Age-related increase of PCDD/F-levels in human blood — a study with 95 unexposed persons from Germany

Schrey, P.[A], Wittsiepe, J.[A], Ewers, U.[A], Exner, M.[A], Selenka, F.[B]

[A] Hygiene Institut des Ruhrgebiets, Institut für Umwelthygiene und Umweltmedizin an der Ruhr-Universität Bochum, W-4650 Gelsenkirchen, Germany

[B] Ruhr Universität Bochum, Institut für Allgemeine- und Umwelthygiene, W-4630 Bochum, Germany

Summary. Levels of PCDD and PCDF were determined in whole blood of 95 persons, with no known exposure, living in a rural area of Northrhine-Westfalia, Germany. Age ranged between 12 and 82 years with at least 5 female and 5 male persons per age-decade.
The mean values of the PCDD/F-blood levels of the youngest and oldest subcohort were 16.6 and 64.5 pg/g I-TEQ (lipid basis) or 87.7 and 407 fg/g I-TEQ (whole-weight basis) and show therefore a 3.9- or 4.6-fold increase with age. For statistical evaluation the results of isomer specific determination on lipid basis[pg/g] and on whole-weight basis [fg/g] were correlated with age [a] using a multiplicative model: $CONCENTRATION\ IN\ BLOOD = A \cdot AGE^{B}$. The age-related increase of human blood levels, calculated as International Toxicity Equivalents, can therefore be described by the following equations: TEQ [pg/g (lipid basis)] = $1.52 \cdot AGE^{0.871}$ or TEQ [fg/g (whole-weight basis)] = $6.06 \cdot AGE^{0.965}$. The 95%-prediction-limits are given by $0.878 \cdot AGE^{0.871}$ and $2.62 \cdot AGE^{0.871}$ (F) or $3.33 \cdot AGE^{0.965}$ and $11.0 \cdot AGE^{0.965}$ (W), respectively. A statistically significant increase ($\alpha < 0.01$) was found for the following congeners (factors *A* and *B* for concentrations on lipid basis (F) and on whole-weight basis (W) are given in parenthesis): 2,3,7,8-TetraCDD *(F: 0.122, 0.945; W: 0.483, 1.04)*, 1,2,3,7,8-PentaCDD *(F: 1.07, 0.738; W: 4.37, 0.826)*, 1,2,3,4,7,8-HexaCDD *(F: 0.714, 0.814; W: 2.84, 0.910)*, 1,2,3,6,7,8-HexaCDD *(F: 2.16, 0.798; W: 8.67, 0.892)*, 1,2,3,7,8,9-HexaCDD *(F: 1.01, 0.577; W: 3.96, 0.676)*, 1,2,3,4,6,7,8-HeptaCDD *(F: 8.46, 0.596; W: 33.6, 0.692)*, OctaCDD *(F: 91.7, 0.401; W: 371, 0.492)*, 2,3,7,8-TetraCDF *(F: 0.172, 0.490; W: 0.749, 0.568)*, 2,3,4,7,8-PentaCDF *(F: 0.619, 1.04; W: 2.43, 1.14)*, 1,2,3,4,7,8-HexaCDF *(F: 0.551, 0.794; W: 2.21, 0.888)*, 1,2,3,6,7,8-HexaCDF *(F: 0.794, 0.791; W: 3.24, 0.880)* and 2,3,4,6,7,8-HexaCDF *(F: 0.461, 0.534; W: 1.86, 0.625)*. No correlation with age was found for 1,2,3,4,6,7,8-HeptaCDF and for 1,2,3,7,8-PentaCDF and OctaCDF, which were found in low concentrations. 1,2,3,7,8,9-HexaCDF and 1,2,3,4,7,8,9-HeptaCDF could be determinated only in a few cases near the detection limit.
With regard to the age-range of the group the relative increase between 10- and 80-year-old persons can be calculated by 8^{B}. The age-related increase was mostly pronounced for

2,3,4,7,8-PentaCDF *(F: 8.7; W: 10.7)* and 2,3,7,8-TetraCDD *(F: 7.1; W: 8.7)*. For higher chlorinated congeners, e. g. OctaCDD *(F: 2.3; W: 2.8)*, the age-related increase was considerably lower.

