RESULTS OF THE THIRD ROUND OF THE WHO-COORDINATED EXPOSURE STUDY ON THE LEVELS OF PCBs, PCDDs AND PCDFs IN HUMAN MILK

FX Rolaf van Leeuwen[1] and Rainer Malisch[2]

[1]National Institute of Public Health and the Environment, P.O.Box 1, NL-3720 BA Bilthoven, The Netherlands (coordinator of the 3rd round on behalf of WHO)

[2]State Institute for Chemical and Veterinary Analysis of Food, Bissierstrasse 5, Freiburg, Germany (reference laboratory of the 3rd round of the WHO study)

Introduction

Chlorinated hydrocarbons such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are globally distributed in the environment, and people are exposed to them from numerous sources, of which foodstuffs are, by far, the most important one. These compounds are lipid-soluble, poorly eliminated and therefore accumulate in human adipose tissues. They can pass through the placenta causing exposure of the foetus, and their existence in human milk causes additional exposure of infants during the lactating period.

Breast milk contains many lipid soluble compounds that are also present in mother's adipose tissue. It can be assumed that the levels of PCDDs, PCDFs and PCBs in breast milk are representative for those in plasma, serum lipid and adipose tissue. Therefore levels of these contaminants in human milk do reflect the body burden and can thus be used as an indicator for the overall exposure of (part of) the general population. The advantage of studying levels of PCDDs, PCDFs and PCBs in human milk is based upon the non-invasive character of the method and the high content of fat, making the extraction method easier and the precision of the measurements higher.

Since the mid-eighties, the WHO Regional Office for Europe, in collaboration with other international organisations and national institutions, has coordinated a comprehensive programme on possible health risks of PCBs, dioxins and furans, especially in infants, due to exposure through contaminated breast-milk, and aiming to prevent and control environmental exposure to these chemicals. International exposure studies on levels of these contaminants in breast milk were of special interest for this programme. The first WHO-coordinated exposure study took place in 1987-1988 (1), the second round in 1992-1993 (2).

Nineteen countries participated in the second round, in which concentrations of PCBs, PCDDs and PCDFs were determined in milk samples collected in a total of 47 areas. In industrialised countries mean levels ranged from 10-35 pg I-TEQ/g, and in developing countries mean levels were less than 10 pg I-TEQ/g milk fat. Compared to the outcome of the first round, a trend towards declining levels of PCDDs/PCDFs and PCBs in breast milk was found, with largest declines in countries with the highest initial levels. For PCBs rather high exposure levels were found in certain areas in Europe (2).

The third round of the WHO-coordinated exposure study has been initiated in March 2000. In order to collect data on more countries, also beyond the European region, the study was organised in collaboration with the International Programme on Chemical Safety (IPCS) and the WHO Global Environment Monitoring System/Food Contamination Monitoring and Assessment (GEMS Food).

The current exposure study has the following aims: a) produce reliable and comparable data on

levels of PCBs, PCDDs and PCDFs in human milk for further improvement of the health risk assessment in infants; b) determine time trends in exposure levels in the countries and areas already studied in the first and second round of the study in the period 1987-88 and 1992-1993, respectively; c) provide a better overview of exposure levels in various countries and geographical areas; d) identify highly exposed local populations in relation to their daily intake for guidance on risk management actions, including epidemiological follow-up studies; e) promote, if necessary, additional national studies to be closely linked with the present study through the use of the same protocol.

Methods

Protocol

To ascertain comparability of results, a protocol for the study was developed that was followed by each of the participating countries. This protocol was only slightly modified compared to the one used in the previous round, and dealt primarily with number and type of samples, selection of donors, collection, storage and pooling of samples, and shipping of samples to the reference laboratory. In addition, a questionnaire was distributed that served as a basis to interview donating mothers in order to collect relevant, demographic data.

Milk from well-defined groups of 10 mothers, living in areas with different exposure levels, was collected and pooled. It was the aim of the study to include at least two different groups from each country in the study; for example, an expected high- and low-exposure group. Inclusion of additional groups from areas with potential high exposure or specific food consumption habits was recommended.

