# PCDD/F and dioxin-like PCB in human blood and milk from German mothers

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## Introduction

Human biomonitoring of polychlorinated dibenzo-p-dioxins and dibenzofuranes (PCDD/F) and polychlorinated biphenyls (PCB) is done by analyzing both blood and milk samples. With reference to calculation of Toxicity Equivalents (TEq) as published by the World Health Organization (WHO) in 1998 [1] determination of 17 PCDD/F congeners together with 4 non- and 8 mono-ortho PCB congeners is the preferred method.
In contrast to data on PCDD/F only little is known on background levels of dioxin-like PCB in human blood or milk samples. In the present study we report on PCDD/F and PCB levels in human blood samples of pregnant women living in an industrialized area of Germany and of human milk samples from the same women taken in the first weeks after birth. The investigations demonstrate the current background levels found in Germany, make a contribution for the assessment of pre- and postnatal exposure of infants and show correlations between the two matrices.

## Methods and Materials

### *Study group*

Participants were 169 pregnant women aged between 19 and 42 years at time of birth living in an industrialized area of Germany. Most of the subjects were german (142), 18 mothers were of turkish, 6 of east european or asian, 1 of african and 2 of other origin. In view of exposition to PCDD/F, PCB and other persistant organic pollutants it is significant, that 135 of them (79.9 %) have never lived outside West-Germany for more than 3 months and 140 (82.8 %) never outside West-Europe.

***Sampling***

It was planned to take the blood samples (about 50 ml) in the 32nd week of pregnancy. However in 11 cases blood samples were taken after birth. Sampling of milk (about 150 ml) was planned for the 2nd week after birth. This was achieved for more than the half of all cases, in other cases either sampling period was later or took longer. All in all, blood and milk samples were taken between September 2000 and January 2003.

***Analysis***

Blood samples were analyzed at the Department of Hygiene of the Ruhr-University Bochum, Germany (RUB) and milk samples at the Chemical and Veterinary Control Laboratory, Münster, Germany (CVUA), both with their established methods.

***Analysis of human blood samples at RUB***

*Extraction*

Homogenized whole blood (50 ml) is diluted with deionized water (50 ml) and shaken overhead for 30 min. Extraction procedure is as follows: addition of 50 ml aqueous saturated ammonium sulfate solution, shaking for 1 min, addition of 50 ml of absolute ethanol, shaking for 1 min, twofold extraction with 100 ml of hexane. The hexane layer is dried with anhydrous sodium sulfate and evaporated at 40 °C under vacuum to constant weight. The residue (about 250 mg), which represents the fat content, is 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 (aliquot B).

*Sample clean up*

Aliquot A is fortified with 17 $^{13}C_{12}$-labelled 2378-chlorosubstituted PCDD/F congeners (25 or 50 pg/sample) 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 is 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 reconstituted by addition of 10 µl of toluene, containing 25 pg $^{13}C_{12}$-1234-TCDD as external standard.

Aliquot B is 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. Clean up is done by column chromatography using Florisil. At the end 0.50 ng $^{13}C_{12}$-labelled PCB 47 and 20 µl of dodecane are added. The extract is evaporated under a nitrogen stream to a final volume of 20 µl.

*HRGC/HRMS analysis*

The analytical instrument system consists 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) are 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 are recorded for labelled and native congeners.

Blood analyses are performed in series of 4 or 5 samples and one blank. The standard deviation of the method is lower than 10 % for most congeners and up to 25 % for congener concentrations near the detection limit. The recovery rate is typically in the range of 70 - 95 % and the detection limits (S:N = 3:1) are < 1 pg/g on lipid basis. Samples of pooled human blood are regularly analyzed for internal quality control.

***Analysis of human milk samples at CVUA***

*Extraction*

The extraction of lipophilic persistent halogenated pollutants along with fat starting with at least 50 ml of milk is performed after addition of potassium oxalate with ethanol, ether and pentane. The extract is washed with water and dried over sodium sulphate. After careful solvent evaporation, gravimetric lipid determination is performed.