Introduction. PCDD and PCDF, predominantly 2,3,7,8-chlorosubstituted isomers, are known to accumulate in the food chain and therefore in man. As a result of their long biological half-life and their lipophilic nature they accumulate in human tissues, especially in those with a high fat content. Typical pattern and concentrations due to background-exposure have been found in several organs, adipose tissue, human milk and blood [1-3].
In most epidemiological studies blood samples are used to estimate human exposure, because procuring human blood is less invasive than procuring tissues and can be obtained from all persons in contrast to human milk samples. To have a good knowledge of parameters influencing human background exposure-levels is important both for risk assessment and for planning and realization of epidemiological studies.
In this study the influence of age on the levels of PCDD/F in human blood was observed in a group of unexposed persons living in Kreis Steinfurt, a rural area of Northrine-Westfalia, Germany. All persons were asked for personal data, habits of life, food consumption, number of breastfed infants, possible contact with PCDD/F-sources, physical condition, and other relevant data using a standardized questionnaire.

Methods. A method which has been developed for the determination of PCDD/F in human serum [4] was modified for analysis of whole blood samples. 50 ml of homogenized whole blood diluted with 50 ml deionized water is spiked with 100 µl of an internal standard solution containing 16 $^{13}C_{12}$-labelled PCDD/F-isomers and shaken overhead for 30 min. Extraction-procedure: addition of 50 ml of 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, which represents the fat content, is weighed and redissolved in hexane for sample clean up. The clean up is performed by standard methods using modified silicagels and activated charcoal [5]. After addition of 2 µl of dodecane the final sample extract is evaporated under a nitrogen stream to dryness and reconstituted to 10 µl with toluene, containing $^{13}C_{12}$-1,2,3,4-TetraCDD as external standard.
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 [*MS:* SIR, Resolution 10,000 at 10%, EI+, 40 eV, PFK lock mass check, observation of 2 ions for native and labelled isomers, setting of 5 time windows; *GC:* column: J&W Scientific, DB-5, 60 m, 0,1 µm; temperature program: 200°C (3 min), 5°C/min, 220°C (16 min), 5°C/min, 235°C (7 min), 5°C/min, 330°C (9 min); injector program: 70°C (60 s), 12°C/s, 330°C (10 min), split off (1 min); split on (2 min); injection volume: 2 µl].
The blood analyses were performed in series of 4 samples and 1 blank. Each third series containes additionally 1 pool blood sample for internal quality control. The standard deviation of the repeated pool blood analysis is smaller 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%. The detection limits (S:N = 3:1) are < 1 pg/g on lipid basis.

Results and discussion. Basic statistical results of the PCDD/F blood determinations of the population studied are presented in table 1, both on lipid basis and on whole-weight basis. For most congeners the blood levels increase with age. In order to fit the data set and for statistical evaluation the following multiplicative model was used: $CONCENTRATION\ IN\ BLOOD = A \cdot AGE^{B}$. Calculated factors A and B, correlation coefficients r and the 95%-prediction-limits for A are listed in table 2. The correlation is highly significant for most of the 2,3,7,8-chlorosubstituted PCDD/F except for some of the higher chlorinated PCDF (see table 2). Isomers increasing with age show an appreciable smaller range of variation for younger people. The relative increases with age are not equal for all congeners (see fig. 2). It shows a decrease with the chlorination grade. The age-related increase was mostly pronounced for 2,3,4,7,8-PentaCDF and 2,3,7,8-TetraCDD and only low for OctaCDD (see fig. 1). With regard to the age-range of the group and considering the lipid based PCDD/F-levels in blood the age-related increase is 8.7-fold for 2,3,4,7,8-PentaCDF, 7.1-fold for 2,3,7,8-TetraCDD and 2.3-fold for OctaCDD. The correspondings on whole-weight basis are 10.7, 8.7 and 2.8, respectively. A twofold increase of age is associated with a significant increase of the PCDD/F-levels in blood which can be described by the increase factors 2^{B} given in table 2. Regarding human exposure to PCDD/F expressed as I-TEQ (lipid basis) in relation to age the regression curve and the 95%-prediction-limits are shown in figure 1. The fitted line shows a more than 6-fold increase between 10 and 80 year-old persons and a nearly 2-fold increase with doubling of age.

