Non-additive induction of altered hepatic foci in female Spraque Dawley rats by a mixture of dioxin-like compounds

Simone A. van der Plas, Abraham Brouwer
Department of Toxicology, Agricultural University Wageningen, Tuinlaan 5, 6703 HE Wageningen, The Netherlands.
Marie Haag-Grönlund, Lars Wärngård, Ulf G. Ahlborg*
Institute of Environmental Medicine, Karolinska Institute, Box 210, S-17177 Stockholm, Sweden. * Deceased

1. Introduction

The toxic equivalency factor (TEF) concept has been developed for risk management of complex mixtures of dioxins and PCBs, assuming additive effects of the Ah receptor agonists. There is some debate on the predictive value of the TEF concept for carcinogenicity; both *in vivo* and *in vitro* studies indicate also a tumor promotion potential for several non-planar PCBs [1,2]. Besides, there is some evidence for antagonistic interactions between dioxin-like compounds and several PCB congeners[3].

The overall goal of our project is to investigate the tumor promotion potential of complex mixtures of dioxins and PCBs relevant for human exposure and to validate the usefullness of the TEF approach. In this paper we present data on the tumor promotion potential of a defined mixture of PCDD, PCDF and PCB congeners representing 95% of the total TCDD toxic equivalence in fish oil, using the altered hepatic foci model introduced by Pitot et al (1978)[4].

2. Materials and methods

Test compounds
3,3',4,4',5-Pentachlorinated biphenyl (PCB 126), 2,3',4,4',5-pentachlorinated biphenyl (PCB 118), and 2,3,3',4,4',5-hexachlorinated biphenyl (PCB 156) were kindly provided by Prof. Å. Bergman, Wallenberg laboratory, University of Stockholm, Sweden. 1,2,3,7,8-Pentachlorinated-dibenzo-*p*-dioxin (PeCDD) was from Wellington Laboratories (Canada), 2,3,4,7,8-pentachlorinated-dibenzofuran (PeCDF) was a gift from Prof. S. Safe and 2,3,7,8-tetrachlorinated-dibenzo-*p*-dioxin (TCDD) was from RADIAN CIL, inc.(USA). All compounds had a purity > 99%. For animal exposure all compounds were dissolved in corn oil. The following solutions were used; corn oil as a negative control, TCDD as a positive control and a PCB/dioxin mix (composition shown in table 1). The PCB/dioxin mix covers 95% of the TEQs in Swedish herring.

table 1 Composition of the PCB/dioxin mixture

Congener	Relative level of congeners in PCB/dioxin mix	TEF[5]
TCDD	1	1
PeCDD	3.3	1
PeCDF	17	0.1
PCB 126	61	0.1
PCB 118	12800	0.0001
PCB 156	1888	0.0005

Animal treatment

Female Spraque Dawley rats, 3-4 weeks old, were obtained from Møllegaard Breeding Centre Ltd. (Denmark) and housed under standard conditions (12 hr light/dark cycle, temperature 22°C, humidity 55%). After 3 weeks of acclimatisation the initiation treatment was started by removing $^2/_3$ of the liver (partial hepatectomy; PH) followed by a single injection (i.p.) of 30 mg/kg N-nitrosodiethylamine (NDEA; Fluka) 24 hours after PH. The promotion treatment was started 6 weeks after initiation and the following doses were used: corn oil group (n=18) 1 ml/kg body weight/week, TCDD group (n=12) 1 μg TEQ/kg body weight/week and the PCB/dioxin mix group (n=10) 0.93 μg TEQ/kg body weight/week. The test compounds were administered once a week by subcutaneous injection for 20 weeks. The first injection (loading dose) was 5 times higher then the dose given the following 19 weeks. Body weight, food and water consumption were measured every week. One week after the last exposure the animals were sacrificed and blood plasma, the liver and other organs were collected. Samples taken from the liver were fixed in ice-cold acetone and embedded in paraffin. Sections of this tissue were stained for gluthatione-S-transferase-p (GST-p) positive foci and analysed according to a method described by Flodström et al (1988)[6].

