Toxicological interactions between 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF in rat.

Niklas Johansson[1,2], Fredrik Wærn[1], Christina Trossvik[1], Ellu Manzoor[1], Ulf G. Ahlborg[1], and Helen Håkansson[1].

[1] Institute of Environmental Medicine, Karolinska Institutet, Box 210, S-171 77 Stockholm, Sweden
[2] Swedish Environmental Protection Agency, S-106 48 Stockholm, Sweden

1. Introduction

In order to extend the risk assessment for TCDD to include PCDDs and PCDFs and to express the total toxic effect of a mixture as a single number, the Toxic Equivalency Factor (TEF) concept has been developed[1,2,3]. One prerequisite for this model is that the contribution from all congeners is strictly additive and that there are no antagonistic or synergistic effects. Only a few *in vivo* studies have been made to investigate such interactions.

2. Material and methods

The objective of the study was to investigate possible additive, antagonistic and/or synergistic effects between different dioxins. Interactions between substances are preferably studied in systems where the concentrations of the substances in question are varied simultaneously and in a systematic way. This approach requires a statistical experimental design. Several protocols to handle such situations have been developed. These comprise factorial designs, fractional factorial designs and composite designs. For this study, we chosed a central composite face centred (CCF) design[4,5]. This design is used to develop elaborate models consisting of linear and cross-terms and also quadratic terms which makes it possible to detect nonlinear interactions, if present.

***Table 1**. The 15 different mixtures of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF given to each group (µg/kg). The control group received the vehicle (corn oil) only. The administration was made by gavage within 24 hours post partus.*

Group no	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
TCDD	0	0.1	0.1	0.58	0.58	0.58	0.1	0.1	3.33	0.58	0.58	3.33	0.1	3.33	3.33	3.33
PeCDD	0	0.2	1.16	1.16	0.2	1.16	0.2	6.67	0.2	1.16	6.67	1.16	6.67	0.2	6.67	6.67
PeCDF	0	0.2	1.16	0.2	1.16	1.16	6.67	0.2	0.2	6.67	1.16	1.16	6.67	6.67	0.2	6.67
TEQ	0	0.3	1.3	1.3	1.3	1.7	3.5	3.5	3.5	4.5	4.5	4.5	6.8	6.8	6.8	10

The dosages, based on the statistical experimental design mentioned above is given in table 1. Data were collected for a number of endpoints, including body and organ weights, hepatic CYP1A1-induction and vitamin A levels.

The statistical analysis included Principal Component Analysis (PCA) and Partial Least Squares (PLS), as included in the statistical packages Modde and Simca-S (Umetri AB, Umeå, Sweden). PCA is a multivariate data analytical method designed to highlight the systematic variation in a data matrix[6]. PCA combines the variables included to a few underlying dimensions thus summarizing the systematic information present in the data matrix. The PLS method[6)] is suited for correlation of systematic variation in one data matrix Y (dependent variables) to systematic variation in an other 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.

3. Results and discussion

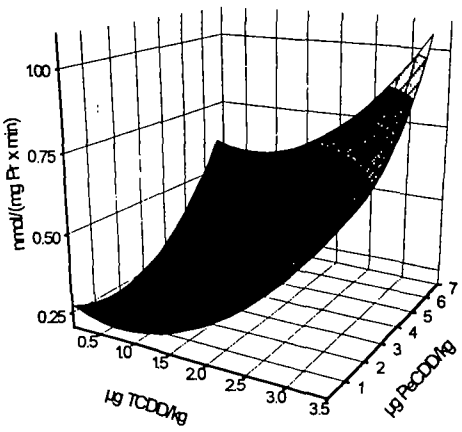
Clear effects were seen in both dams and their offspring. The responses were more pronounced in the offspring compared to the adult rat. Increased renal vitamin A and increased hepatic EROD-activity showed the strongest correlation with the exposure variables in both the dams and the offspring. The following results are a few examples selected to illustrate the study:

PeCDD was found to have between 50 and 90 percent of the effect found for TCDD in the dams with respect to hepatic EROD induction and renal vitamin A, while PeCDF had between 20 and 60 percent of the effect of TCDD. The coefficients (scaled and centred) are given in table 2. The coefficient represents the effect that the individual congener has on the statistical model. The quotients gives the relative potency of the congeners compared with TCDD. The relative potency of PeCDD for the two strongest variables, EROD and renal vitamin A, was in the range of 0.9 to 0.5 and for PeCDF is the corresponding numbers 0.6 and 0.2.

