PCDD/F, PCB AND ORGANOCHLORINE PESTICIDES IN HUMAN BLOOD OF PREGNANT WOMEN FROM GERMANY

Wittsiepe J [1], Schrey P [1], Lemm F [1], Eberwein G [2], and Wilhelm M [1]

[1] Department of Hygiene, Social and Environmental Medicine, Ruhr-University Bochum, Universitätsstraße 150, D-44801 Bochum, Germany

[2] North Rhine-Westphalia State Environment Agency, Wallneyer Straße 6, D-45133 Essen, Germany

Introduction

In 2000 we initiated the Duisburg birth cohort study to investigate the influence of pre- and postnatal exposure to Polychlorinated Dibenzo-p-dioxins and Dibenzofurans (PCDD/F) and Polychlorinated Biphenyls (PCB) on neurodevelopment of infants. The first results on the PCDD/F and PCB levels in blood and human milk of mothers, from who both blood and milk samples were available, have been published recently [1, 2].
Here we report on PCDD/F and PCB levels in blood of all mothers participating in the study. Additionally results on organochlorine pesticides in blood and more detailed analysis of influencing factors on levels of persistant organic pollutants (POP) in blood are given. In detail the POPs included in the present study compose of PCDD/F, non-ortho, mono-ortho and indicator PCB (#138, #153 and #180), Hexachlorobenzene (HCB), α-, β-, and γ-Hexachlorocyclohexane (HCH), 4,4'-Dichlorodiphenyltrichlorethane (DDT) and 4,4'-Dichlorodiphenyldichloroethene (DDE).

Methods and Materials

Study group and Sampling

Participants were 226 pregnant women aged between 19 and 41 years (median: 31, mean 30.8) living in an industrialized area of Germany. Blood samples (50 ml) were in most cases taken in the 32nd week of pregnancy, the total sampling period was September 2000 to November 2002. For survey of personal data, residential and nursing behaviour and other relevant data, the women were interviewed using standardized questionnaires.The participation in the study was voluntary. The evaluation was carried out with anonymous personal data. The examination program was approved by the ethics committee of the Ruhr-University Bochum in May 2000 (registry No. 1478).
Most of the subjects were of german (193), 21 mothers were of turkish, 7 of east european or asian, 1 of african and 3 of other origin. In view of exposure to POPs it is significant, that 189 of them (83.6 %) have never lived outside West-Germany and 194 (85.8 %) never outside Western Europe for more than 3 months.

Analysis

Extraction

Homogenized whole blood (50 ml) was diluted with deionized water (50 ml) and shaken for 30 min. Extraction procedure was as follows: addition of 50 ml aqueous saturated ammonium sulfate solution, shaking for 1 min, addition of 50 ml absolute ethanol, shaking for 1 min, twofold extraction with 100 ml of hexane. The hexane layer was dried with anhydrous sodium sulfate and evaporated at 40 °C under vacuum to constant weight. The residue (about 250 mg), which represented the fat content, was weighed, redissolved in hexane and divided into two aliquots of 90 % for determination of PCDD/F and non-ortho PCB (aliquot A) and 10 % for determination of mono-ortho and indicator PCB, HCB, HCH and DDT/DDE (aliquot B).

Sample clean up

Aliquot A was fortified with 17 $^{13}C_{12}$-labelled 2378-chlorosubstituted PCDD/F congeners and 4 $^{13}C_{12}$-labelled non-ortho PCB congeners (#77, 81, 126 and 169) each at concentrations of 25 or 50 pg/sample. Clean up was performed by standard methods using modified silicagels, alumina and activated charcoal. After addition of 2 µl of dodecane, the final sample extract was evaporated under a nitrogen stream to dryness and redissolved by addition of 10 µl of toluene, containing 25 pg $^{13}C_{12}$-1234-TCDD as external standard.
Aliquot B was spiked with 8 $^{13}C_{12}$-labelled mono-ortho (#105, 114, 118, 123, 156, 157, 167, 189) and 6 $^{13}C_{12}$-labelled indicator (#28, 52, 101, 138, 153, 180) PCB congeners each at concentrations of 0.50, 1.25 or 2.5 ng/sample, $^{13}C_6$-HCB, $^{13}C_6$-α-, β-, and γ-HCH and $^{13}C_{12}$-4,4'-DDT at concentrations of 1.0 ng/sample and with $^{13}C_{12}$-4,4'-DDE at concentrations of 8.0 ng/sample. Clean up was carried out by column chromatography using

Florisil. At the end 0.50 ng $^{13}C_{12}$-labelled PCB 47 and 20 µl of dodecane were added. The extract was evaporated under a nitrogen stream to a final volume of 20 µl.