Statistics

Foci data were log transformed to obtain a better normal distribution. To analyse group differences a one-way analysis of variance and the Student-Newman-Keuls multiple range test were performed.

3. Results

There were no significant differences between the groups in body weight, food and water consumption and liver/body weight ratio. The exposure groups had significant higher liver weights compared to the corn oil group (data not shown).

Table 2 represents the data on GST-p positive foci. All animals showed large numbers of foci per cm^3 of the liver with only a small statistical significant difference between the corn oil and the TCDD group. However the average foci volume and the percentage of the liver occupied by GST-p positive foci was much higher in both the PCB/dioxin mix

and the TCDD group compared to the corn oil group. Although nearly equivalent doses were used in the PCB/dioxin mix group and the TCDD group, the average foci volume and the percentage of the liver occupied by GST-p positive foci was significantly lower in the PCB/dioxin mix group.

table 2 Effect of TCDD and PCB/dioxin mix on the number and foci volume in the liver

	number of foci per cm^3 liver		average foci volume in mm^3		volume fraction of the liver occupied by foci in %	
group	mean	se	mean	se	mean	se
Corn oil	3721.79	285.75	2.93	0.36	2.01	0.26
PCB/dioxin mix	4181.94	403.59	4.86$^{\blacktriangledown *}$	0.35	3.76$^{\blacktriangledown *}$	0.44
TCDD	5049.07$^{\blacktriangledown}$	446.24	10.78$^{\blacktriangledown}$	1.90	8.66$^{\blacktriangledown}$	1.42

$^{\blacktriangledown}$ Significantly different from the corn oil group ($p<0.05$)
* Significantly different from the TCDD group ($p<0.05$)

4. Discussion

Although both treatment groups showed a significant increase in average foci volume and percentage of liver occupied by foci, there is a striking difference between the TCDD and the PCB/dioxin group. The TCDD and the PCB/dioxin group did not receive exactly the same dose based on TEF values (respectivily 1 and 0.93 μg TEQ/kg bw/week) but the dissimilarities in the GST-p positive foci between the groups can not be explained by this difference in exposure levels.

One possible explanation could be a different body disposition of the congeners present in the PCB/dioxin mix compared to TCDD. Co-exposure of PCBs, PCDDs and PCDFs can modulate body disposition of congeners. However, mixed dosage of 2,3,4,7,8-PeCDF or PCB 156 and PCB 153 has been reported to increase liver retention of these compounds in C57BL/6J mice after a single injection, compared to animal groups dosed with the individual compounds[8,9]. Subchronic co-exposure to TCDD and PCB 126 did not appreciably alter the retention of either compounds in the liver[7]. These findings do not support the idea that the difference in average foci volume and the percentage of the liver occupied by GST-p positive foci between the PCB/dioxin mix and the TCDD group could be explained by a decreased liver retention of congeners in the PCB/dioxin mix group. The definite role of liver retention will await analysis of individual congener liver concentrations, which is still in progress.

An alternative explanation is that the TEF values used in this experiment, which are based on literature data[5], are not necessarily applicable for tumor promotion studies. 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF and PCB 126 have been demonstrated to be potent tumor promotors[1,7,10], whereas PCB 118 seems to be less potent in vivo[11]. The

toxic equivalency factors calculated on the basis of tumor promotion potential may differ from the values used from literature.

Interactions between PCB congeners is reported several times[3] and could probably be another possibility to explain the observed differences.

In conclusion, there is a significant difference in effect between the TCDD and the PCB/dioxin group which can not be explained by different dose regimes of the animals. Several other factors may play a role, such as a change in body disposition by co-exposure of PCB, PCDF and PCDD congeners and a discrepancy between the actual tumor promotion potential and the TEF values used in this experiment. Also antagonistic interactions within the mixture can not be excluded.

