Interlaboratory Quality Assessment on Levels of PCBs, PCDDs and PCDFs in Human Milk and Blood Plasma

Summary report of the *Fourth Round of WHO-coordinated study*

The WHO Regional Office for Europe has been coordinating a comprehensive project on polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), aiming to prevent and control exposure to these chemicals and to assess their possible health risks, especially for infants exposed through consumption of contaminated breast milk. Because only limited data allowing estimation of exposure through consumption of breast milk were available, the Regional Office initiated a series of international studies on the concentrations of PCBs, PCDDs and PCDFs in human milk.

To help ensure the reliability and comparability of analytical data from exposure studies, and also to encourage the development and improvement of analytical methodologies and analytical capacity, WHO/EURO has, since 1987, coordinated a series of analytical quality assessment studies. The first of these studies took place in 1986-1987 (WHO Environmental Health Series No 34). The second study was completed in 1988-1989 with the participation of nineteen laboratories (WHO *Environment and Health Series No 37, 1991).* Thirty laboratories were involved in the third round study, which also included analysis in cow's milk and fish (*Environmental Health in Europe Series No 2, 1995: Quality assessment of PCBs, PCDDs and PCDFs analyses: Third Round of WHO-coordinated Study).*

The fourth study, was conducted by the WHO European Centre for Environment and Health, Bilthoven Division with the assistance of a coordinating committee whose members were: Dr Jacob de Boer from the National Institute for Fisheries Research, Netherlands, Dr Jørgen S Carlé from SCANMECO A/S, Denmark, Dr Martin Nygren from the National Defence Research Establishment, Sweden, Dr James Startin from the Central Science Laboratory, United Kingdom, Dr Michal Krzyzanowski from ECEH, Bilthoven Division, Netherlands, Dr Erkki Yrjänheikki from the Ministry of Social Affairs and Health, Finland and Dr F.X. Rolaf van Leeuwen from ECEH, Bilthoven Division, Netherlands

The study was run between February 1996 and April 1997 and was finalised during a consultation meeting held in Prague, Czech Republic, 24-26 November 1997. The meeting was organised by WHO/ECEH in collaboration with the Faculty of Medicine, Charles University of Prague to evaluate and discuss the results of the study and to agree on the final acceptance criteria to be used to select laboratories from which analytical data can be collected for future WHO-coordinated studies to assess exposure of the general population to dioxins and related compounds. The meeting was attended by 26 experts from 14 countries as well representatives from 20 out of the 24 participants laboratories and 3 observers and 4 members of staff from WHO European Centre for Environment and Health, Bilthoven Division.

The specific objectives of this study were:

a) to assess the analytical quality of the participating laboratories, based on statistical evaluation of the between-laboratory comparability and within-laboratory medium-term reliability of the analytical data on all of the individual PCDD, PCDF and PCB congeners included in the study

b) to encourage laboratories to further develop and improve their methodologies

c) to identify laboratories, from which results can be accepted by WHO for exposure assessment studies, by assessing the results of a selected set of the most toxic and/or abundant congeners

d) to develop an international network for analytical service and expert advice.

Study design

The study included assessment of the accuracy and precision of measurements of each of the compounds shown in Table 1. The acceptability of laboratories for WHO exposure assessment studies was determined from data on a subset of these compounds, and was decided separately for compounds with biological activities mediated through the Ah receptor, classified as Group I, and for PCBs commonly regarded as indicator compounds, classified as Group II (see Table 1). Group I consisted of 3 PCDDs, 1 PCDF and 5 PCBs, selected since they each contribute 3% or more to the total TEQ in typical human tissues, when using I-TEFs for PCDD/Fs and WHO-TEFs for PCBs, and their sum accounts for about 90% of the total TEQ for all congeners. Group II consisted of the 3 most abundant congeners of the conventional marker PCBs.

The coordinating committee selected human milk and human blood plasma as the matrices for study in this round. Participants were instructed to measure the concentrations of relevant analytes by reference to their own, in-house, analytical reference standard materials, and using their own internal standard materials where these were required. Participants had a free choice of analytical methods.