HRGC/HRMS analysis

The analytical instrument system consisted of a VG AutoSpec high-resolution mass spectrometer and a Hewlett Packard 5890 series II gas chromatograph equipped with a Gerstel KAS 2 vaporization system and a J&W Scientific DB-5MS GC column of 60 m length and 0.1 µm film thickness. Both cleaned extracts (aliquot A injection volume 4 µl; aliquot B 1 µl) were measured in single ion recording mode with 5 or 4 mass optimized functions at a resolution of 8,000 - 10,000 at 10 %. Each two mass fragments were recorded for labelled and native congeners.

Statistical methods

All statistical calculations were performed with the statistics package STATISTICA (data analysis software system), version 7.1, StatSoft, Inc. (2005).

Results and Discussion

Description

Descriptive statistical data on blood levels of PCDD/F and PCB calculated as WHO-TEq-values on lipid base and on levels of indicator PCB and organochlorine pesticides on whole blood volume base are given in Table 1. Levels found are in general in good agreement with literature data on PCDD/F, dioxin-like PCB, indicator PCB or organochlorine pesiticides human biomonitoring data from Germany (mainly milk) with regard to the sampling years and the age range of the participants [3-7]. Except for two women levels of indicator PCB were in the range of German reference values [8] (Figure 4 a)- c)). For HCB (Figure 4 d)), β-HCH (Figure 4 e)) and 4,4'-DDE (Figure 4 f)) 0, 12 or 8 exceedings of reference values were found, respectively.

Table 1: Descriptive statistical data on levels of PCDD/F, PCB and organochlorine pesticides in human blood of pregnant women from Germany (N=226, study period 09/2000 to 11/2002)

Parameter	Minimum	5. Perc.	Median	95. Perc.	Maximum	Arithmetic mean
[pg WHO-TEq/g lipid base]						
PCDD/F	2.73	6.89	15.38	31.72	55.07	16.76
non-ortho PCB	0.56	1.48	4.23	10.08	21.47	4.99
mono-ortho PCB	0.41	1.62	5.58	12.45	32.27	6.20
PCB	1.40	3.71	10.34	21.82	42.23	11.19
PCDD/F+PCB	4.34	10.21	25.96	51.95	97.30	27.95
[µg/l whole blood]						
PCB #138	0.0077	0.056	0.18	0.39	0.91	0.199
PCB #153	0.016	0.099	0.32	0.76	2.4	0.366
PCB #180	0.011	0.076	0.29	0.76	5.1	0.370
HCB	0.036	0.062	0.15	0.32	0.53	0.163
α-HCH	0.00009	0.00029	0.00095	0.0045	0.036	0.00162
β-HCH	0.0045	0.026	0.067	0.33	1.3	0.110
γ-HCH	0.00022	0.00080	0.0016	0.0049	0.037	0.00231
4,4'-DDT	0.00060	0.0019	0.018	0.052	0.52	0.0291
4,4'-DDE	0.10	0.19	0.54	2.0	9.1	0.785

Parameters of influence

As known from our own studies and literature data blood levels of PCDD/F, PCB and organochlorine pesticides depend on several parameters. Frequently discussed or confirmed parameters are age, time of examination, number of previous births and nursing behaviour of previous birth childs, smoking, body-mass-index, decrease or increase of weight, migration history, diet, having self been breastfed, occupational exposure and environmental exposure in context with the residential area.

