INTERACTIVE EFFECTS OF DIFFERENT POLYCHLORINATED BIPHENYLS IN RAT

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Introduction

To facilitate the risk assessment of PCBs and related substances, the toxic equivalency factor (TEF) concept has been introduced. One of the assumptions for inclusion of dioxin-like substances in the TEF scheme is demonstrated additivity between the toxicities of individual congeners (1). Additivity as the only type of interaction for PCB mixtures has, however, been questioned, particularly for mixtures containing *ortho*-substituted PCBs. Synergism has been reported for plasma thyroxin levels, immunotoxic effects, altered hepatic foci, liver porphyrin levels and induction of liver CYP1A activities for combinations of non-planar PCBs and planar PCBs or TCDD (2-6). Antagonism between *ortho*-substituted PCBs and TCDD has also been suggested for induction of hepatic CYP1A and CYP2B, hepatic retinoid levels, immunotoxic effects and teratogenic effects (4, 6-10).

A problem with the majority of the published data is that the traditional study design precludes a proper statistical analysis of the interacting effects. In the present study, we have used a statistical design suitable for identifying chemical interactions to investigate interactive effects between three PCB congeners representing three types of chlorine substitution in the *ortho*-position; the non-*ortho*-substituted 3,3',4,4',5-pentachlorobiphenyl (PCB126), the mono-*ortho*-substituted 2,3,3',4,4'-pentachlorobiphenyl (PCB105) and the di-*ortho*-substituted 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153).

Materials and methods

Female Sprague-Dawley rats, 4-5 weeks old, weighing approximately 120 g and with free access to a standard pellet diet and tap water were observed daily for general appearance, and body weight was recorded once a week. The rats were allowed an acclimatisation of one week prior to treatment. The animals were partially (2/3) hepatectomized and initiated with a single administration of 30 mg NDEA/kg body weight (ip). Five weeks after the initiation procedure, and the promotion treatment started. The test compounds were administered by (sc) injections once a week for 20 weeks. The multivariate analysis is preferably performed with an experimental design in which the exposure variables are changed simultaneously in a systematic way. Three doses were therefore used for each congener in the 15 combinations presented in Table 1.

The doses for individual congeners were selected to correspond to TEQs of 0.0133 (low dose), 0.0933 (middle dose) and 0.667 (high dose) µg/kg/week. The REPs used for calculations of the TEQs were 0.1 for PCB126, 0.0002 for PCB105 and 0.00006 for PCB153 based on tumour

promotion data (11, 12). Thus, a combination of the highest doses for all three congeners corresponds to a TEQ of 2 µg/kg/week.

TABLE 1
Administered Doses of PCB 126, PCB 105 and PCB 153 (µg/kg/week) to Rats in Various Treatment Groups

Treatment group (PCB 126/105/153)[a]	No. Of animals[b]	PCB 126	PCB105	PCB 153
LLL	5	0.13	66	220
LLH	5	0.13	66	11003
LMM	5	0.13	467	1556
LHL	5	0.13	3302	220
LHH	5	0.13	3302	11003
MLM	5	0.93	66	1556
MML	5	0.93	467	220
MMM	15	0.93	467	1556
MMH	5	0.93	467	11003
MHM	5	0.93	3302	1556
HLL	5	6.6	66	220
HLH	5	6.6	66	11003
HMM	5	6.6	467	1556
HHL	5	6.6	3302	220
HHH	5	6.6	3302	11003

[a] L, low dose; M, medium dose: H, high dose.
[b] For Plasma retinol and all-*trans* retinoic acid six animals were analysed in group MMM and three animals were analysed in remaining groups.

One week after the end of the promotion period, the animals were killed. Liver, thymus, spleen, lungs, heart, kidneys and adipose tissue from the ovaries were removed and the organs were weighed. Samples were taken from the liver as described earlier (13).

