Serum/whole blood PCDD/F-data from different labs originating from the same persons

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Abstract

Polychlorinated dibenzo-p-dioxins and dibenzofurans were measured in whole blood and in serum in samples gathered within one year of 54 persons. 38 of these samples were of the same day. TCDD- and I-TEQ-levels were well correlated between both matrices (r=0.93-0.97). The concentration of TCDD in serum was significantly lower than that in whole blood (regression coefficient ~0.8). The I-TEQ-levels did not show this difference. Different sampling points had no influence on the comparability of the two methods.

Introduction

The way of determination of polychlorinated dibenzodioxins and -furans (PCDD/Fs) in human samples has changed since first measurments in 1986 (1, 2). Its extreme lipophilicity was the reason that the first determinations of PCDD/Fs in this material took place in fatty tissue, a procedure that restricted access to higher numbers of determinations. With growing sensitivity of the analytical tools it was possible to measure PCDD/Fs in blood lipids. It was shown that its concentration in fatty tissue and in whole blood, related to its lipid contents, were highly correlated (3, 4, 5,6). But it was still necessary to have samples of about 50-200ml of full blood to analyze for all 2,3,7,8- chlorinated congeners of PCDD/Fs. Now it is possible to measure PCDD/Fs in much lower quantities of serum/plasma and whole blood. To our knowledge that is the first paper that studies the two detection methods (in whole blood and in serum) for their comparability.

Methods

A pesticide producing plant (γ-hexachlorocyclohexane, 2,4,5-trichlorophenoxyacetic acid and others) was closed in 1984 for its contamination with PCDD/Fs. 192 of its former workers were investigated in 1992 to 1994 for their health status according to a social contract. On this occasion the body burden of PCDD/Fs was analyzed in 135 of the investigated workers. Two analyses (one at the ERGO laboratory in Hamburg, FRG, and one at the CDC in Atlanta, GA, USA) were performed in 72 persons because of separate studies and parallel processes for worker's compensation. Of these 72 double analyses 54 were performed within one year. 38 of these 54 were based on specimens of one blood draw. Either heparinized samples were directly stored at -20°C (whole blood) or serum was separated by centrifugation before storing at -20°C.

The analysis in whole blood (at the ERGO laboratories) was nearly identical to that used in the WHO interlaboratory validation study, round III (7). High resolution gas chromatography/high resolution mass spectrometry was performed in duplicate for each sample.
The analysis in serum (at the CDC) was performed according to (8,9)
I-TEQs were calculated according to the NATO recommendation (10).

Statistical analysis
Statistical analysis was done for the levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and for the calculated TEQs. The data of the group of 54 as well as of that of 38 persons (see above) were described by means, medians, standard deviations, 5%- and 95%-quantiles and by their minima and maxima. Regression analysis was performed using the regression model

$$\textit{concentration (whole blood)} = a * \textit{concentration (plasma)} + b$$

with a slope a (regression coefficient) and a constant b. Pearson's correlation coefficient r was calculated. Furthermore it was checked whether the referring regression coefficient was different from 1.

Results

Descriptive statistics is shown in table 1. Mean and median of TCDD in whole blood are somewhat higher than in serum in both groups. I-TEQs in serum and whole blood do not show this difference.

Table 1: TCDD and I-TEQ-levels in whole blood and in plasma in specimens of 38 persons, obtained at the same date, and of 54 persons, obtained within 1 year.

	TCDD (pg/g fat) (whole blood)	TCDD (pg/g fat) (serum)		TEQ (pg/g fat) (whole blood)	TEQ (pg/g fat) (serum)
	specimens of 38 persons gained at the same date				
mean	59.99	52.19		153.45	161.86
median	45.10	38.25		126.21	127.58
standard dev.	60.27	52.19		115.45	150.25
5%-quantile	6.93	4.95		33.67	23.39
95%-quantile	196.06	192.60		362.22	363.83
minimum	2	0		23.86	18.86
maximum	277.9	207		618.69	783.15
	specimens of 54 persons, gained within 1 year				
mean	76.01	64.25		181.18	185.00
median	45.1	34.25		123.47	122.04
standard dev.	110.23	86.74		171.46	191.34
5%-quantile	4.91	2.85		31.56	23.49
95%-quantile	282.13	214.7		521.21	616.48
minimum	2	0		19.60	18.86
maximum	674	493		803.81	912.3