*Determination of PCDD/F and non-ortho PCB*

An aliquot of approx. 2 g of fat is spiked with 17 $^{13}C_{12}$-labelled PCDD/F each at a concentration of 25 pg/sample and 3 $^{13}C_{12}$-labelled non-ortho PCB standards each at a concentration of 100 pg/sample. Fat and non stable compounds are removed on a silica gel column coated with sulphuric acid. The hexane eluate containing the PCDD/F and PCB is carefully reduced by rotary evaporation and applied to a florisil column to separate the PCB from PCDD/F.
While the PCB fraction is further cleaned up on a Chromosorb WHP/Charcoal SP-1 column, the PCDD/F elute is applied to a Carbopack C/Celie 545 column to remove any remaining non planar compounds.
The resulting extracts are analysed by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) at a resolution of R=10.000. Each batch of six samples is accompanied by a laboratory blank as well as a quality control pool.

*Determination of mono-ortho and indicator PCB*

An aliquot of approx. 0.25 g of fat is spiked with 8 $^{13}C_{12}$-labelled mono-ortho PCB standards each at a concentration of 500 pg/sample and 6 $^{13}C_{12}$-labelled indicator PCB standards each at a concentration of 5 ng/sample. Fat and non stable compounds are removed on a silica gel column coated with sulphuric acid. The hexane eluate is carefully reduced by rotary evaporation and finally a gentle stream of nitrogen to a volume of 100 µl. This extract is directly applied to HRGC/HRMS analysis at a resolution of R=10.000. Each batch of six samples is accompanied by a laboratory blank as well as a quality control pool.

**Results and Discussion**

Descriptive statistical data on levels of PCDD/F, dioxin-like PCB and calculated TEq-values in human blood and milk are given in Table 1. PCDD/F levels found are in good agreement with literature data on PCDD/F [2, 3] or dioxin-like PCB [4, 5] human biomonitoring data from Germany with regard to the sampling years and the age range of the participants.

