# Tumour Promotion Induction by Complex Mixtures of 2-4 and 0-1 *ortho* Polychlorinated Biphenlys in Female Sprague Dawley Rats

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## Introduction

The Toxic Equivalency Factor (TEF) concept has been developed for risk management of complex mixtures of polychlorinated biphenyls (PCBs), polychlorodibenzo-*p*-dioxins (PCDDs) and other polyhalogenated aromatic hydrocarbons (PHAHs), assuming that all co-planar PHAH congeners act through the same dioxin-like Ah receptor based mechanism of action, and that the effects of individual compounds are additive[1,2]. There is some debate on the role of the Ah receptor and thus the predictive value of the TEF concept for carcinogenicity. A tumour promotion potency of several non-dioxin-like di-*ortho* PCBs has been reported as well as several non-additive effects by co-exposure of PCB congeners.

The overall goal of our project is to investigate the tumour promotion potential of complex mixtures of dioxins and PCBs relevant for human exposure and to validate the usefulness of the TEF approach, using an altered hepatic foci model in female rats[3]. In a first experiment the tumour promotion potential of a defined mixture of PCDD, PCDF and PCB congeners representing over 90% of the total TCDD toxic equivalence (TEQ) in fish oil was tested. It was shown that the tumour promotion potential of the mixture was lower than expected on the basis of the TEF concept and that these findings probably had a toxicokinetic basis[4]. In this paper data are presented on the tumour promotion potential of the non-dioxin-like (2-4 *ortho*) and the dioxin-like (0-1 *ortho*) PCB fraction, isolated from the commercial mixture Aroclor 1260.

**Materials and Methods**

*Chemicals* The commercial PCB mixture Aroclor 1260 was fractionated into a 0-1 *ortho*, dioxin-like fraction (~3.1% of the total mass), a 1-2 *ortho* fraction (~6.6% of the total mass) and a 2-4 *ortho* non-dioxin-like fraction (~90% of the total mass) of PCBs using charcoal columns according to a method described by Athanasiadou *et al.*[5] with slight modifications. For the animal experiment the 0-1 *ortho* and the 1-2 *ortho* PCB fractions were combined and dissolved in corn oil. A small amount of the fractions was dissolved in DMSO to test the dioxin like potency of the fractions in the AhR-dependent H4IIE-Luc reporter gene assay (CALUX) as described by Murk *et al.*[6,7].

*Animal experiment* For the tumour promotion study female Sprague Dawley rats (Møllegaard Breeding Centre Ltd., Denmark) were initiated by partial ($^2/_3$) hepatectomy followed by a diethylnitrosamine injection (i.p. 30 mg/kg bw) 24 hours after hepatectomy. After 6 weeks of recovery the promotion treatment was started by giving a loading dose of the experimental compound which was 5 times the maintaining dose given subsequently for the following 19 weeks. The animals were sacrificed one week after the last exposure. The PCB fractions were tested separately and combined (n=10) in concentrations as indicated in table 1. A corn oil (n=18) and a TCDD group (n=10) were incorporated as a negative and positive control respectively.

*Foci analysis* Liver slices were stained for glutathione-p positive foci as described by Haag-Grönlund *et al.*[8]. Foci were analysed with an image analyser as described by Flodström *et al.*[9] whereby the smallest group of GST-p positive cells scored as a focus had a radius of 35 µm (cut off limit).

*Statistics* All data were statistically analysed using SPSS 7.5. Data were tested on normality and homogenicity and log transformed if necessary. Statistical differences were tested with an ANOVA followed by a Tukey test.