Multivariate modelling and analysis were used to extract information from the data. Partial Least Squares (PLS) analysis was used for these calculations. The PLS method (14) is suited for correlation of systematic variation in one data matrix **Y** (dependent variables) to systematic variation in another data matrix **X** (independent variables). PLS modelling consists of simultaneous projections of both **X** and **Y** spaces on low dimensional hyper planes with the purpose to predict **Y** from **X**. PLS simultaneously calculates latent variables for the two matrices and a relation between them. Each model dimension in PLS consists of an **X** score vector, a **Y** score vector, an **X** loading vector, a **Y** loading vector and an **X** weight vector. The latter is computed to achieve maximal correlation between the score vectors.

Results and Discussion

The percentage of the liver occupied by foci was significantly increased (3-8 fold) compared to controls in four dose groups. All these groups were administered high or medium doses of PCB126. The mean foci volume was significantly increased in three of the treatment groups administered the high dose of PCB126. The number of foci/cm^3 of liver was significantly increased in three dose groups compared to controls.

Liver retinoid concentration was significantly decreased (67-75%) in groups which received the high dose of PCB126. Significantly increased renal retinoid concentration (8-13 fold) was observed in all groups treated with the highest dose of PCB126.

The activity of both EROD and PROD was markedly affected. EROD increased up to 116 times and PROD increased up to 33 times compared to controls. The highest EROD levels were observed in groups administered high or medium doses of PCB126. The highest PROD levels were found in groups dosed with high or medium levels of PCB153.

The PLS calculation included 11effect variables, i.e. foci volume fraction (VF), concentrations of retinoids in the liver (LIA) and kidneys (KIA), concentrations of plasma retinol (RET), EROD, PROD, ASAT, GGT, body weight gain (BWG) and relative weights of thymus (RTH) and liver (RLI). The calculation resulted in four significant PLS components explaining some 42% of the total variation in effect. To investigate the relationship between exposure variables and effect variables, the first PLS component was plotted against the second (Figure 1).

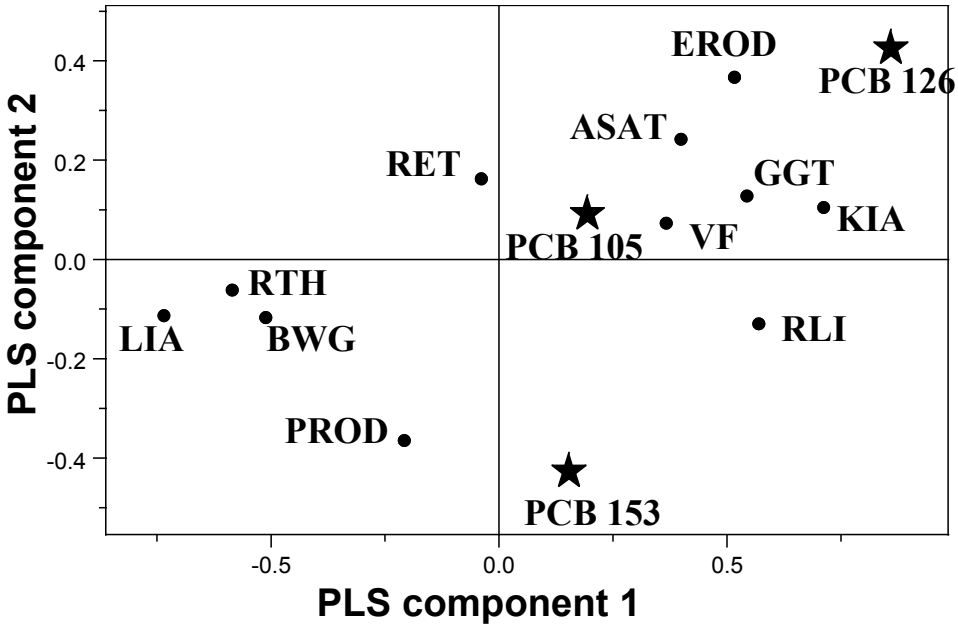

Figure 1. Partial least square (PLS) loading plot for the first and second PLS components. For explanation of abbreviations see text.