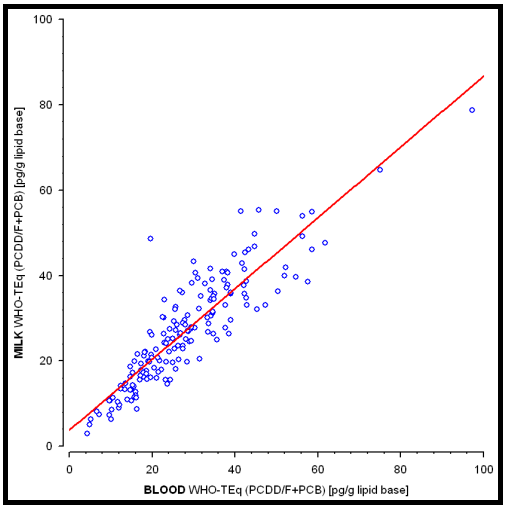


**Figure 1:** Correlation of PCDD/F- and PCB-concentrations (expressed as WHO-TEq on lipid base) in human milk and blood

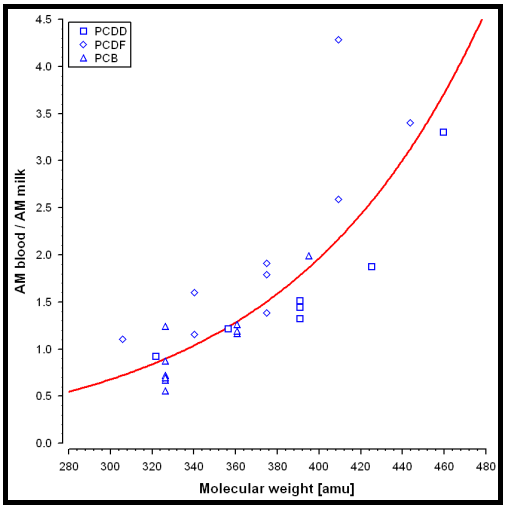


**Figure 2:** Dependence of the distribution of PCDD/F and PCB between human blood and human milk on molecular weight

A good correlation between lipid-adjusted PCDD/F and PCB levels in blood and milk was found for nearly all congeners as demonstrated in Figure 1 for the calculated WHO-TEq. To compare the levels in blood and milk the ratios of the arithemetic means of both data sets as well as correlation calculations were done for each congener and the TEq-values (see Table 1). For the different congeners different distributions in the two body compartments were found. In the primary compartment blood higher chlorinated substances are found in higher concentrations when compared to milk (e. g. OctaCDD 3.3fold), whereas lower chlorinated congeners such as pentachlorinated biphenyls are predominantly enriched in milk in concentrations about 1.5fold higher than found in blood. Besides the different lipophilicity of the substances, the molecule diameter and molecular weight influencing the membrane permeability might be responsible for this observation**.** Figure 2 **s**hows the dependence of the distribution between blood and milk on the molecular weight.

To our knowledge, the present study is the first investigation on a larger study group where blood and milk samples from the same donors were analyzed. Similar trends on the different distributions between blood and milk with regard to PCDD/F and dioxin-like PCB have been found when comparing samples from different donors or of analyzed pool samples [5-8].

The slightly different congener patterns observed in blood and milk result in a different contribution of the individual congeners to the calculated WHO-TEq values. In both matrices, blood and milk, only 4 congeners (12378-PeCDD, 23478-PeCDF, 33'44'5-PeCB (# 126) and 233'44'5-HxCB (# 156)) contribute the main share (see Figure 3). The mean share of PCDD/F to total WHO-TEq is 60 % in blood and 52 % in milk, the shares of non-orthe PCB and mono-ortho PCBs are 18 % and 22 % (blood) or 27 % and 21 % (milk), respectively.

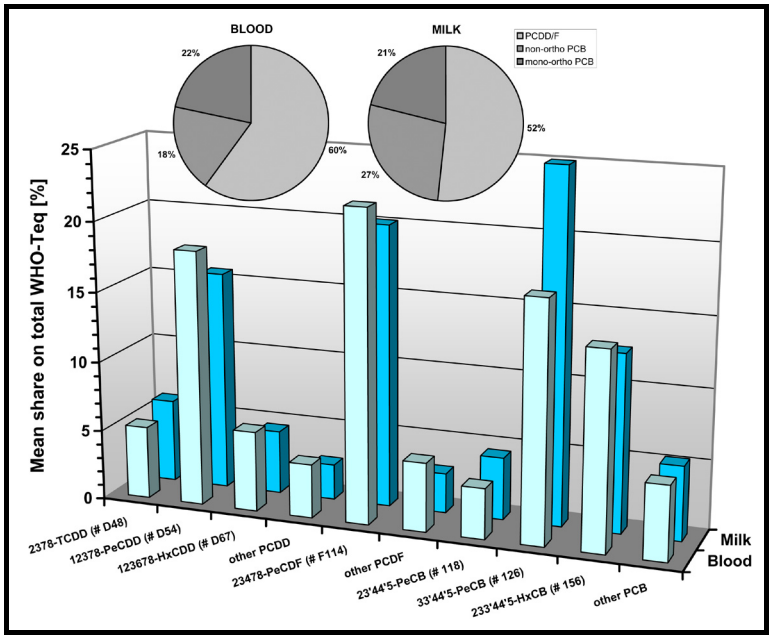


**Figure 3:** Mean share of different PCDD/F- and PCB-congeners on total WHO-TEq in blood and milk

**Table 1:** Descriptive statistical data on PCDD/F and PCB concentrations [lipid base] in human blood and milk from German mothers collected from the same donors (study period 09/2000 to 01/2003)