Participants were required to perform at least one procedural blank determination, including all of the reagents, solvents and internal standard solutions used in the analysis, with each set of samples and each matrix.

For each matrix, eight different pools were prepared. Each laboratory received one sub-sample from each pool, divided into two separate sets of four samples. Participants were required to report results for the first set of four samples before receiving the second set. The design thus allowed assessment of within-laboratory medium-term consistency without requiring replicate analysis of identical samples.

To allow assessment of any inaccuracy associated with the use of inaccurate reference standards, the study also involved measurement of the concentration of each analyte in a set of supplied solutions. These analyte solutions were included with the first shipment of samples.

Questionnaires on the methodology used were distributed to the participating laboratories with the request to return them to the WHO/ECEH office in Bilthoven. All of the methods used followed the general steps of extraction and fat determination, clean up (and in many cases fractionation), and finally gas chromatographic separation and determination. Many of the laboratories that analysed both milk and blood used different extraction methods for the two types of matrix. With only a few exceptions, laboratories that analysed more than one class of compound did so with a single extraction method and with some common cleanup steps, usually followed by some form of fractionation and further class-specific cleanup. Determinative methods were generally identical for both matrices.

During the consultation it was noted that results for the fat content of plasma apparently falls into two different groups. It was pointed out that in plasma unlike milk fat is a complex mixture of materials of different polarities and that unless a standardised definition or

standardised methods are used the results of the fat determinations were not comparable and were dropped from the analysis

Statistical analysis

Statistical calculations were completed for each substance in each matrix and pool, and included the following steps:

a) removal of obvious outliers
b) removal of unlikely results falling more than 3 standard deviations from the mean
c) calculation of the mean and standard deviation of the remaining values
d) setting of an assigned value for each analyte and pool as the mean from step c
e) calculation of the relative deviation |r| for each reported concentration, defined as the difference of an individual result from the assigned value as a proportion of the assigned value – a between-laboratory measure
f) calculation of relative deviations for the weighted sum of Group I substances and of the unweighted sum of Group II substances in each pool – a between-laboratory measure
g) calculation of z-scores (deviation from the mean in units of standard deviation) for the weighted sum of Group I substances and of the unweighted sum of Group II substances in each pool; followed by the calculation of an aggregated test value (t) for all pools as $\Sigma z/n^{1/2}$ – a between-laboratory measure
h) normalisation of raw results for the differences between pools by subtraction from data for a particular pool of the difference between the assigned value for that pool and the mean value for all pools
i) calculation from the normalised results of the coefficient of variation (CV) for each substance and laboratory across all pools – a within-laboratory measure
j) calculation from the normalised results of the CV for the weighted sum of Group I substances and of the unweighted sum of Group II substances for each laboratory across all pools – a within-laboratory measure
k) For the calculation of the between laboratory comparability and the within laboratory consistency, the results (i.e. the analyte detected and analysed, or sums for Group I and II) from at least 5 (out of 8) samples must have been reported.

Criteria of acceptance

Acceptance was based on the analysis of the TEF-weighted sum of the compounds in Group I and the unweighted sum of the compounds in Group II, as well as on repeatability and reproducibility of the determination of the individual compounds of Group I and II.

Acceptance criteria as given in the study protocol were:
For Group I substances and Group II substances:
- Full reporting of all relevant measurements in all 8 pools.
- Within-laboratory consistency giving a CV <0.1 for the appropriate summed concentration and a CV <0.15 for individual congeners.
- Between-laboratory agreement giving, for the appropriate summed concentrations, |r|<0.3 and T <2.58.

For fat determinations:
- Full reporting of all relevant measurements in all 8 pools and relative deviation |r| <0.1.

After discussion it was agreed that the criterion for within-laboratory consistency for individual substances in Group I, for both milk and plasma, was excessively demanding compared with the criteria for weighted sum (which is of more practical importance) and a revised value of CV <0.3 was agreed by the consultation instead of CV <0.15.

