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Evaluation of Toxic Equivalent Quantity of Dioxins in Human Milk Using Different Toxicity Equivalence Factors

K. Saito,[1] A. Ohmura,[2] M. Takekuma,[2] M. Fukui,[1] Y. Iwasaki,[1] R. Ito,[1] Y. Matsuki,[3] H. Nakazawa[1]

[1] Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan
[2] Biological Effect Research Group, Saitama Institute of Public Health, 639-1 Kamiokubo, Sakura-ku, Saitama-shi 338-0824, Japan
[3] Institute of Food Hygiene, Japan Food Hygiene Association, 2-5-47 Tadao, Machida, Tokyo 194-0035, Japan

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The contamination of food and the ecosystem by dioxins and its resultant effects on our health have been drawing much attention from the public. Thus, the investigation of human exposure to dioxins is an urgent and important task for the government. Since "The Law Concerning Special Measures against Dioxins" took effect in Japan in 1999, the number of substances to be sampled and measured has been increasing. We have been part of the development of a Japanese official analytical method for dioxins in human milk and blood, and food. In addition, we have participated in a collaborative study of dioxins in food as well as human milk and blood in order to validate the method, and have contributed to improving public health. However, there remain some problems in the assessment of dioxin levels in human milk and blood and other samples. So far, the toxicity evaluation of dioxins has been done by using only 2,3,7,8-substituted congeners, called "toxic equivalency factors (TEFs)" (Van Zorge et al. 1989). Several TEFs have been reported, including those from Switzerland (1982), Denmark (1984), FRG (1985), Ontario (1985), US EPA (1987, 1989), Nordic-TEF (1988), NATO/CCMS (I-TEF) (1988), Netherlands (1989), WHO-TEF (1993) and WHO-TEF (1998), which are widely used for calculating the toxicity equivalent quantity (TEQ) (Van den Berg et al. 1998).

Differences have been observed in the literature TEQ values particularly in the 1990s, mainly because many regulatory bodies and researchers used different TEFs in their calculations (Schecter et al. 1989). Therefore, it is difficult to compare hitherto documented research results and to evaluate the TEQs obtained from the different TEFs. In this regard, we examined whether it was possible to compare the TEQs obtained by using old TEFs with that obtained by using the latest TEF, in the analysis of human milk. In addition, we attempted to derive a factor with high reliability for the mutual correction of the TEQs.

MATERIALS AND METHODS

All dioxin standards were from Wellington Laboratories (Canada) and were diluted with decane to the appropriate concentrations. Most of the organic

Correspondence to: K. Saito

solvents, such as hexane, acetone, dichloromethane, toluene, diethyl ether, ethanol and methanol, were of dioxin analysis grade and were from Kanto Kagaku (Tokyo, Japan) or Wako Pure Chemicals (Osaka, Japan). Decane was of reagent grade and was redistilled prior to use, because decane of dioxin analysis grade was not available. All other chemicals were of PCB analysis grade or high quality reagent grade.

The multi-layered silica gel column packed in a disposable cartridge tube was from Supelco (USA). It consisted of the following layers: 0.9 g of silica gel, 3 g of 2% KOH/silica gel, 0.9 g of silica gel, 4.5 g of 44% H_2SO_4/silica gel, 6 g of 22% H_2SO_4/silica gel, 0.9 g of silica gel and 3 g of 10% $AgNO_3$/silica gel. Six grams of sodium sulfate was manually added on top of the $AgNO_3$/silica gel layer in the column. The column was washed with 100 mL of hexane prior to use.

Human milk samples were collected from 150 Japanese primiparae. The sample pretreatment for dioxin analysis was carried out in accordance with a slightly modified method from the official manual of the Ministry of Health, Labour and Welfare, Japan. Briefly, approximately 50 g of milk sample was used for the analysis. Fat was extracted from the sample according to a previously described procedure (Saito et al. 2003a). A stable isotope of each congener of the PCDD/Fs and coplanar PCBs (Co-PCBs) was added as surrogate after the fat was extracted from the sample. The fat was dissolved in ca. 2 mL of hexane, and then the whole mixture was applied to the multi-layered silica gel column. After eluting the column with 160 mL of hexane, the eluate was concentrated to ca. 1 mL. This was subjected to chromatography on an activated carbon/silica gel column (0.5 g of activated carbon/silica gel was pre-packed in a manner similar to that described previously (Saito et al. 2003a, 2003b)), and was subsequently fractionated as follows. First, 10 mL of hexane was added to elute most of the non-planar PCBs, and then 40 mL of a mixed solvent of dichloromethane/hexane (25:75, v/v) was added to elute 8 mono-ortho PCBs and 2 di-ortho PCBs. Finally, 100 mL of toluene was added to elute 17 PCDD/Fs and 4 non-ortho PCBs.