| N=169 | BLOOD | | | | | | MILK | | | | | | AM blood/ AM milk | CORRELATION | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MIN | P5 | MED | P95 | MAX | AM | MIN | P5 | MED | P95 | MAX | AM | | r | constant | slope |
| Age [years] | 19 | 23 | 32 | 39 | 41 | 31.24 | 19 | 23 | 32 | 40 | 42 | 31.85 | 0.98 | — | — | — |
| **PCDD** [pg/g lipid base] | | | | | | | | | | | | | | | | |
| 2378-TetraCDD (# D48) | 0.085 | 0.29 | 1.3 | 3 | 4.9 | 1.43 | 0.075 | 0.54 | 1.5 | 3 | 5.3 | 1.57 | 0.92 | 0.68 | 0.67 | 0.63 |
| 12378-PentaCDD (# D54) | 0.65 | 1.6 | 4.7 | 10 | 16 | 5.06 | 0.55 | 1.4 | 4 | 7.6 | 11.6 | 4.17 | 1.21 | 0.77 | 1.11 | 0.61 |
| 123478-HexaCDD (# D66) | 0.46 | 1.2 | 3.8 | 7.9 | 12 | 4.12 | 0.17 | 0.79 | 2.7 | 5.4 | 10.2 | 2.86 | 1.44 | 0.79 | 0.56 | 0.56 |
| 123678-HexaCDD (# D67) | 1.5 | 4.2 | 15 | 32 | 64 | 16.44 | 0.68 | 2.9 | 11.6 | 24.1 | 42.6 | 12.45 | 1.32 | 0.83 | 2.29 | 0.62 |
| 123678-HexaCDD (# D67) | 0.6 | 1.1 | 3.2 | 7.5 | 12 | 3.57 | 0.29 | 0.8 | 2.3 | 4.7 | 7.5 | 2.36 | 1.51 | 0.8 | 0.57 | 0.50 |
| 1234678-HeptaCDD (# D73) | 1.9 | 7.1 | 22 | 59 | 100 | 26.30 | 1.9 | 4 | 12.6 | 29.7 | 65.5 | 14.09 | 1.87 | 0.86 | 1.82 | 0.47 |
| OctaCDD (# D75) | 23 | 94 | 220 | 560 | 1100 | 268.43 | 9.2 | 22.5 | 70.4 | 178 | 538 | 81.25 | 3.30 | 0.77 | 8.33 | 0.27 |
| **PCDF** [pg/g lipid base] | | | | | | | | | | | | | | | | |
| 2378-TetraCDF (# F83) | 0.060 | 0.085 | 0.255 | 1 | 1.6 | 0.35 | 0.05 | 0.1 | 0.28 | 0.69 | 1.5 | 0.31 | 1.10 | 0.49 | 0.20 | 0.34 |
| 12378-PentaCDF (# F94) | 0.055 | 0.075 | 0.295 | 0.79 | 1.15 | 0.33 | 0.05 | 0.05 | 0.19 | 0.42 | 0.57 | 0.21 | 1.60 | 0.37 | 0.14 | 0.19 |
| 23478-PentaCDF (# F114) | 1.7 | 4.5 | 12 | 24 | 44 | 12.54 | 1.4 | 3.4 | 10.1 | 20.6 | 26.6 | 10.90 | 1.15 | 0.84 | 2.27 | 0.69 |
| 123478-HexaCDF (# F118) | 1.1 | 2.2 | 4.5 | 8.7 | 13 | 4.99 | 0.58 | 1.1 | 2.7 | 5.2 | 7.3 | 2.80 | 1.79 | 0.75 | 0.72 | 0.42 |
| 123678-HexaCDF (# F121) | 1.1 | 2.1 | 4.2 | 9 | 12 | 4.73 | 0.52 | 0.97 | 2.4 | 4.6 | 6.6 | 2.48 | 1.91 | 0.74 | 0.61 | 0.39 |
| 234678-HexaCDF (# F130) | 0.195 | 0.44 | 1.5 | 3.1 | 4.1 | 1.56 | 0.24 | 0.37 | 1 | 2.4 | 3.5 | 1.12 | 1.38 | 0.62 | 0.35 | 0.50 |
| 123789-HexaCDF (# F124) | 0.035 | 0.048 | 0.215 | 0.65 | 0.85 | 0.27 | 0.025 | 0.025 | 0.05 | 0.1 | 0.19 | 0.05 | — | — | — | — |
| 1234678-HeptaCDF (# F131) | 0.94 | 2.35 | 4.7 | 20 | 230 | 9.29 | 0.03 | 0.94 | 1.9 | 7.4 | 79.9 | 3.59 | 2.59 | 0.99 | 0.37 | 0.35 |
| 1234789-HeptaCDF (# F134) | 0.050 | 0.08 | 0.31 | 0.8 | 2.8 | 0.37 | 0.05 | 0.05 | 0.08 | 0.18 | 0.24 | 0.09 | 4.28 | 0.21 | 0.08 | 0.03 |