For selection of donating mothers the following criteria were applied: a) they should be primiparae, b) healthy, c) exclusively breastfeeding one child (i.e. no twins), and d) residing in the area for about 5 years.

At least 50 ml of milk was collected from each mother. The portions collected during each feeding were added to the collecting bottle and stored in the home freezer until the total volume has been collected. Individual samples were homogenised (shaking for 10 minutes) before pooling. Pooling of 10 individual samples produced a total of 500 ml of pooled milk, available for analysis.

Pooled samples were identified by an unique code and appropriate description, and shipped to the reference laboratory in dry ice. If receipt by the reference laboratory could not be guaranteed within 2 days, and thawing of samples had to be expected, $K_2Cr_2O_7$ (0.1% w/w) was added.

Analysis

All samples were analysed by the reference laboratory of the current study, thereby guaranteeing the comparability of results form different countries. Solvent extraction, clean-up and determination by GC-MS of three groups of compounds (PCDDs/PCDFs, coplanar PCBs and marker PCBs) was carried out following the same procedure as used for the WHO interlaboratory quality assessment study (3). Details of the procedure are described elsewhere in this volume (4). For PCDDs, PCDFs and dioxin-like PCBs the results of the analysis are expressed as pg WHO-TEQ/g fat, using the WHO-TEFs as derived in 1997 (5). The data represent upper-bound concentrations; the mean of the difference between upper and lower-bound concentrations is 0.2 %.

A rigid quality control programme was carried out, including blank samples, spiked vegetable oil samples on different levels, and five different kinds of quality control samples (two butter samples and two egg samples of different levels of contamination and WHO pooled breast milk samples which remained from the WHO interlaboratory quality assessment study (3)). Samples were analysed in a way which can best be described as “overlapping sandwich method”: a large portion of the samples was analysed as duplicate analyses, with the duplicate analyses being performed in sequences with samples from other countries and with different quality control samples. This guarantees that the

results of all samples from different countries have the same reliability, even in the situation where receipt of the various samples by the reference laboratory spanned a period of more than one year.

Results and Discussion

Eighteen countries (Brazil, Bulgaria, Croatia, Czech Republic, Egypt, Finland, Hungary, Ireland, Italy, New Zealand, Norway, Romania, Russia, Slovak Republic, Spain, Sweden, The Netherlands, Ukraine) sent samples for analyses to the reference laboratory before 31-12-2001. In addition, two samples from Australia were received by the laboratory in April 2002. For these two samples the rigid quality control programme could not be finished before submission of this manuscript, and therefore the presented results of the Australian samples should be considered as preliminary. Samples from Germany and Philippines are expected to be received by the reference laboratory on short notice. All together, pooled samples were collected from 62 different areas. The number of areas per country varied from 1-9.

Table 1. Levels of PCDDs, PCDFs and dioxin-like PCBs in human milk (2001/2002)

Country	PCDDs/PCDFs WHO-TEQ pg/g fat		PCBs WHO-TEQ pg/g fat		Number of pools
	median	*range*	*median*	*range*	
Australia	5.65	5.50 – 5.79	3.09	2.48 – 3.69	2
Brazil	3.93	2.73 – 5.34	1.81	1.30 – 12.30	9
Bulgaria	6.14	5.08 – 7.11	4.21	3.74 – 4.70	3
Croatia	6.40	5.99 – 6.80	7.17	6.82 – 7.52	2
Czech Republic	7.78	7.44 – 10.73	15.24	14.32 – 28.48	3
Egypt	22.79	17.16 – 51.50	6.01	4.43 – 8.26	7
Finland	9.44	9.35 – 9.52	5.85	5.66 – 6.03	2
Hungary	6.79	5.26 – 7.46	2.87	2.38 – 4.24	3
Ireland	6.91	6.19 – 8.54	4.66	2.72 – 5.19	3
Italy	12.66	9.40 – 14.83	16.29	11.02 – 19.33	4
New Zealand	6.86	6.08 – 7.00	3.92	3.50 – 4.71	3
Norway	7.30	7.16 – 7.43	8.08	6.56 – 9.61	2
Romania	8.86	8.37 – 12.00	8.06	8.05 – 8.11	3
Russia	8.88	7.46 – 12.93	15.68	13.38 – 22.95	4
Slovak Republic	9.07	7.84 – 9.87	12.60	10.72 – 19.49	4
Spain	11.90	10.41 – 18.32	11.65	9.96 – 16.97	3
Sweden	9.58	–	9.71	–	1
The Netherlands	18.27	17.09 – 21.29	11.57	10.90 – 13.08	3
Ukraine	10.04	8.38 – 10.16	19.95	14.10 – 22.00	3