In calculating the TEF-weighted summed concentrations for substances comprising Group I the revised TEFs proposed by a recent WHO consultation (Stockholm, Sweden, 15-18 June 1997) were used; thus 1,2,3,7,8-PeCDD was given a TEF of 1 instead of the previous factor of 0.5. In addition PCB 170 was included in Group I with a weight of 0.0001 since, although it is no longer assigned a TEF, data on this substance was requested as part of the study design.

Results from the laboratories

The overall summary of the results with the number of laboratories that met each of the criteria for acceptance as described above is given in Table2. According to the protocol of the study results of participating laboratories were only considered for statistical analysis when they fulfilled the criterion of full reporting of all the relevant compounds in Group I and Group II (see Table 1) in all pools (8/8).

Laboratories, which the analytical results for dioxin-like compounds, marker PCBs and fat in human milk and in plasma can be accepted by WHO, are indicated bellow.

LABORATORIES WHICH MET CRITERIA FOR ANALYSES OF PCDDS, PCDFS AND DIOXIN-LIKE PCBS IN HUMAN MILK

(listed in alphabetical order of countries)

Laboratory	**Contact information**
AXYS Analytical Services/Axys Varilab P.O. Box 2219 Sidney BC V8L 3S8 Canada	Telephone: +1 250 655 5800 Telefax: +1 250 655 5811 E-mail: dthomas@axys.com (Mr David Thomas)
Health Canada Bureau of Chemical Safety Banting 2203 D, Ross Ave. Tunney's Pasture Ottawa, Ontario K1A OL2 Canada	Telephone: +1 613 9570978 Telefax: +1 613 9414775 E-mail: Jake_ryan@hc-sc.gc.ca (Dr John Jake Ryan)
Chemisches und Veterinäruntersuchungsamt Freiburg (State Institute for Chemical and Veterinary Analysis of Food) Bissierstr. 5 D-79114 Freiburg Germany	Telephone +49 761 8855133 +49. 761 8855 0 (switchboard) Telefax: : +49 761 8855100 E-mail: malisch@clua.cvuafr.bwl.de (Dr Rainer Malisch)

Total 3 laboratories

LABORATORIES WHICH MET CRITERIA FOR ANALYSES OF MARKER PCBS IN HUMAN MILK

(listed in alphabetical order of countries)

Laboratory	**Contact information**
Flemisch Institute for Technological Research (VITO) Gebouw LMB, Boeretang 200 B-2400 Mol Belgium	Telephone: +32 14 335020 Telefax: +32 14 319472 E-mail: VCLEUVER@VITO.BE (Dr Rudy Van Cleuvenbergen)
National Public Health Institute Division of Environmental Health Laboratory of Chemistry P.O. Box 95 FIN-70701 Kuopio Finland	Telephone: +358 17 201346 Telefax: +358 17 201265 E-mail:
Chemisches und Veterinäruntersuchungsamt Freiburg (State Institute for Chemical and Veterinary Analysis of Food) Bissierstr. 5 D-79114 Freiburg Germany	Telephone +49 761 8855133 Telefax: +49 761 8855100 E-mail: malisch@clua.cvuafr.bwl.de (Dr Rainer Malisch)
Norwegian College of Veterinary Science Dept. of Pharmacology & Toxicology P.O. Box 8146, Dep. N-0033 Oslo Norway	Telephone: +47 22 96 4960 Telefax: +47 22 59 7089 E-mail: Anuschka.Polder@veths.no (Dr Anuschka Polder)
Institute of Preventive and Clinical Medicine Laboratory of Toxic Organic Pollutants Limbova 14 83301 Bratislava The Slovak Republic	Telephone: +421 7 59369218 Telefax: +421 7 59369217 E-mail: Kocan@upkm.sk (Dr Anton Kocan)
Institute of Environmental Chemistry University of Umeå c/o FOA ABC-Skydd Cementvägen 20 S-901 87 Umeå Sweden	Telephone: +46 90 786 7622 Telefax: +46 90 186155 E-mail:

LABORATORIES WHICH MET CRITERIA FOR ANALYSES OF MARKER PCBS IN HUMAN MILK (CONT'D)

National Food Administration
P.O. Box 622
S-751 26 Uppsala
Sweden

Telephone: +46 18 171462
Telefax: +46 18 105848
E-mail: saat@slv.se
(Dr Samuel Atuma)

total 7 laboratories

LABORATORIES WHICH MET CRITERIA FOR ANALYSES OF PCDDS, PCDFS AND DIOXIN-LIKE PCBS IN HUMAN PLASMA

(listed in alphabetical order of countries)

Laboratory	**Contact information**
AXYS Analytical Services/Axys Varilab P.O. Box 2219 Sidney BC V8L 3S8 Canada	Telephone: +1 250 655 5800 Telefax: +1 250 655 5811 E-mail: dthomas@axys.com (Mr David Thomas)

total 1 laboratory

LABORATORIES WHICH MET CRITERIA FOR ANALYSES OF MARKER PCBS IN HUMAN PLASMA

(listed in alphabetical order of countries)

Laboratory	**Contact information**
AXYS Analytical Services/Axys Varilab P.O. Box 2219 Sidney BC V8L 3S8 Canada	Telephone: +1 250 655 5800 Telefax: +1 250 655 5811 E-mail: dthomas@axys.com (Mr David Thomas)
National Public Health Institute Division of Environmental Health Laboratory of Chemistry P.O. Box 95 FIN-70701 Kuopio Finland	Telephone: +358 17 201346 Telefax: +358 17 201265
National Institute of Public Health and the Environment (RIVM) Laboratory for Organic-Analytical Chemistry P.O. Box 1 / postbak 11 NL-3720 BA Bilthoven The Netherlands	Telephone: +31 30 274 2871 Telefax: +31 30 274 4424 E-mail: Djien.Liem@rivm.nl (Dr A.K.Djien Liem)

total 3 laboratories

LABORATORIES WHICH MET CRITERIA FOR ANALYSES OF FAT IN HUMAN MILK

(listed in alphabetical order of countries)

Laboratory	**Contact information**
Flemisch Institute for Technological Research (VITO) Gebouw LMB, Boeretang 200 B-2400 Mol Belgium	Telephone: +32 14 335020 Telefax: +32 14 319472 E-mail: VCLEUVER@VITO.BE (Dr Rudy Van Cleuvenbergen)
Enviro-Test Laboratories 9936-67 Avenue Edmonton, Alberta T6E OP5 Canada	Telephone: +1 780 413 5236 Telefax: +1 780 437 2311 E-mail: LEANNE@envirotest.com (Ms Leanne Lawrence)
Laboratory for Biochemistry and Organic-Analytical Chemisry Institute for Medical Research and Occupational Health Ksaverska cesta 2 P.O. Box 291 HR-10001 Zagreb Croatia	Telephone: +385 1 4673 188 Telefax: +385 1 4673 303 E-mail: bkrautha@imi.hr (Dr Blanka Krauthacker)
Danish Veterinary and Food Administration Division of Chemical Contaminants Mørkhøj Bygade 19 DK-2860 Søborg Denmark	Telephone: +45 33 956000 Telefax: +45 33 956001 E-mail: Tce@fdir.dk (Dr Tommy Cederberg)
Chemisches und Veterinäruntersuchungsamt Freiburg (State Institute for Chemical and Veterinary Analysis of Food) Bissierstr. 5 D-79114 Freiburg Germany	Telephone: +49 761 8855133 Telefax: +49 761 8855100 E-mail: malisch@clua.cvuafr.bwl.de : (Dr Rainer Malisch)
Dr E. Wessling Chemisches Laboratorien GmbH Labor Altenberge Oststrasse 6 D-48341 Altenberge Germany	Telephone: +49 2505 89144 Telefax: +49 2505 89119 E-mail: Labor@wessling-gruppe.de (Dr Hans-Joachim Mosche)