To show several influencing parameters we used a multiple regression model including an intercept. Within the model the logarithmic (ln) transformed values of the POP concentrations in blood on lipid or whole blood vol-

ume basis were used as dependent variables to ensure a normal distribution. Continuous predictor variables were age [years], time of examination [month], total lactation period of before breastfed children [month] and body-mass-index before pregnancy [kg/m^2]. The migration history was included in the model as a categorical factor, whether the women had ever lived outside Western Europe for more than 3 month or not.
The number of previous births was found not to describe POP exrection as good as the total lactation period and was therefore excluded. Data on the current diet, like frequency of fish, meat, milk and egg consumption, were included in the model in a first approach, but they showed in most cases no influence and were therefore eliminated in the final model. At the current level of examination exact and reliable data on smoking behaviour in the past, changes of weight, data on diet concerning the whole lifespan, having self been breastfed as a child and environmental exposure were not available or the number of cases was too low. An occupational exposure could be excluded on the basis of the questionaire.

The results of the multiple regression model are shown in Table 2. Together with simple regression models they can be summarized as follows:

Age

Blood levels of most PCDD/F and PCB congeners, of the calculated WHO-TEq values and of HCB (see Figure 1 and Figure 4 a) - d)) increase with age. Also the scatter widths increases with age. For HCH and DDT/DDE this was not observed (Figure 4 e) and f)). Within the multiple regression model β-HCH and DDT show a low increase with age.

Time trend

Since beginning of routine human biomonitoring of PCDD/F and PCB in Germany in the mid of the 1980th a coninuous decreasing time trend has been observed until the late 1990th. As known from other studies this clearly trend might now have stopped. Using the multiple regression model we observed only a low decrease during the study period (2000-2002).

Total lactation period

Breast feeding can be seen as a detoxification mechanism of the mother for many lipophilic persistent organochlorines. Over the summarized total lactation period a clearly decrease of human blood levels can be observed. This is statistical significant in the model for all above mentioned POP concentrations, except of α- and γ-HCH. The dependence of PCDD/F and PCB blood levels on age and on the summarzized total lactation preiod is shown in Figure 2.

Body-Mass-Index

In general no dependence of POP levels on body-mass-index was found within the multiple regression model, except of a low positive effect for non-ortho PCB WHO-TEq levels, HCB and β-HCH.

Migration history

Human exposure to PCDD/F and PCB is in most high industrialized countries higher in comparison to lower industrialized ones. In return for, exposure to organochlorine pesticides is often higher in less developed countries, where these chemicals are still in use. Within the regression model this is reflected by the influence of the migration history on the human POP blood levels. Women who had ever lived outside Western Europe show lower PCDD/F and PCB blood levels and higher HCH (except of γ-HCH, which has a very short half-life) and DDT/DDE levels in comparison to women who had never lived outside Western Europe. Figure 3 shows this dependence for PCDD/F + PCB and β-HCH.

Conclusion

Besides age, as shown in our pilot study on PCDD/F at the beginning of the 1990th [9, 10], the total lactation period and the time trend are the main parameters influencing levels of organochlorine POPs in human blood samples of pregnant women from Germany. In special cases the migration history can also be a major influencing parameter. For assessment of human biomonitoring data these parameters have to be considered. Reference values should be considered and if necessary adapted regularly.

Acknowledgements

The Duisburg birth cohort study was financially supported by the Ministry of environment and conservation, agriculture and consumer protection of the state of North Rhine-Westphalia, Germany, and coordinated by the North Rhine-Westphalia State Environment Agency, Germany. The technical assistance of Heidemarie Joppich, Kerstin Schmitz, Bibiane Erlenkämper and Jochen Scheld is greatfully acknowledged.