| OctaCDF (# F135) | 0.065 | 0.225 | 0.65 | 4 | 180 | 2.95 | 0.1 | 0.1 | 0.34 | 3.6 | 25.4 | 0.87 | 3.40 | 0.94 | 0.47 | 0.13 |
| **non-ortho PCB** [pg/g lipid base] | | | | | | | | | | | | | | | | |
| 33'44'-TetraCB (# 77) | 3.0 | 4.1 | 7 | 15 | 88 | 8.72 | — | — | — | — | — | — | — | — | — | — |
| 344'5-TetraCB (# 81) | 0.105 | 0.305 | 1.2 | 3.4 | 8.2 | 1.49 | — | — | — | — | — | — | — | — | — | — |
| 33'44'5-PentaCB (# 126) | 2.5 | 12 | 41 | 97 | 210 | 47.83 | 9.6 | 20.4 | 67.3 | 134 | 291 | 71.76 | 0.67 | 0.73 | 19.85 | 1.09 |
| 33'44'55'-HexaCB (# 169) | 3.2 | 12 | 35 | 88 | 200 | 41.53 | 1.7 | 7.6 | 28.9 | 69.1 | 128 | 33.47 | 1.24 | 0.85 | 5.85 | 0.67 |
| **mono-ortho PCB** [ng/g lipid base] | | | | | | | | | | | | | | | | |
| 233'44'-PentaCB (# 105) | 0.12 | 0.41 | 1.4 | 2.8 | 5.7 | 1.47 | 0.24 | 0.72 | 1.8 | 3.7 | 4.66 | 2.04 | 0.72 | 0.85 | 0.00 | 0.96 |
| 2344'5-PentaCB (# 114) | 0.043 | 0.13 | 0.43 | 0.98 | 1.7 | 0.47 | 0.025 | 0.19 | 0.63 | 1.4 | 2.1 | 0.68 | 0.69 | 0.81 | 0.00 | 1.12 |
| 23'44'5-PentaCB (# 118) | 0.64 | 2.9 | 9.2 | 20 | 36 | 10.30 | 1.5 | 4.2 | 11 | 20.8 | 28.99 | 11.85 | 0.87 | 0.91 | 0.00 | 0.89 |
| 2'344'5-PentaCB (# 123) | 0.015 | 0.039 | 0.13 | 0.28 | 0.51 | 0.14 | 0.016 | 0.05 | 0.23 | 0.52 | 0.91 | 0.26 | 0.55 | 0.52 | 0.00 | 1.04 |
| 233'44'5-HexaCB (# 156) | 0.37 | 1.4 | 7.2 | 19 | 49 | 8.42 | 0.12 | 1.2 | 6.4 | 14.1 | 27.1 | 7.22 | 1.16 | 0.91 | 0.00 | 0.67 |
| 233'44'5'-HexaCB (# 157) | 0.035 | 0.26 | 1.1 | 2.4 | 5.7 | 1.19 | 0.025 | 0.22 | 0.87 | 1.8 | 3 | 0.94 | 1.26 | 0.90 | 0.00 | 0.64 |
| 23'44'55'-HexaCB (# 167) | 0.15 | 0.55 | 2.1 | 5.7 | 9.4 | 2.44 | 0.05 | 0.49 | 2 | 4.3 | 7.8 | 2.05 | 1.19 | 0.88 | 0.00 | 0.69 |
| 233'44'55'-HeptaCB (# 189) | 0.031 | 0.18 | 1.1 | 2.6 | 9.5 | 1.25 | 0.025 | 0.2 | 0.38 | 1.4 | 9.8 | 0.63 | 1.99 | 0.85 | 0.00 | 0.83 |
| **TEq** [pg/g lipid base] | | | | | | | | | | | | | | | | |
| WHO-TEq (PCDD/F) | 2.73 | 6.32 | 15.32 | 31.72 | 55.07 | 16.79 | 1.80 | 4.80 | 13.30 | 23.90 | 34.70 | 13.84 | 1.21 | 0.83 | 2.74 | 0.66 |
| WHO-TEq (non-ortho PCB) | 0.56 | 1.36 | 4.53 | 10.50 | 21.47 | 5.20 | 0.98 | 2.20 | 7.00 | 14.00 | 30.40 | 7.51 | 0.69 | 0.76 | 1.72 | 1.11 |
| WHO-TEq (mono-ortho PCB) | 0.41 | 1.36 | 5.90 | 13.74 | 32.27 | 6.38 | 0.23 | 1.60 | 5.60 | 11.30 | 19.70 | 5.91 | 1.08 | 0.92 | 1.36 | 0.71 |
| WHO-TEq (PCB) | 1.40 | 3.71 | 10.81 | 22.04 | 42.23 | 11.57 | 1.21 | 4.00 | 13.00 | 24.50 | 50.10 | 13.43 | 0.86 | 0.91 | 1.71 | 1.01 |
| WHO-TEq (PCDD/F+PCB) | 4.34 | 10.06 | 26.37 | 54.65 | 97.30 | 28.36 | 3.01 | 8.70 | 26.40 | 49.20 | 78.70 | 27.27 | 1.04 | 0.89 | 3.73 | 0.83 |