Table 2. *Scaled and centred coefficients and their relationships of the PLS-model for the 33 dams. The coefficients represents the effect of the individual congeners on the statistical model. The quotients then gives the relative potency of PeCDD and PeCDF compared with TCDD on the variables.*

	Relative liver weight	Relative thymus weight	Hepatic vitamin A conc.	Renal vitamin A conc.	Pulmonary vitamin A conc.	EROD-activity
Coefficient for TCDD	0.07	-0.27	-0.15	0.33	-0.22	0.24
Coefficient for PeCDD	0.18	-0.03	-0.13	0.15	-0.05	0.21
Coefficient for PeCDF	0.14	0.01	-0.09	0.08	-0.01	0.14
PeCDD/TCDD-quotient	2.5	0.1	0.9	0.5	0.2	0.9
PeCDF/TCDD-quotient	2.0	-0.05	0.6	0.2	0.04	0.6
"Predictability"	0.19	0.25	0.25	0.72	0.19	0.73

(a) EROD-activity



(b) Renal vitamin A concentration

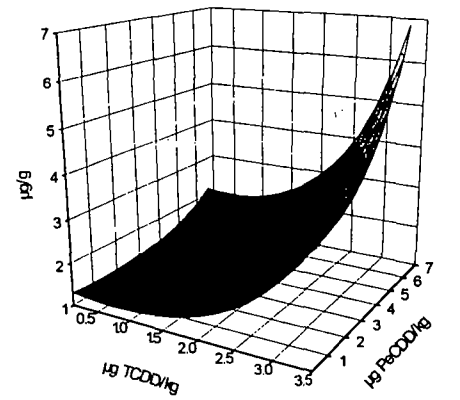


Figure 1. *Response surfaces generated with the final model for the dams. The surfaces illustrates the effect on hepatic EROD-induction (a) and renal vitamin A (b) as a result of combined exposure to TCDD and PeCDD. The PeCDF is set to zero*

PeCDF gave minor responses in the offspring compared with the effects of TCDD while the potency of PeCDD was about 50 to 60 percent compared to TCDD.

Interactive effects, apart from pure additivity, were of limited importance. The data also indicate that the relative potency of TCDD, PeCDD and PeCDF to induce hepatic EROD activity and increase the renal vitamin A concentration is dose-dependent.

Nonlinear relationships between exposure and response variables were detected. To investigate these further, a response surface was generated by the model. Figure 1a and 1b illustrate the combined response of the strongest exposure variables (TCDD and PeCDD) on the two best correlated exposure variables, EROD induction and renal vitamin A concentration, respectively, when the PeCDF is set to zero. The surface for EROD induction (Figure 1a) is not dramatically curved, but a non linear relationship, seen as a curvature, is clearly distinguished and the combined effect is close to additive. The shape of the surface for renal vitamin A (Figure 1b) is similar to that for EROD. PeCDD and PeCDF were, however, less potent in affecting renal vitamin A than inducing the EROD activity. TCDD in combination with PeCDD reveals a clear supra-additive interaction. On the other hand, the combined effect of the two penta substituted congeners was sub-additive.

To verify these findings, the effect on EROD-induction of different combinations of dioxins and PCBs have been investigated in cell cultures. The results from these studies show similarities with the *in vivo* studies. However there are a number of striking differences that might have toxicokinetical explanations. Results from these *in vitro* studies also support that the relative potency of different dioxins and PCBs (compared to TCDD) to induce EROD activity is dose-dependent. Before efforts are made to set dose-dependent TEFs, the importance of this dependency within commonly found exposure ranges must be investigated.

4. References

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