TUMOUR PROMOTION BY COMPLEX MIXTURES OF DIOXIN AND NON-DIOXIN LIKE POLYHALOGENATED AROMATIC HYDROCARBONS

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Introduction

Polychlorinated Biphenyls (PCBs), -dibenzo-p-dioxins (PCDDs), -dibenzofurans (PCDFs) and related polyhalogenated aromatic hydrocarbons (PHAHs) are ubiquitously present in the environment. Human and wildlife exposure almost exclusively occurs to complex mixtures of these classes of compounds as are present in food and environmental matrices. Most toxicology data are however derived from studies performed on single congeners, or simple mixtures of these chemicals. In risk assessment of dioxins and dioxin-like chemicals it is assumed that individual congeners will contribute to the total relative toxic potency in an additive manner. This is also an important assumption in the toxic equivalency factor approach for dioxins and dioxin-like compounds.

In our laboratory we have investigated the integrated effects of complex mixtures of dioxins and dioxin-like compounds, using the tumour promotion model of Pitot (1). In addition, we have investigated the relative contribution of dioxin-like (0-1 ortho) and non-dioxin like (2-4 ortho) PCB congeners to the total tumour promotion potential of PCBs (2,3). In this paper an overview of the findings on toxicokinetics, biochemical parameters and on altered hepatic foci will be presented.

Experimental procedures

PHAH mixtures

The following mixtures of PHAHs were generated and used in two independent in vivo tumour promotion experiments:

Dioxin-like PHAH mixture: a semi-synthetic mixture of the following congeners was established, in a relative concentration ratio as present in Swedish herring. 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, PCB 126, PCB 118, PCB 156 in a weight based ratio of respectively 1, 3.3, 17, 61, 12800 and 1888. This PHAH mixture was tested with or without addition of PCB 153 (rel ratio 20000).

PCB-based mixtures: Aroclor 1260 was chosen as the basis for preparing fractions that contained all dioxin-like PCB congeners: PCB 0-1 ortho-fraction and a fraction containing no dioxin-like PCBs: PCB 2-4 ortho fraction. The Aroclor 1260 was separated in these PCB 0-1 ortho and PCB 2-4 ortho fractions, using the method described by Athanasiadou et al (1991, (4)), with modifications.

The composition and quantity of the individual congeners in the mixtures was checked by GC-ECD and GC-MS analysis at the DLO-National Institute for Fisheries Research (RIVO) laboratory in IJmuiden, The Netherlands.

Animal experiments
The treatment protocol used in these studies was based on the altered hepatic foci (AHF) tumour promotion protocol introduced by Pitot et al. (1978 (1). Young female Sprague Dawley rats were initiated by partial hepatectomy followed by an ip injection with N-nitrosodiethylamine (NDEA). Six weeks after initiation the promotion treatment was started by weekly subcutaneous (sc) injections with test compounds during 20 weeks.

The following dose groups were included:
Experiment I: PHAH mixture: corn oil (vehicle control); 2,3,7,8-TCDD (1 µg/kg bw/week, positive control); PHAH-mixture at a dose level of 2, 1, or 0.5 µgTEQ/kg bw/week.
Experiment 2: PCB mixtures: corn oil (vehicle, negative control); 2,3,7,8-TCDD (1 µg/kg bw/week, positive control); 0-1 ortho fraction (1 mg/kg b.w/week); 2-4 ortho fraction (1, 3 or 9 mg/kg bw/week); 0-4 ortho re-mix fraction (10 mg/kg b.w./week), Aroclor 1260 (10 mg/kg bw/week) and PCB 153 (1 and 9 mg/kg bw/week).
At the end of this 20-week period liver, blood and other organs were collected.

For further information regarding details of these studies, see Van der Plas et al., 1999 (2), 2000(3)).

Sections of parts of the livers were fixed and stained for gluthatione-S-transferase-p (GST-p) positive foci. Foci were analysed using a Leica Aristoplan microscope connected to a Quantimed 570 Image Processing and Analysis system. A total sectional area of approximately 3 cm^2 was analysed for each animal.

Biochemical parameters: EROD, retinoid levels in plasma and liver; thyroid hormone levels in plasma.

Results and Discussion
In both studies no PHAH-related clinical signs of toxicity were observed throughout the studies. In addition, no indications for liver toxicity were found, based on the absence of an increase in plasma aspartate aminotransferase (AST) and alanine aminotransferase (AST) levels, as well as on liver histopathology examination.

Altered hepatic foci
Experiment 1: The mean foci volume as well as the fraction of the liver occupied by GST-P-positive foci (AHF) was significantly increased in all PHAH treatment groups. However, the increase in mean foci volume and volume fraction for the PHAH semi-synthetic mixture (without PCB153) was significantly smaller than in the TEQ equivalent dosed TCDD group. These results suggest an apparent antagonistic interactive effect of congeners in the PHAH mixture used. Possible explanations for the difference in AHF between TCDD and the TEQ-equivalent PHAH mixture are the TEF values applied to calculate the toxic potency of the mixture and secondly by kinetic factors that have a profound effect on the hepatic disposition of the various individual

PHAH congeners in the mixture, resulting in a very different relative composition in the liver as compared to the mixture that was dosed to the animals. In fact, results on relative disposition of congeners in the liver could mostly explain the observed differences between 2,3,7,8-TCDD and the PHAH-mixture. Therefore, overall the conclusion from this experiment is, that the TEF approach predicts the tumour promotion potency for dioxin-like mixtures of PHAHs accurately.

Experiment 2: The 0-1 ortho and especially the 2-4 ortho fraction of PCBs were analysed extensively for composition and occurrence of congeners by GC-ECD and GC-MS. Importantly, no indication could be found for the presence of dioxin-like congeners in the 2-4 ortho fraction (based on both GC-MS and on Ah-receptor reporter gene assay (DR-CALUX) data). Also in this experiment significant increases were observed in mean foci volume and volume fraction in groups treated with 2,3,7,8-TCDDand surprisingly also in the 2-4 ortho (9 mg) fraction but not in the 0-1 ortho fraction at a dose level of 1 mg/kg bw/week. In fact, when comparing the increase in AHF caused by Aroclor 1260 as well as the reconstituted 0-4 ortho mixture, with the 0-1 and the 2-4 ortho fractions, more than 80% of the total AHF effect was contributed by the non-dioxin like 2-4 ortho fraction. This contrasts to our and others expectations, e.g., that the dioxin-like 0-1 ortho fraction would contribute the most to the total AHF response, while the 2-4 ortho fraction was expected not, or only very limited to contribute to the AHF response of PCB mixtures. This study therefore indicates that the tumour promotion potential of non-dioxin like congeners of PCBs is more important than the dioxin-like congeners. This may have some serious implications for the risk assessment of PCB mixtures.

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