a) PCDD/F b) PCB

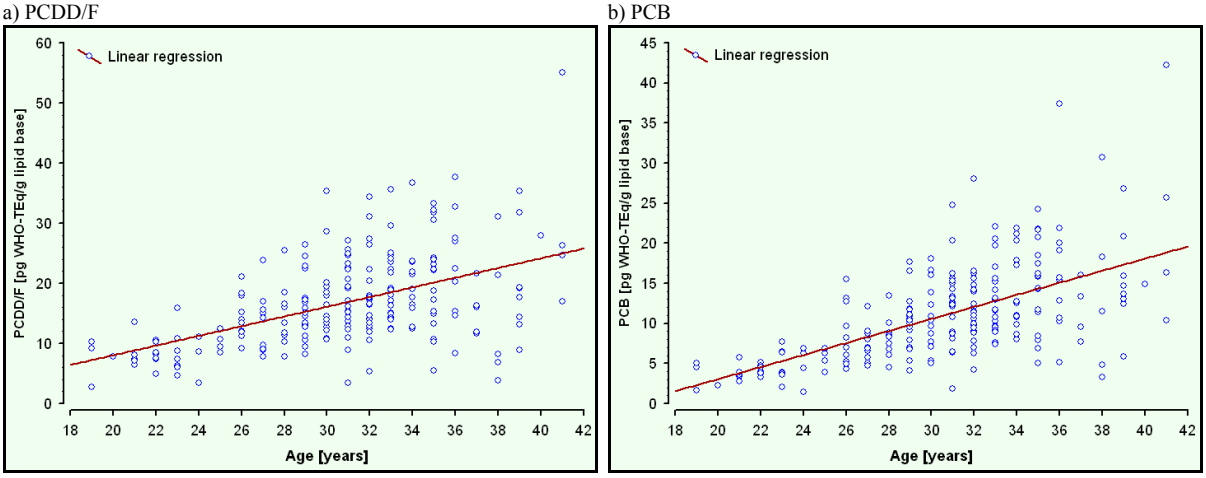

Figure 1: Dependence of levels of PCDD/F and PCB in human blood [pg WHO-TEq/g lipid base] on age: a) PCDD/F, b) PCB

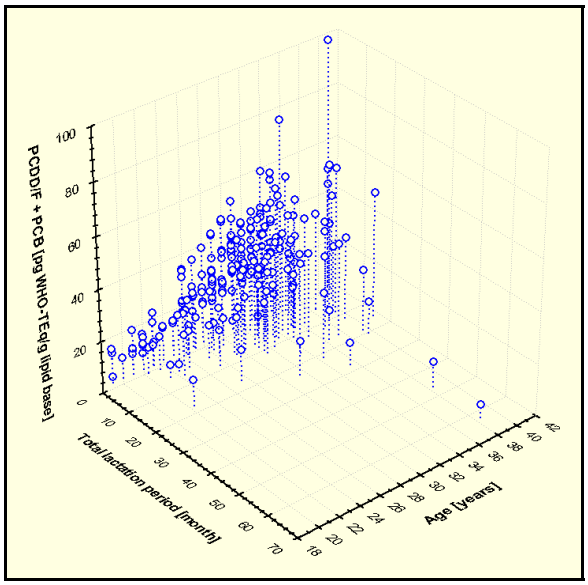

Figure 2: Dependence of levels of PCDD/F and PCB in human blood [pg WHO-TEq/g lipid base] on age and total lactation period

a) PCDD/F + PCB b) β-HCH

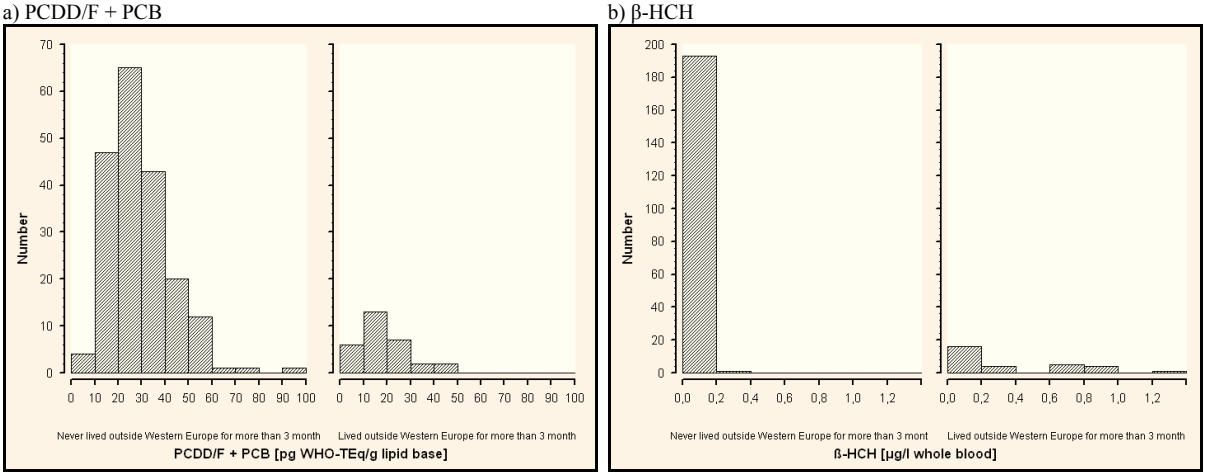

Figure 3: Dependence of levels of PCDD/F + PCB [pg WHO-TEq/g lipid base] and β-HCH [µg/l whole blood] in human blood on migration history: a) PCDD/F + PCB, b) β-HCH