LABORATORIES WHICH MET CRITERIA FOR ANALYSES OF FAT IN HUMAN MILK (CONT'D)

Laboratory	Contact information
Staatliches Veterinär- und Lebensmitteluntersuchungsamt Potsdam P.O. Box 601452 D-14467 Potsdam Germany	Telephone: +49 331 5688 342 Telefax: +49 331 5688 226/346 E-mail: Thomas.wiesmueller@svla.brandenburg.de (Dr Thomas Wiesmüller)
National Institute of Public Health and the Environment (RIVM) Laboratory for Organic-Analytical Chemistry P.O. Box 1 / postbak 11 NL-3720 BA Bilthoven The Netherlands	Telephone: +31 30 274 2871 Telefax: +31 30 274 4424 E-mail: Djien.Liem@rivm.nl (Dr A.K.Djien Liem)
Consejo Superior de Investigaciones Cientificas (CSIC) Dept. of A.I. and Environmental Chemistry Institute of Organic Chemistry Juan de la Cierva, 3 - 28006 Madrid Spain	Telephone: +34 915622900 Telefax. +34 915644853 E-mail: mariche@iqog.csic.es (Dr Maria José Conzalez)

Total 9 laboratories

Table 1. Compounds assessed in the fourth round of interlaboratory quality assessment studies. Compounds used for the assessment of acceptance by WHO for exposure assessment studies are indicated in bold type and by * for Group I and ** for Group II

PCDDs	PCDFs	Non-ortho PCBs	Other 'planar' PCBs	Marker PCBs
2,3,7,8-TCDD*	2,3,7,8-TCDF	PCB 77	PCB 105	PCB 28
1,2,3,7,8-PeCDD*	1,2,3,7,8-PeCDF	**PCB 126***	PCB 114	PCB 52
1,2,3,4,7,8-HxCDD	**2,3,4,7,8-PeCDF***	PCB 169	**PCB 118***	PCB 101
1,2,3,6,7,8-HxCDD*	1,2,3,4,7,8-HxCDF		PCB 123	**PCB 138****
1,2,3,7,8,9-HxCDD	1,2,3,6,7,8-HxCDF		**PCB 156***	**PCB 153****
1,2,3,4,6,7,8-HpCDD	1,2,3,7,8,9-HxCDF		**PCB 157***	**PCB 180****
OCDD	2,3,4,6,7,8-HxCDF		PCB 167	
	1,2,3,4,6,7,8-HpCDF		[1]**PCB 170***	
	1,2,3,4,7,8,9-HpCDF		PCB 189	
	OCDF			

[1]PCB 170 was included in Group I with a weight of 0.0001 but it is no longer assigned a TEF (revised TEFs proposed by WHO consultation, Stockholm, Sweden, 15-18 June 1997)

Table 2. Number of laboratories that met each of the criteria for acceptance in the fourth round study Interlaboratory Quality Assessment on Levels of PCBs, PCDDs and PCDFs in human Milk and Blood Plasma

WHO - Criteria	Human Milk	Plasma
GROUP I Compounds		
Number of participants*	11	7
8 out of 8	9	3
T<2.58 (TEQ)	8	4
|r|<0.3 (TEQ)	9	4
CV<0.1 (TEQ)	4	4
CV<0.3 (congeners)	4	2
All criteria	3	1
GROUP II Compounds		
Number of participants*	19	15
8 out of 8	17	14
T<2.58 (TEQ)	15	10
|r|<0.3 (TEQ)	10	9
CV<0.1 (TEQ)	9	6
CV<0.15 (congeners)	7	5
All criteria	7	3
FAT		
Number of participants	22	15
8 out of 8	20	14
|r|<0.1	9	Deleted

* The number of participants refers to laboratories that reported data for all compounds of Group I or Group II ('full reporting of data').