The observed increase of PCDD/F in human blood reflects the accumulation of these substances in the human body and may also be influenced by higher background exposure to PCDD/F in the past. The data suggest that the biological half-life of some congeners may be considerably higher than calculated from previous studies on highly exposed subjects. For the most toxic isomers, 2,3,7,8-TetraCDD and 2,3,4,7,8-PentaCDF, and some others a steady increase was observed over the whole age-range (see fig. 2). The different age-related increases of the PCDD/F congeners in blood may result from their different toxicocinetic behaviour (rates of metabolism, membrane permeance resulting in different distribution volumes in the body, binding to biomolecules) and/or different routes of intake such as ingestion, dermal absorption or inhalation. The increase of the scattering range in the higher age-groups may be explained by different forms of nutrition, different background exposure sources and/or different individual biochemical factors.

Conclusion. This study presents data on the PCDD/F-levels in individual human blood samples obtained from a selected group of 95 subjects with no known exposure. A significant age-related increase was observed for most congeners as well as for the I-TEQ values. Our data indicate that the PCDD/F-levels in human blood are strongly influenced by age. With respect to the assessment of individual exposure and further epidemiological studies this has to be taken into account.

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Table 1: Basic statistical data on levels of PCDD/F in human blood of unexposed persons from Germany

	N	LIPID BASIS [pg/g]					PERCENTILES			WHOLE-WEIGHT BASIS [fg/g]					PERCENTILES		
			MIN.	MAX.	MEAN	MEDIAN	25.	75.	95.		MIN.	MAX.	MEAN	MEDIAN	25.	75.	95.
2378-TetraCDD	95	++++	1.2	12	4.62	4.4	2.5	6.1	10	++++	5.5	93	27.1	24	13	35	58
12378-PentaCDD	95	+++	5.6	44	18.0	17	11	22	32	+++	28	340	104	94	66	130	200
123478-HexaCDD	95	+++	3.9	38	16.3	15	9.7	22	30	+++	20	230	94.4	94	52	120	190
123678-HexaCDD	95	+++	12	110	45.9	46	31	56	87	+++	59	730	266	250	170	340	520
123789-HexaCDD	95	++	2.9	22	9.26	8.6	6.2	12	16	++	16	150	53.5	49	34	67	94
1234678-HeptaCDD	95	++	21	210	87.2	86	51	110	160	++	95	1400	504	500	270	660	960
OctaCDD	95	+	140	950	446	420	280	580	880	+	790	5700	2550	2500	1600	3200	4900
2378-TetraCDF	51 [1]	+	0.16	5.9	1.37	1.1	0.71	1.6	3.8	++	1.1	38	8.07	6.7	4.1	9.5	22
12378-PentaCDF	56 [2]	-	0.30	1.1	0.64	0.63	0.50	0.78	1.0	+	1.7	7.1	3.77	3.8	2.9	4.4	6.3
23478-PentaCDF	95	++++	6.7	110	34.3	32	17	46	73	++++	33	840	201	170	93	270	470
123478-HexaCDF	95	+++	3.9	29	11.5	11	7.0	15	22	+++	20	230	67.5	59	37	89	130
123678-HexaCDF	95	+++	5.3	42	16.5	16	10	21	32	+++	25	330	95.9	88	50	120	200
234678-HexaCDF	95	++	0.62	7.9	3.67	3.3	2.4	4.6	7.5	++	2.7	58	21.3	19	13	27	42
123789-HexaCDF	2 [3]																
1234678-HeptaCDF	95	-	6.8	45	15.4	14	12	17	23	-	35	170	86.0	82	64	100	140
1234789-HeptaCDF	38 [2]	-	0.28	1.1	0.48	0.43	0.34	0.54	1.1	-	1.5	6.4	2.84	2.5	2.1	3.2	5.4
OctaCDF	70 [2]	-	0.24	3.3	1.10	0.96	0.72	1.4	2.3	-	1.4	20	6.23	5.4	4.2	7.5	12
Total PCDD	95	+	194	1320	633	621	430	824	1120	++	1050	7810	3630	3460	2400	4680	6600
Total PCDF	95	++	27.7	207	84.7	81.2	53.0	111	153	+++	143	1590	489	434	293	618	965
Total PCDD/F	95	+	223	1480	718	706	479	914	1240	++	1190	9020	4120	4070	2630	5230	7370
German TEq	95	+++	6.061	52.08	21.81	21.65	13.15	27.28	40.98	+++	31.26	401.4	126.9	120.3	69.33	165.7	258.2
International TEq	95	+++	11.16	113.6	42.67	40.77	25.03	54.40	82.07	++++	57.49	872.8	248.9	234.1	134.3	325.5	517.6

