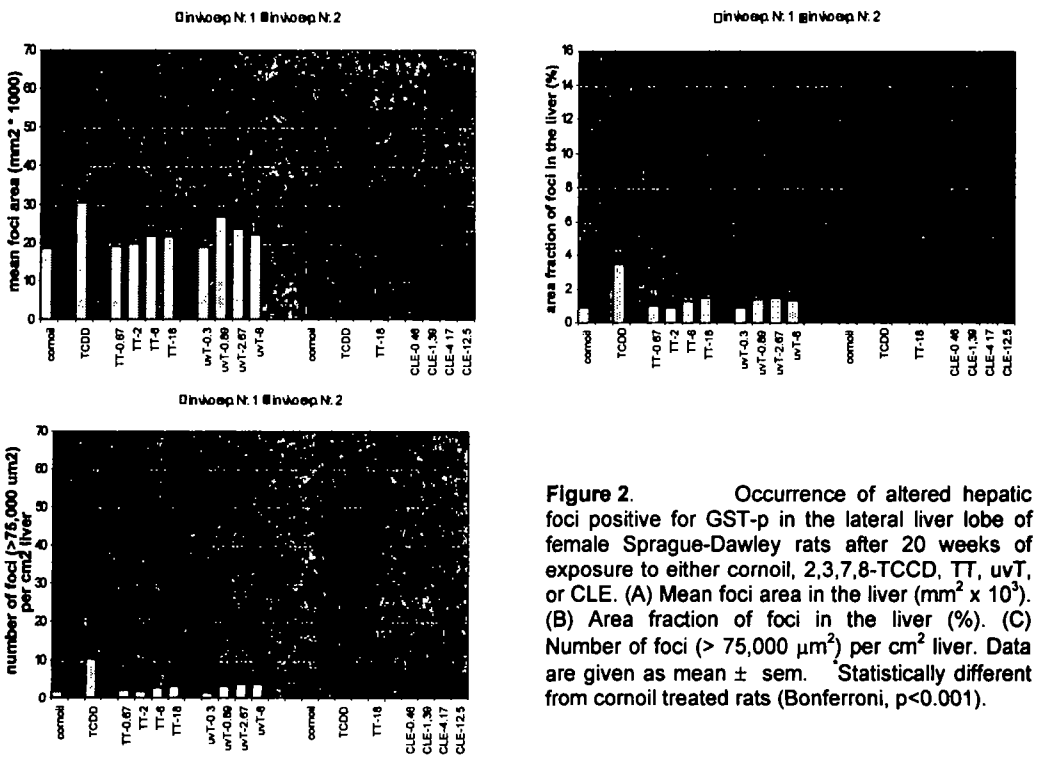


Figure 2. Occurrence of altered hepatic foci positive for GST-p in the lateral liver lobe of female Sprague-Dawley rats after 20 weeks of exposure to either cornoil, 2,3,7,8-TCCD, TT, uvT, or CLE. (A) Mean foci area in the liver ($mm^2 \times 10^3$). (B) Area fraction of foci in the liver (%). (C) Number of foci (> 75,000 μm^2) per cm^2 liver. Data are given as mean ± sem. *Statistically different from cornoil treated rats (Bonferroni, $p<0.001$).

- Technical toxaphene and both toxaphene derived mixtures inhibit intercellular communication in Hepa 1c1c7 cells in a dose- and time-dependent manner.
- Based on the time dependent decrease in intercellular communication, the mechanism of inhibition of intercellular communication of the tested toxaphene (derived) mixtures most probably differs from that of TCDD.
- The maximal inhibition of intercellular communication as observed for TT, uvT, and CLE are in the same order of magnitude.
- The lower observed EC_{50} value for toxaphene extracted from cod liver suggest that the tumor promoting potency of residual toxaphene has increased due to metabolism in fish
- 2,3,7,8-TCDD enhances the promotion of GST-p positive AHF significantly in female Sprague-Dawley rats.
- Technical toxaphene, uv-irradiated toxaphene and residual toxaphene extracted from cod liver did not significantly enhance the promotion of GST-p positive AHF. However, a slight dose dependent increase in the area fraction of AHF and the number of large AHF (>75,000 μm^2) could be observed.
- The present data might indicate that the concentrations for TT, uvT and CLE used in this in vivo experiment are close to the lowest observed effect level for tumor promotion in female Sprague-Dawley rats.

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