MIN = minimun; P5 = 5. percentile; MED = median; P95 = 95. percentile; MAX = maximum; AM = arithmetic mean; CORRELATION = results of Pearson correlation MILK = constant + slope * BLOOD; r = coefficient of regression;Concentrations below the detection limit were calculated using half the value of the detection limit.

As known from other studies PCDD/F as well as PCB levels in human blood depend on the age of the subjects [2, 9]. Levels in human milk are in addition mainly influenced by the number of breastfed children and length of nursing period [10]. Figure 4 shows the age-dependence of PCDD/F and PCB, calculated as WHO-TEq, in human blood and milk. Since the age-dependence is not the same for all congeners [9] and both matrices, the distribution between blood and milk and the share on TEq will also change with age.

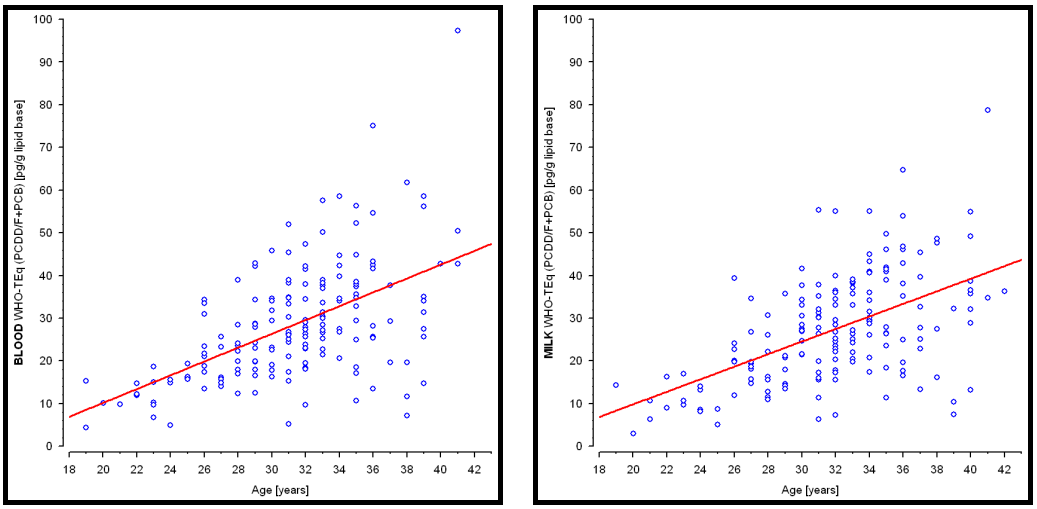


**Figure 4:** Age-dependence of PCDD/F- and PCB-concentrations (expressed as WHO-TEq on lipid base) in human blood (left) and human milk (right)

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