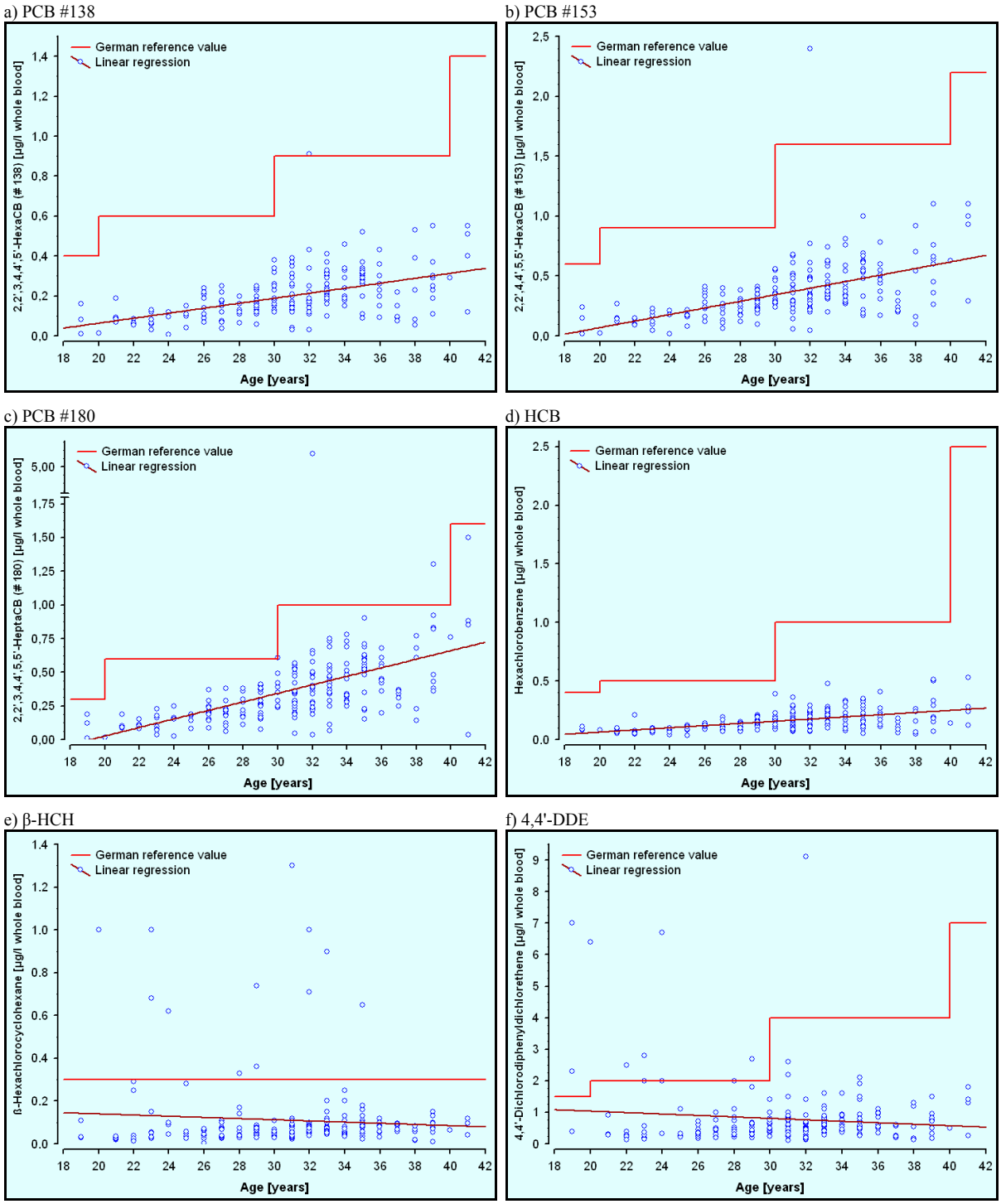

Figure 4: Dependence of levels of persistent organic pollutants in human blood [µg/l whole blood] on age and comparison with German reference values [8]:
a) PCB #138, b) PCB #153, c) PCB #180, d) HCB, e) β-HCH, f) 4,4'-DDE