Table 2. Levels of indicator PCBs in human milk (2001/2002)

Country	Sum indicator PCBs ng/**g fat**	
	median	*range*
Australia	30	25 – 35
Brazil	16	10 – 96

Bulgaria	42	32 – 52
Croatia	135	121 – 150
Czech Republic	502	496 – 1009
Egypt	116	97 – 140
Finland	91	84 – 98
Hungary	34	29 – 59
Ireland	61	41 – 64
Italy	253	195 – 323
New Zealand	37	30 – 41
Norway	119	106 – 132
Romania	173	165 – 198
Russia	138	83 – 311
Slovak Republic	443	331 – 621
Spain	399	278 – 469
Sweden	146	–
The Netherlands	191	178 – 210
Ukraine	136	102 – 148

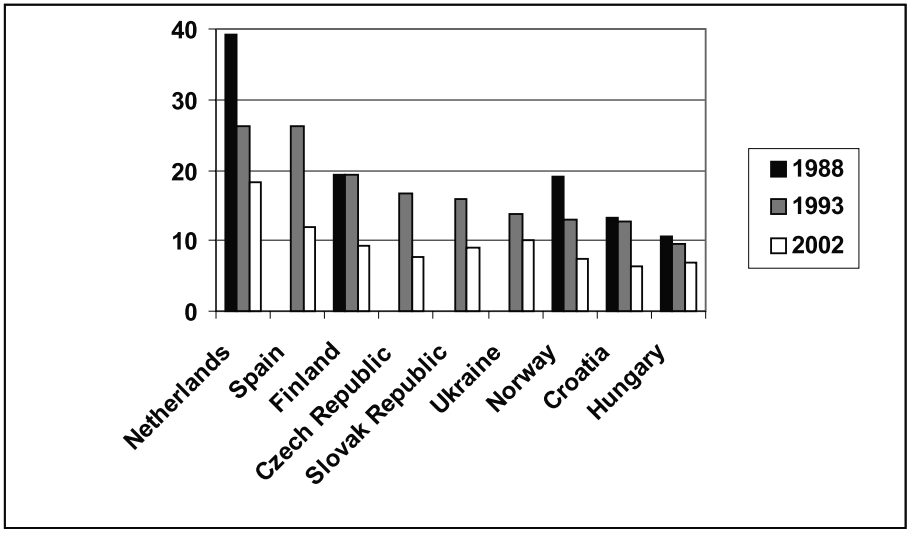

Fig.1. Temporal trends of levels of PCDD/PCDF in human milk

The results of the third round as given in Table 1, show that, in general, variation between countries is much higher that within countries. In some countries specific sites of contamination could be identified. In Europe, industrialised countries like the Netherlands, Italy and Spain show relatively high levels of PCDDs and PCDFs (median values range from about 12-18 pg WHO-TEQ/g fat). Low PCDD/PCDF levels have been found in Bulgaria, Croatia, Hungary, and Ireland (median values range from about 6-7 pg WHO-TEQ/g fat). Outside Europe, very high PCDD/PCDF levels have been found in Egypt (median 22,8 pg WHO-TEQ/g fat), whereas Brazil shows the lowest PCDD/PCDF levels in this study (median about 3.7 pg WHO-TEQ/g fat), followed by Australia and New Zealand.