Regression analysis correlation coefficients are equivalent in the the group of 38 as in that of 54 persons for TCDD (0.965 and 0.973) and I-TEQ (0.934 and 0.928). The regression coefficient *a* of the comparison of TCDD in whole blood and in serum is significantly ($p<0.05$) different from 1 in both groups 0.836 and 0.766), for I-TEQ only in the group of 38 persons (1.216), not for that of 54 persons (1.033). The constant *b* was never found to be significantly different from 0 (2.04 and 6.03 for TCDD and -24.68 and -1.18 for I-TEQ). Scatterplots for the group of 38 persons are given in figures 1 and 2.

Discussion

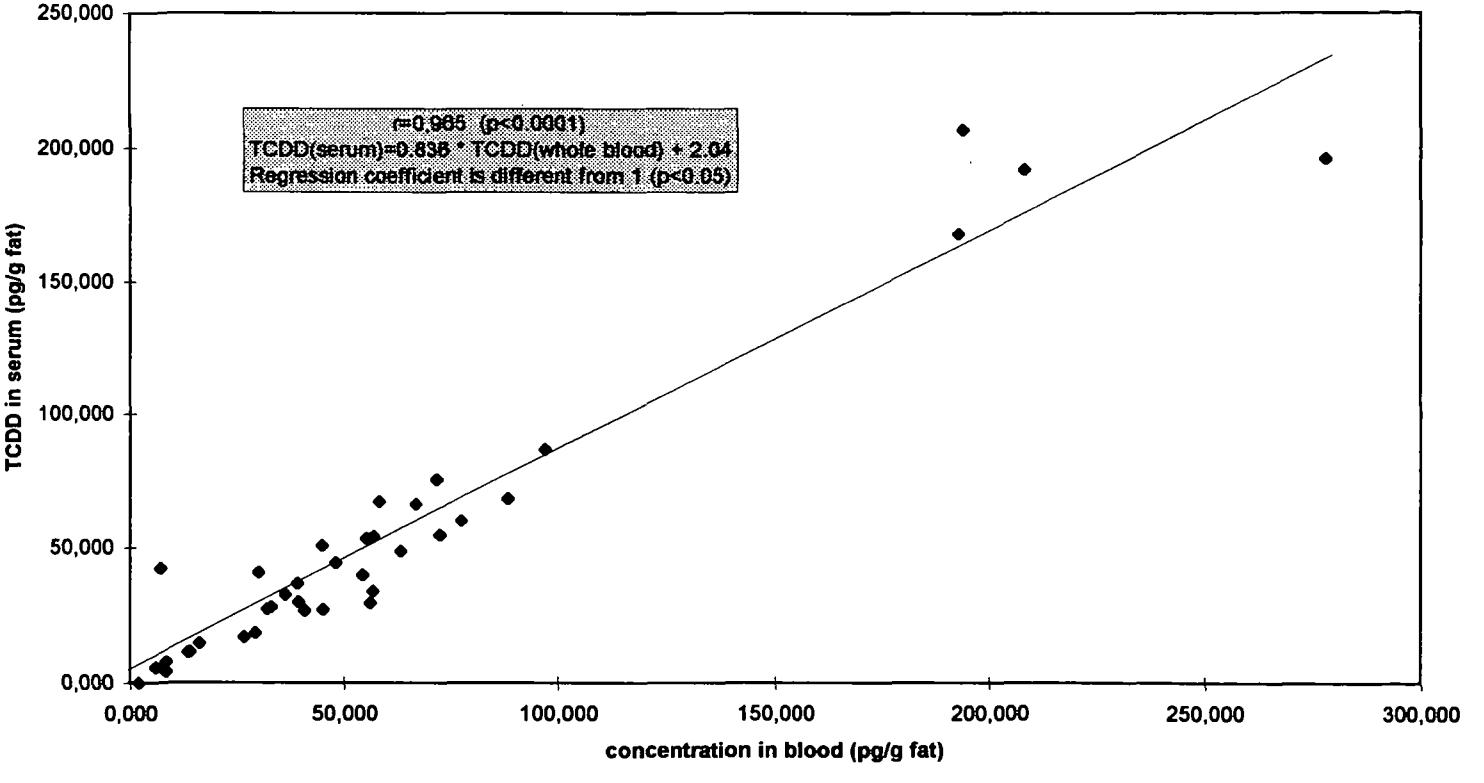
Determination of PCDD/Fs in whole blood and in serum are highly correlated. It is remarkable that the descriptive statistics as well as the regression analysis show a tendency towards lower TCDD levels in plasma than in whole blood analysis (regression coefficient ~0.8). This phenomenon is not found in I-TEQ levels. The descriptive statistics show essentially equal values for mean, median and the 5%- and 95%-quantils in the group of 54 and in the group of 38 persons. Additionally in the group of 54 persons the slope of the regression function is close to 1 (1.033). There is no apparent explanation for the lower levels of TCDD in plasma. Different minimal detection levels were not the reason, as only one person had TCDD not detectable in plasma and the corresponding concentration in whole blood was low as well (2 pg/g fat).
The very similar correlation coefficients show that different sampling time points (e.g. within 1 year) per se do not influence on the comparability of the two analytical methods respectively the two different matrices. The samples we analyzed were gathered within one year, so the half life time of TCDD being about 7 years it was appropriate to compare directly these values. For a comparison of specimens, which are largely different in their sampling dates, adjustments relative to half life are of course necessary (11).