Table 2: T-values of multiple regression on the influence of age, time of examination, total lactation period, body-mass-index and residential habits on levels of PCDD/F and PCB [lipid base] and indicator PCB and organochlorine pesticdes [whole blood volume base] in human blood

Parameter	Intercept	Age [years]	Time of examination [month]	Total lactation period [month]	Body-Mass-Index before pregnancy [kg/m^2]	Living outside Western Europe *) [categorical]	Multiple R
log nat. concentration [pg WHO-TEq/g lipid base]							
PCDD/F	**4.35**	**11.06**	**-2.94**	**-6.70**	1.01	**-3.55**	**0.6768**
non-ortho PCB	**-3.43**	**8.64**	-0.93	**-2.49**	**2.69**	-1.60	**0.5531**
mono-ortho PCB	**-5.14**	**15.58**	**-2.15**	**-7.10**	-1.07	**-5.50**	**0.7846**
PCB	-1.56	**14.17**	**-1.98**	**-5.72**	0.78	**-3.66**	**0.7377**
PCDD/F+PCB	**5.42**	**12.95**	**-2.71**	**-6.64**	1.01	**-3.62**	**0.7175**
log nat. concentration [µg/l whole blood]							
PCB #138	**-16.39**	**11.37**	-0.43	**-6.22**	0.26	**-5.28**	**0.7003**
PCB #153	**-15.79**	**14.05**	-1.07	**-6.36**	-0.95	**-5.25**	**0.7542**
PCB #180	**-14.16**	**13.11**	-1.16	**-4.17**	-1.49	**-4.34**	**0.7224**
HCB	**-19.87**	**11.05**	-0.56	**-5.47**	**3.92**	-1.39	**0.6495**
α-HCH	**-13.61**	-0.90	**-2.01**	0.33	1.35	**3.68**	**0.3171**
β-HCH	**-11.25**	**4.91**	-0.59	**-3.77**	**2.79**	**8.80**	**0.5635**
γ-HCH	**-19.56**	1.14	1.38	1.00	0.69	0.71	0.1703
4,4'-DDT	**-4.09**	**4.34**	-0.65	**-3.78**	0.52	**7.89**	**0.5037**
4,4'-DDE	**-10.16**	1.39	0.62	**-2.41**	**3.22**	**4.50**	**0.3823**

*) In comparison to women who had never lived outside Western Europe for more than 3 month.
Statistical significant ($p < 0.05$) parameters of regression are marked bold.

References

1. Wittsiepe J, Fürst P, Schrey P, Lemm F, Kraft M, Eberwein G, Winneke G, Wilhelm M *Organohalogen Compd* 2004; 66: 2831
2. Wittsiepe J, Fürst P, Schrey P, Lemm F, Kraft M, Eberwein G, Winneke G, Wilhelm M *Chemosphere* (in press)
3. Wittsiepe J, Schrey P, Ewers U, Selenka F, and Wilhelm M *Chemosphere* 2000; 40:1103
4. Päpke O *Environ Health Perspect* 1998; 106 Suppl. 2, 723
5. Wuthe J, Piechotowski I, Päpke O, Zier B, Gabrio T, Krämer D, Kouros B, Schwenk M, and Pfaff G *Chemosphere* 1996; 32, 567
6. Fürst P and Päpke O *Organohalogen Compd* 2002; 55, 251
7. Fürst P, In: Deutsche Gesellschaft für Ernährung *Ernährungsbericht 2004* 2004, 155
8. Wilhelm M, Ewers U, Schulz C *Int J Hyg Environ Health* 2003; 206: 223
9. Schrey P, Wittsiepe J, Ewers U, Exner M, Selenka F *Organohalogen Compd* 1992, 9: 261
10. Schrey P, Wittsiepe J, Ewers U, Exner M, Selenka F *Bundesgesundhbl* 1993; 36: 455