**Table 1** PHAH mixtures used in the tumour promotion study

| Fraction | given dose (per/kg bw/week) | equivalent to Aroclor amount | equivalent to TCDD ($TEQ_{CALUX}$) |
|---|---|---|---|
| Corn oil | 1 ml | - | no activity |
| 2,3,7,8-TCDD | 1 µg | - | 1 µg |
| 0-2 *ortho* PCBs | 1 mg | 10 mg | 1.1 ng |
| 2-4 *ortho* PCBs | 1 mg | 1.1 mg | no activity |
| | 3 mg | 3.3 mg | no activity |
| | 9 mg | 10 mg | no activity |
| 0-4 *ortho* PCBs | 10 mg | 10 mg | 1.1 ng |
| Aroclor 1260 | 10 mg | 10 mg | 1.3 ng |

**Results and Discussion**

The 2-4 *ortho* fraction tested in the CALUX assay (detection limit 0.5 fmol) didn't show any luciferase activity. This suggests that there are no Ah receptor agonists present in the 2-4 *ortho* PCB fraction or at such low levels that no activity could be measured. The 0-1 *ortho* and the 1-2 *ortho* fraction showed a luciferase activity up to 47% and 10% of the maximum response of 2,3,7,8-TCDD with a total dioxin like potency of 3.1 µg TEQ/g and 0.21 µg TEQ/g respectively. This corresponds with a TEQ based dose for the 0-2 *ortho* fraction of 1.1 ng TEQ/kg bw/week (Table 1) which is only one tenth of the given TCDD dose.

The promotion of GST-p positive foci was enhanced in all PHAH treated groups (Table 2). The increase in the number of foci/cm$^3$ in the PHAH treated groups is probably a result of using a cut off limit. The volume fraction of the liver occupied by foci (VF), which is the most important parameter, is significantly increased in all groups compared to the corn oil group but not in the group treated with the 0-2 *ortho* PCB fraction (Table 2). The 2-4 *ortho* PCB fraction contributed for over 80% to the observed effect on tumour promotion by the 0-4 *ortho* PCB mixture. Although not significant, there is a slight difference between the 0-4 *ortho* PCB fraction and the original, not fractionated, Aroclor 1260 mixture. This is possibly a result of the loss of contaminants present in commercial mixtures like Aroclor, due to the fractionation[5].

**Table 2** Foci induction in female Sprague Dawley rats after 20 weeks exposure

| Fraction (dose/kg bw/week) | | Number of foci (foci/ cm$^3$) | Mean foci volume (mm$^3$ × 10$^3$) | Volume fraction (%) |
|---|---|---|---|---|
| Corn oil | 1 ml | 5132.9 ± 292.3 | 2.3 ± 0.2 | 1.2 ± 0.1 |
| 2,3,7,8-TCDD | 1 µg | 8406.1 ± 663.2[a] | 7.1 ±1.4[a] | 6.3 ±1.5[a] |
| 0-2 *ortho* PCBs | 1 mg | 6568.3 ±778.2 | 2.5 ± 0.3 | 1.6 ± 0.2 |
| 2-4 *ortho* PCBs | 1mg | 7098.5 ± 638.7 | 3.5 ±.04 | 2.5 ± 0.4[a] |
| | 3 mg | 7527.9 ± 673.8 | 3.4 ±0.3 | 2.5 ± 0.3[a] |
| | 9 mg | 7071.2 ± 591.3 | 4.4 ±0.6[a] | 3.1 ± 0.5[a] |
| 0-4 *ortho* PCBs | 10 mg | 8041.2 ± 817.4[a] | 4.5 ± 0.5[a] | 3.7 ± 0.5[a] |
| Aroclor 1260 | 10 mg | 7551.3 ± 706.4 | 6.9 ± 1.5[a] | 4.8 ± 0.8[a] |

Data are expressed as means ± standard error

[a] Significant different from the corn oil group (Tukey $p<0.05$)

Several di-*ortho* PCBs were shown to have a tumour promotion potential [10,11]. Although 2-4 *ortho* chlorinated PCBs are in general considered to be less toxic, their concentration in commercial mixtures and thus the environment is much higher compared to the dioxin like planar PCBs. The results of this study clearly indicate that the 2-4 *ortho* PCB fraction predominantly induced the foci development by the Aroclor 1260 mixture.

**Acknowledgements**
We would like to thank Annelies Landman, Bert Weijers and Gerrit van Tintelen of the centre of small laboratory animals (CKP) for their assistance during the animal experiment. Our collegues from the Toxicology group we would like to thank for their help during the termination of the experiment. This project is funded by the Ministery of Agriculture, Nature Management and Fisheries of the Netherlands.

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