High levels of dioxin-like PCBs were found in human milk from Ukraine, Italy and the Czech Republic (median values range from about 15-20 pg WHO-TEQ/g fat). Low levels (< 4 pg WHO-TEQ/ g fat) were found in pooled samples from Brazil, Hungary, Australia and New Zealand.

Temporal trends in levels of PCDDs and PCDFs in human milk for countries participating in the current study as well as in one of the previous rounds, are indicated in Fig.1. It is obvious, that the declining trend observed before, continues. On average, the decline between the levels found in 2nd round in 1993 and those found in the current study is about 40%.

Also the levels of indicator PCBs in human milk (Table 2) vary widely between the countries, with lowest levels (median: 14 – 37 ng/g fat) found in Brazil, Australia and New Zealand, and highest levels (median: 400 – 500 ng/g fat) in Spain, Slovak Republic and Czech Republic.

In summary, there are only a few countries (Australia, Brazil, Bulgaria, Croatia and Hungary) for which the observed levels for all the three groups of compounds (PCDD/Fs, dioxin-like PCBs and indicator PCBs) are consistently lower than in other countries. Countries like Italy, Spain and The Netherlands consistently show high levels for these three groups of compounds. Other countries show varying profiles, indicating different sources of contamination. In addition, for a number of countries, pools (areas) were identified as being different from the other pools obtained from the same country. Usually these pools show a higher level of contamination, as is obvious from a comparison of the median values and the ranges as given in Table 1 and 2. The most striking examples are Brazil, Czech Republic and Egypt, where areas were identified with a high contamination with dioxin-like PCBs and/ or indicator PCBs, in relation to the samples from other areas in the same country. Analyses of the pattern of the various congeners and the demographic data can provide a clarification for the reason of these differences.

The declining trend in the levels of PCDDs and PCDFs indicates a continuing decline of exposure of the general population as a result of emission reduction measures that have been taken in the past. It is, however, recognised that breast-fed infants are still exposed to high intakes of these compounds (on a body weight basis higher than adults), although for a small proportion of their lifespan. However, taking into account the well-proven and accepted benefits of breastfeeding for developing infants, WHO repeatedly and strongly recommended that breastfeeding be encouraged and promoted, particularly in view of the declining trend in levels of these compounds in human milk (6,7,8). The results of the current study support that recommendation.

Acknowledgement

We would like to thank Mrs. Tritschler and Mr. Huber for their technical support and the reliable performance of all steps of the comprehensive analytical method and quality control, and Mr. Winterhalter for running the high resolution mass spectrometer.

References

1. Environmental Health Series No 34 (1989): Levels of PCBs, PCDDs, and PCDFs in breast milk, WHO Regional Office for Europe, Copenhagen, Denmark.
2. Environmental Health in Europe No. 3 (1996): Levels of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in human milk: Second round of WHO-coordinated exposure study), WHO Regional Office for Europe, Copenhagen, Denmark.
3. Interlaboratory Quality Assessment of Levels of PCBs, PCDDs and PCDFs in Human Milk and Blood Plasma (2000), WHO Report EUR/00/5020352, WHO Regional Office for Europe, Copenhagen, Denmark.

4. Malisch R and van Leeuwen FXR (2002) Third round of WHO-coordinated exposure study: Analytical procedure for determination of PCDDs, PCDFs and PCBs in human milk, submitted to Dioxin 2002, see corresponding volume of Organohalogen Compounds.
5. Van den Berg M, Birnbaum L, Bosveld BTC, Brunström B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FXR, Liem AKD, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk, D, Tillitt D, Tysklind M, Younes M, Waern F and Zacharewski T (1998) Environ. Health Perspect. 106 (12), 775-792 .
6. Environmental Health Series No 29 (1988) Assessment of health risks in infants associated with exposure to PCBs, PCDFs and PCDFs in breast milk, WHO Regional Office for Europe, Copenhagen, Denmark.
7. Brouwer A, Ahlborg UG, van Leeuwen FXR and Feeley MM (1998) Chemosphere, 34, 1627-1643.
8. Van Leeuwen FXR and Younes MM, Eds. (2000) Food Additives and Contaminants Vol 17 Number 4, 223-369.