4. Acknowledgements
We would like to thank Maria Faassen-Peters and Gunilla Scheu for their vital help during and after the animal experiment.

5. References

[1] Hemming H., S. Flodström, L. Wärngård, Å. Bergman, T. Kronevi, I. Nordgren and U.G. Ahlborg (1993): Relative tumour promoting activity of three polychlorinated biphenyls in rat liver. European Journal of Pharmacology 248, 163-174.

[2] de Haan L.H.J., S. Halfwerk, S.E.L. Hovens, B. de Roos, J.H. Koeman. and A. Brouwer (1996): Inhibition of intercellular communication and induction of ethoxyresorufin-*O*-deethylase activity by polychlorobipenyls, -dibenzo-*p*-dioxins and -dibenzofurans in mouse hepa1c1c7 cells. Environmental Toxicology and Pharmacology 1, 27-37.

[3] Aarts J.M.J.G., M.S. Denison, M.A. Cox, M.A.C. Schalk, P.M. Garrison, K. Tullis, L.H.J. de Haan and A. Brouwer (1995): Species-specific antagonism of Ah receptor action by 2,2',5,5'-tetrachloro- and 2,2',3,3',4,4'-hexachlorobiphenyl. European Journal of Pharmacology Environmental Toxicology and Pharmacology Section 293, 463-474.

[4] Pitot H.C., L. Barsness and T. Goldsworthy (1978): Biochemical characterisation of stages of hepatocarcinogenesis after a single dose of diethylnitrosamine. Nature 271, 456-457.

[5] Ahlborg U.G., G.C. Becking, L.S. Birnbaum, A. Brouwer, H.J.G.M. Derks, M. Feeley, G. Golor, A. Hanberg, J.C. Larsen, A.K.D. Liem, S.H. Safe, C. Schlatter, F. Waern, M. Younes and E. Yrjänheikki (1994): Toxic equivalency factors for dioxin-like PCBs. Chemosphere 28, 1049-1067.

[6] Flodström S., L. Wärngård, S. Ljungquist and U.G. Ahlborg (1988): Inhibition of metabolic cooperation in vitro and enhancement of enzyme altered foci incidence in rat liver by the pyrethroid insecticide fenvalerate. Archives of Toxicology 61, 218-223.

[7] Hemming H., Y. Bager, S. Flodström, I. Nordgren, T. Kronevi, U.G. Ahlborg and L. Wärngård (1995): Liver tumour promoting activity of 3,4,5,3',4'-pentachlorobiphenyl and its interaction with 2,3,7,8-tetrachlorodibenzo-p-dioxin. European Journal of Pharmacology Environmental Toxicology and Pharmacology Section 4, 241-249.

[8] de Jongh J., F. Wondergem, W. Seinen and M. van den Berg (1993): Toxicokinetic interactions between chlorinated aromatic hydrocarbons in the liver of the C57BL/6J mouse: 1. Polychlorinated biphenyls (PCBs). Archives of Toxicology 67, 453-460.

[9] de Jongh J., R. Nieboer, I. Schröders, W. Seinen and M. van den Berg M. (1993): Toxicokinetic interactions between chlorinated aromatic hydrocarbons in the liver of the C57BL/6J mouse: 2. Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs). Archives of Toxicology 67, 598-604.

[10] Waern F., S. Flodström, L. Busk, T. Kronevi, I. Nordgren and U.G. Ahlborg (1991): Relative tumour promotion activity and toxicity of some polychlorinated dibenzo-p-dioxin- and dibenzofuran congeners in female Sprague-Dawley rats. Pharmacology and Toxicology 694, 450-458.

[11] Haag-Grönlund M., L. Wärngård, S. Flodström, G. Scheu, T. Kronevi, U.G. Ahlborg and R. Fransson-Steen (unpublished results): Promotion of altered hepatic foci by 2,3',4,4',5,-pentachlorobiphenyl in Sprague-Dawley female rats. Fundamental and Applied Toxicology.