Increase with age:

++++ Very strong increase with age ($2^B > 1.90$).
+++ Strong increase with age ($1.65 < 2^B \leq 1.90$).
++ Medium increase with age ($1.45 < 2^B \leq 1.65$).
+ Low increase with age ($2^B \leq 1.45$).
- No significant correlation with age on the 99%-level.

1) Calculation without non-detects and not quantified samples due to blank problems.
2) Calculation without non-detects.
3) 123789-HexaCDF was only found in 2 samples with concentrations near the detection limit.

Table 2: Correlation of PCDD/F-levels in human blood of unexposed persons from Germany with age

	N	LIPID BASIS [pg/g]						WHOLE-WEIGHT BASIS [fg/g]					
		Statistical Model: CONCENTRATION = A·AGE ^{B}			95%-Prediction-limits		Increase factor	Statistical Model: CONCENTRATION = A·AGE ^{B}			95%-Prediction-limits		Increase factor
		r	A	B	A_{Lower}	A_{Upper}	2^B	r	A	B	A_{Lower}	A_{Upper}	2^B
2378-TetraCDD	95	0.8472 [1]	0.122	0.945	0.066	0.223	1.93	0.8420 [1]	0.483	1.04	0.244	0.954	2.06
12378-PentaCDD	95	0.7999 [1]	1.07	0.738	0.608	1.88	1.67	0.8125 [1]	4.37	0.826	2.38	7.99	1.77
123478-HexaCDD	95	0.7782 [1]	0.714	0.814	0.365	1.40	1.76	0.7988 [1]	2.84	0.910	1.41	5.71	1.88
123678-HexaCDD	95	0.8250 [1]	2.16	0.798	1.24	3.78	1.74	0.8415 [1]	8.67	0.892	4.83	15.6	1.86
123789-HexaCDD	95	0.6719 [1]	1.01	0.577	0.528	1.93	1.49	0.7155 [1]	3.96	0.676	2.02	7.77	1.60
1234678-HeptaCDD	95	0.5500 [1]	8.46	0.596	3.36	21.3	1.51	0.5901 [1]	33.6	0.692	12.8	88.3	1.62
OctaCDD	95	0.4370 [1]	91.7	0.401	39.5	213	1.32	0.5090 [1]	371	0.492	159	867	1.41
2378-TetraCDF	51 [5]	0.3666 [2]	0.172	0.490	0.046	0.649	1.40	0.4160 [2]	0.749	0.568	0.199	2.82	1.48
12378-PentaCDF	56 [6]	[4]					(1)	0.3464 [3]	1.39	0.250	0.717	2.71	1.19
23478-PentaCDF	95	0.8566 [1]	0.619	1.04	0.326	1.17	2.06	0.8590 [1]	2.43	1.14	1.22	4.86	2.20
123478-HexaCDF	95	0.8278 [1]	0.551	0.794	0.319	0.954	1.73	0.8298 [1]	2.21	0.888	1.20	4.06	1.85
123678-HexaCDF	95	0.8174 [1]	0.794	0.791	0.450	1.40	1.73	0.8242 [1]	3.24	0.880	1.75	6.00	1.84
234678-HexaCDF	95	0.5628 [1]	0.461	0.534	0.207	1.03	1.45	0.5914 [1]	1.86	0.625	0.780	4.45	1.54
123789-HexaCDF	2 [7]												
1234678-HeptaCDF	95	[4]					(1)	[4]					(1)
1234789-HeptaCDF	38 [6]	[4]					(1)	[4]					(1)
OctaCDF	70 [6]	[4]					(1)	[4]					(1)
Total PCDD	95	0.5625 [1]	93.9	0.493	44.9	197	1.41	0.6208 [1]	378	0.585	178	803	1.50
Total PCDF	95	0.8077 [1]	5.95	0.695	3.54	9.99	1.62	0.8192 [1]	23.8	0.788	13.6	41.8	1.73
Total PCDD/F	95	0.6100 [1]	97.7	0.517	194	49.2	1.43	0.6620 [1]	393	0.610	194	795	1.53
German TEq	95	0.8489 [1]	0.958	0.818	0.570	1.61	1.76	0.8528 [1]	3.83	0.912	2.17	6.77	1.88
International TEq	95	0.8514 [1]	1.52	0.871	0.878	2.62	1.83	0.8542 [1]	6.06	0.965	3.33	11.0	1.95