Literature cited

(1) Rappe, C.; Nygren, M.; Lindsrom, G.; Hansson, M. Dioxins and dibenzofurans in biological samples of European origin. Chemosphere **1986**, 15, 1635-1639
(2) Beck, H.; Eckart, K.; Mathar, W.; Wittkowski, R. Levels of PCDD's and PCDF's in adipose tissue of occupationally exposed workers. Chemosphere **1989**, 18, 507-516
(3) Patterson, D.G. jr.; Needham,L.L.; Pirkle, J.L.; Roberts, D.W.; Bagby, J.R.; Garrett, W.A.; Andrews, J.S., jr.; Falk, H.; Bernert, J.T.; Sampson, E.J.; Houk, V.N. Correlation between serum and adipose tissue levels of 2,3,7,8- tetrachlorodibenzo-p-dioxin in 50 persons in Missouri. Arch. Environ. Toxicol. **1988**, 17, 139-143
(4) Kahn, P.C.; Gochfeld, M.; Nygren, M.; Hansson, M.; Rappe, C.; Velez, H.; Ghent-Guenther, T.; Wilson, W.R. Dioxins and dibenzofurans in blood and adipose tissue of Agent Orange.exposedVietnam veterans and matched controls. JAMA **1988**, 259, 1661-1667
(5) Gurn, P.; Beck,H.; Flesch-Janys, D.; Jung, D.; Konietzko,J.; Manz, A.; Mathar, W.; Päpke, O. Partitioning of PCDD/Fs between blood and adipose tissue in 9 former chemical workers. 15th International Symposium on Chlorinated Dioxins and Related Compounds, August 21-25, Edmonton, Canada. **1995**, 233-238

(6) Päpke, O.; Ball, M.; Lis, A.; Scheunert, K. PCDD/PCDF in whole blood samples of unexposed persons. Chemosphere **1989**, 19, 1663-1692
(7) WHO Regional Office for Europe. Consultations on the third roundof interlaboratoryquality control studieson levels of PCBs PCDDs and PCDFs in human milk, blood, cow's milk and fish. Volterra, Italy **1992**
(8) Patterson, D.G. jr.; Hampton, L.; Lapeza, C.R. jr.; Belser, W.T.; Green, V.; Needham,L.L. High-resolution gas chromatographic/high resolution mass spectrometric analysis of human serum on a whole weight and lipid basis for 2,3,7,8- tetrachlorodibenzo-p-dioxin. Anal. Chem. **1987**, 59, 2000-2005
(9) Patterson, D.G. jr.; Barr, J.R.; DiPietro, E.S.; Granger, J.; Green, V.; Lapeza, C.R. jr.; Maggio, V.L.; McClure, P.C.; Sirimanne, S.; Turner, W.E. Comprehensive two-dimensional and fast single-dimensional GC-HRMS analysis of human serum for PCDDs, PCDFs, cPCBs, congener PCBs, persistent and non-persistent pesticides, and PAHs. Organohalogen Compounds, **1996**, 27, 309-314
(10) NATO-Report 176. International Toxicity Equivalency Factor (I-TEF) method for risk assessment for complex mixtures of dioxins and relted compounds. North Atlantic Treaty Organization. Committee on the Challenges of Modern Society. Pilot study on international information exchage on dioxin and related compounds. **1988**
(11) Flesch-Janys, D.; Becher, H.; Gurn, P.; Jung, D.; Konietzko, J.; Manz, A.; Päpke, O. Elimination of polychlorinated dibenzo-p-dioxins and dibenzofurans PCDD/F) in occupationally exposed persons. J. Toxicol. Environ. Health **1996**, 47, 363-378