The resulting model illustrates the contribution of the variables to the first and second components in this analysis. As can be seen in Figure 1, the exposure variables contribute to the first PLS component with various strengths; PCB126 contributes more than do PCB105 and PCB153 in the studied dose range. Of the total variability in the kidney and liver retinoid concentrations, 59% and 70% respectively could be explained by the model. The kidney retinoid concentration is strongly positively correlated to PCB126 exposure, especially in the first component. For volume fraction,

only 19% of the total variability within data could be explained. The reason for this low degree of explanation is probably that there is an inherent, high interindividual variability for volume fraction of foci.

No synergistic effect was observed between the compounds in the studied dose ranges. Antagonistic effects were most clearly demonstrated between the non-*ortho* substituted PCB126 and the di-*ortho* substituted PCB153. Some antagonistic interactions were also observed between PCB126 and PCB105 exposure, but between the mono-*ortho* substituted PCB105 and the di-*ortho* substituted PCB153, no significant interactions other than additivity could be detected in the dose ranges applied in this study.

A decrease of liver retinoids by individual non-, mono- and di-*ortho* substituted PCB congeners as seen in this study has previously been reported (15-18). No interaction other than pure additivity was observed between PCB105 and PCB126, but an antagonistic effect of PCB153 exposure was found for the decrease in liver retinoid concentration caused by PCB126.

In conclusion, weak antagonistic interactions were observed between PCB126 and PCB153 exposure for effects on hepatic foci, concentrations of plasma retinol and liver retinoids, induction of CYP2B1/2 and relative liver weight. Weak antagonism was also observed between PCB126 and PCB105 exposure for hepatic foci and plasma retinol concentration. Synergism was not detected for any effects in the dose ranges used in this study.

Acknowledgements
We thank Å Bergman and his collaborators for synthesising the PCBs and the National Institute for Working Life for access to their animal facilities. We are also grateful to Ellu Manzoor and Göran Fex for retinoid analysis. The research was supported by the Swedish Environmental Protection Agency, the Swedish Council for Work Life Research and by Karolinska Institutet.

References

1. Ahlborg UG, Hanberg A and Kenne K; Nord 1992:26, Nordic Council of Ministers, **1992**, ISBN 9291200751
2. Bannister R and Safe S; *Toxicology* **1987***,* 44, 159-169

3. Sargent L *et al; Carcinogenesis* **1991**, 12(5), 793-800
4. van Birgelen, A P J M *et al; Toxicol. Appl. Pharmacol*. **1994**, 126**,** 202-213
5. van Birgelen A P J M *et al; Environ. Health Perspect*. **1996**, 104 (5), 550-557
6. Bager Y *et al; Pharmacol. Toxicol*. **1995**, 77, 149-154
7. Biegel L *et al; Toxicol. Appl. Pharmacol*. **1989**, 97**,** 561-571
8. Morrissey R E *et al; Toxicol. Lett*. **1992**, 60, 19-25
9. van Birgelen, A P J M *et al; Chemosphere* **1992**, 25(7-10), 1239-1244
10. van der Kolk J *et al; Chemosphere* **1992**, 25(12), 2023-2027
11. Hemming *et al; Eur. J. Pharmacol. Environ. Tox. Pharm. Section.* **1993***,* 248, 163-174
12. Hemming *et al; Eur. J. Pharmaco. Environ. Tox. Pharm. Section.* **1995**, 292, 241-249
13. Flodström S *et al; Arch. Toxicol*. **1988**, 61, 218-223
14. Wold S *et al;* Multivariate data analysis in chemistry. in *Chemometrics: Mathematics and Statistics in Chemistry*, **1984**, NATO ASI Series C138, D. Reidel, Dordrecht, The Netherlands.
15. Chen L-C *et al; Toxicol. Appl. Pharmacol*. **1992**, 114, 47-55
16. Chu I *et al; Fundam. Appl. Toxicol*. **1995**, 22**,** 457-468
17. Chu I *et al; Fundam. Appl. Toxicol*. **1994**, 26**,** 282-292
18. Chu I *et al; J. Appl. Toxicol.* **1996**, 16(2), 121-128