[1] Correlation significant on the 99,9%-level.

[2] Correlation significant on the 99%-level.

[3] Correlation significant on the 95%-level.

[4] No significant correlation with age on the 95%-level.

[5] Calculation without non-detects and not quantified samples due to blank problems.

[6] Calculation without non-detects.

[7] 123789-HexaCDF was only found in 2 samples with concentrations near the detection limit.

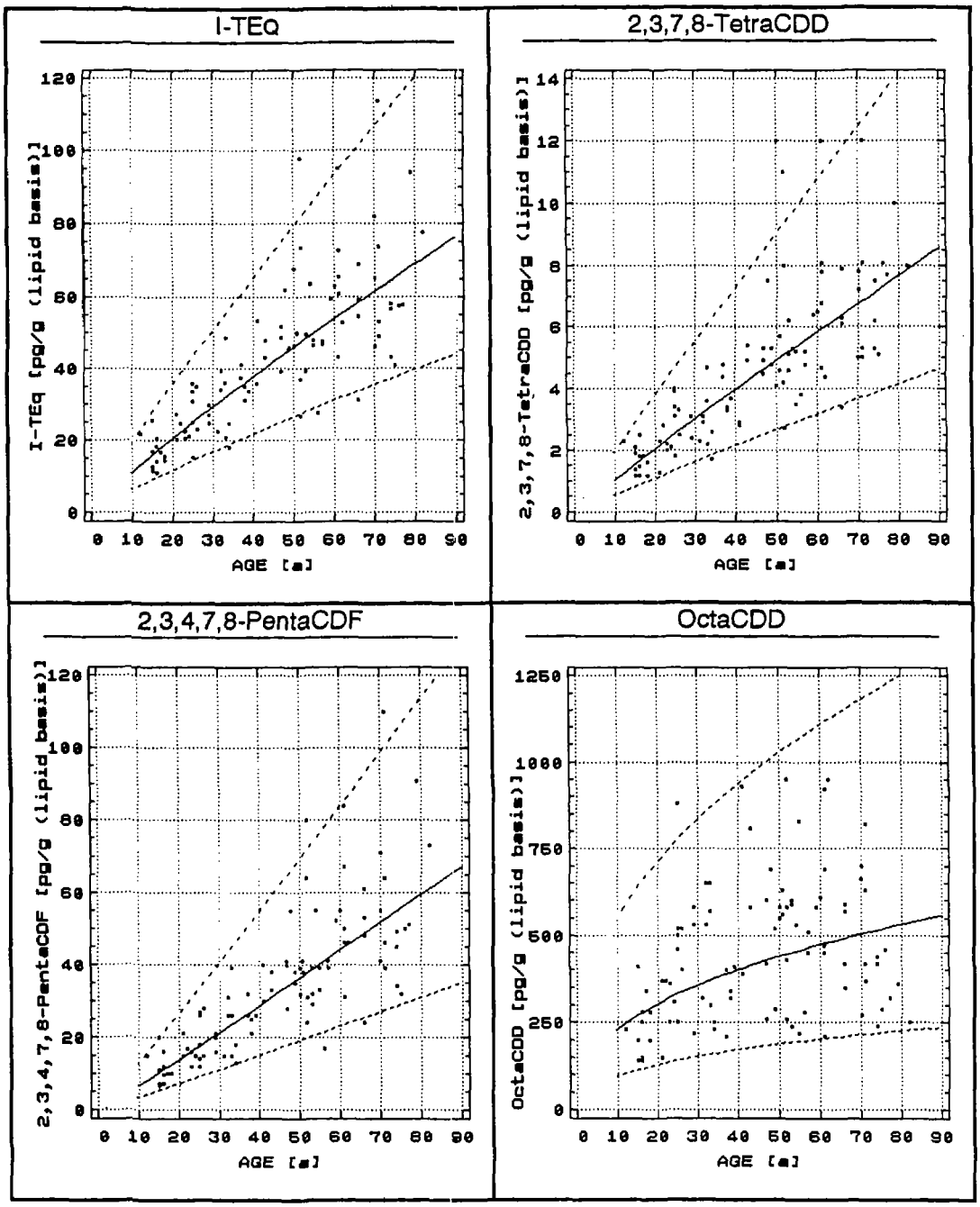

Figure 1: *PCDD/F-levels [pg/g (lipid basis)] in human blood of unexposed persons in relation to age*

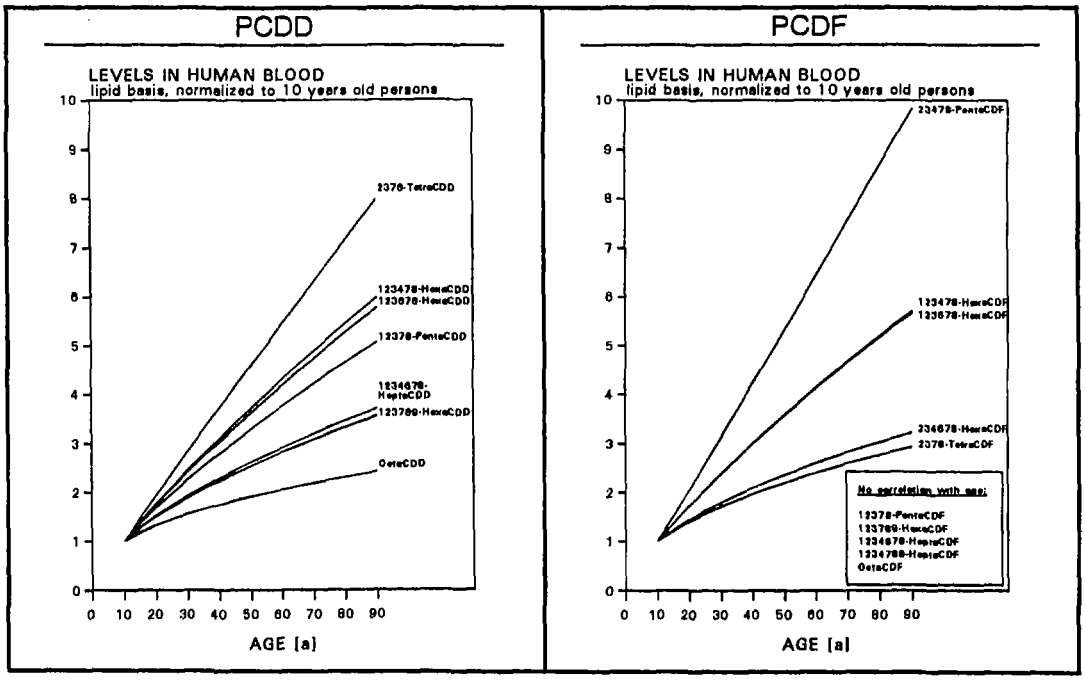

Figure 2: Relative levels of PCDD/F (lipid basis) in human blood of unexposed persons in relation to age

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