Fig.1: TCDD-concentrations in whole blood vs. those in serum of 38 persons. Corresponding specimens are sampled the same day.



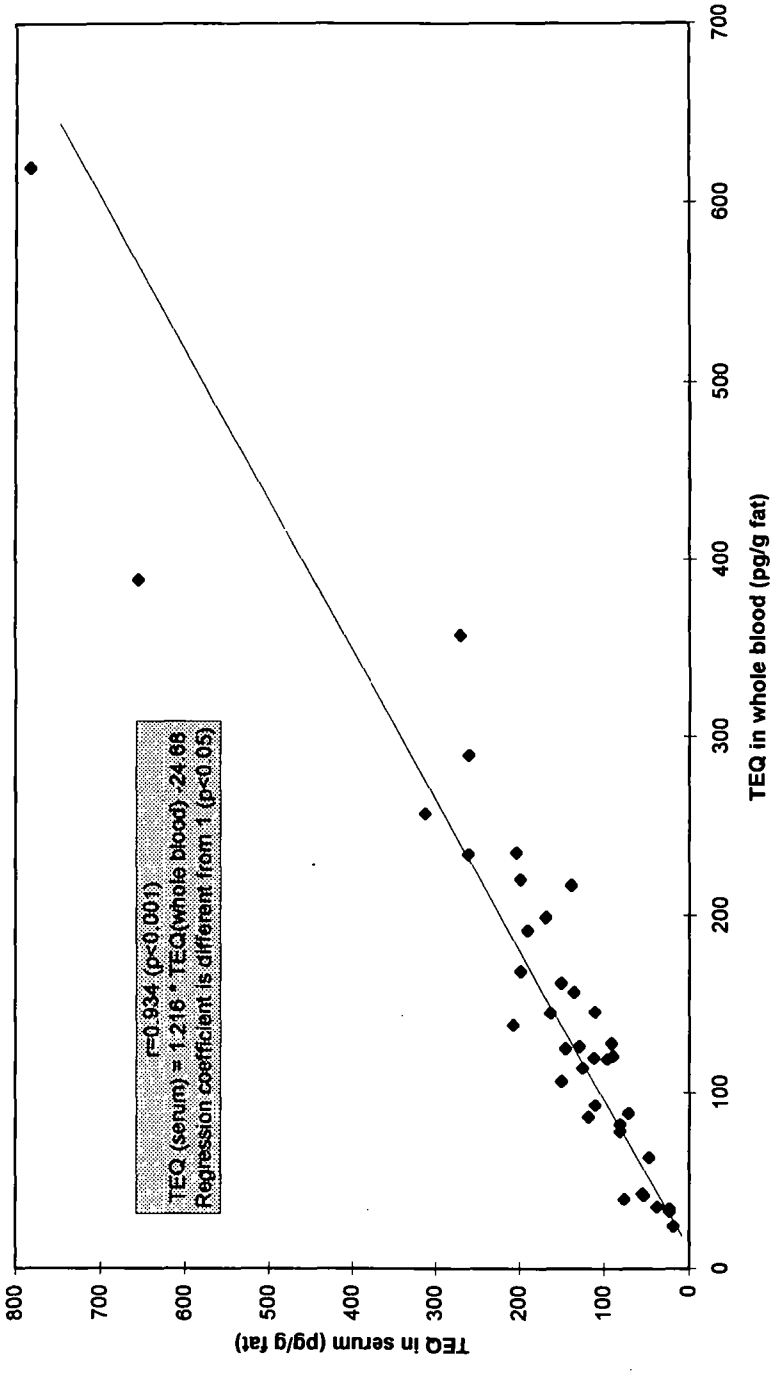


Fig.2: I-TEQ in whole blood vs. that in serum in 38 persons. Corresponding specimens are sampled the same day.