The dioxins were subjected to HR-GC/MS with a JEOL JMS-700 mass spectrometer (JEOL Ltd., Tokyo, Japan) equipped with a DB-17HT capillary column (30 m x 0.25 mm i.d., 0.15 μm film thickness) for PCDD/Fs and non-ortho PCBs, or a DB-5MS capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness) for mono-ortho PCBs and di-ortho PCBs, with helium as the carrier gas at a linear velocity of 35 cm/s in the splitless injection mode (1 μl). The GC program was as follows: 150 °C (1 min) to 220 °C (0 min) at 20 °C/min and subsequently at 4 °C/min to 280 °C, then maintained for 16.5 min at 280 °C, for both DB-17HT and DB-5MS columns. The injector temperature was 280 °C and the GC/MS interface temperature was held at 280 °C. The MS was operated in the selected ion monitoring mode with a mass resolution of 10,000, and the electron impact ionization energy was 38 eV at an ion source temperature of 260 °C. The PCDD/Fs, non-ortho PCBs and mono-ortho PCBs were quantified using a

molecular ion (M^+), an M^{+2} ion or an M^{+4} ion. The TEQ was calculated as described below.

All dioxin congeners having various TEFs, such as I-TEF, WHO-TEF (1993) and WHO-TEF (1998), were measured, and the TEQs were calculated in the following manner.

(1) 17 PCDD/Fs were calculated by using I-TEF, and 3 non-ortho PCBs were calculated by using Ahlborg's TEF (Ahlborg et al. 1994).
(2) 17 PCDD/Fs, 3 non-ortho PCBs, 8 mono-ortho PCBs and 2 di-ortho PCBs were calculated by using WHO-TEF (1993).
(3) 17 PCDD/Fs, 4 non-ortho PCBs and 8 mono-ortho PCBs were calculated by using WHO-TEF (1998).

A comparative study of the correlation between the TEQs was performed, and a factor necessary for the mutual correction of the TEQs was calculated.

RESULTS AND DISCUSSION

Co-PCBs are the so-called dioxin-like PCBs, and are classified into three kinds (non-ortho, mono-ortho and di-ortho PCBs) according to the chlorine atom substitution position. Some TEFs have been proposed for the dioxin-like PCBs (Safe 1990; Ahlborg et al., 1994). TEFs were assigned to only three kinds of non-ortho PCBs in Ahlborg's TEF, while they were assigned to three kinds of non-ortho PCBs, eight kinds of mono-ortho PCBs, and two kinds of di-ortho PCBs in WHO-TEF (1993), and to only four kinds of non-ortho PCBs and eight kinds of mono-ortho PCBs in WHO-TEF (1998).

Because the di-ortho PCBs are not considered to be measurement objects in the currently used official analytical method for WHO-TEF (1998), the fractionation behavior of the PCBs by activated carbon/silica gel column chromatography in the official analytical method was examined. As a result, we found that the first 10 mL of hexane was able to eliminate most of the non-planar PCBs, 8 mono-ortho PCBs and 2 di-ortho PCBs were fractionated into the 40 mL eluate of dichloromethane/hexane (25:75, v/v), and 17 PCDD/Fs and 4 non-ortho PCBs were fractionated into the 100 mL toluene eluate. Therefore, the simultaneous determination of 8 mono-ortho PCBs and 2 di-ortho PCBs was achieved in the second fraction eluted with dichloromethane/hexane (25:75, v/v).

Human milk samples were analyzed using the proposed method, and data analyses were carried out according to the above protocol. The detection limits (pg/g) for the respective analytes were as follows: 0.02 for 4-5CDD/Fs, 0.05 for 6-7CDD/Fs, and 0.1 for OCDD/F, non-ortho PCBs and mono-ortho PCBs.

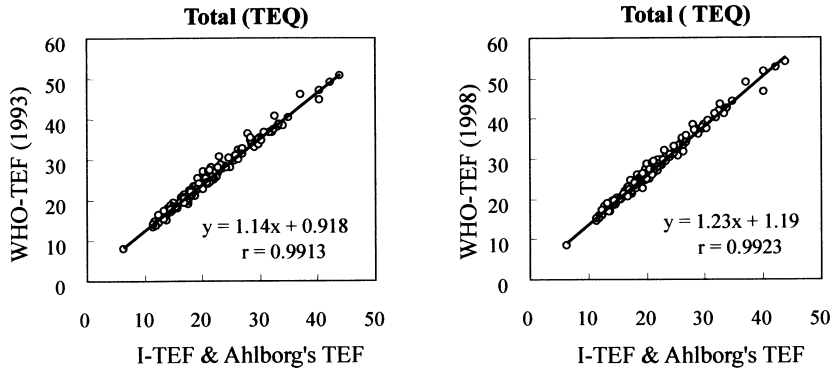

Figure 1. Correlation among the total TEQs (PCDD/Fs + Co-PCBs) in human milk samples obtained by using 3 kinds of TEFs (I-TEF & Ahlborg's TEF, WHO-TEF (1993), and WHO-TEF (1998)).

Table 1. TEQ correction factors obtained by using various TEFs*

	I-TEF**	WHO-TEF (1993)	WHO-TEF (1998)
PCDD/Fs (TEQ)	1	1	1.19
Co-PCBs (TEQ)	1	1.23	1.20
Total TEQ	1	1.14	1.23

*: Correction factor when TEQ obtained by using I-TEF is assumed to be one
**: TEQ of Co-PCBs was calculated by using Ahlborg's TEF

We confirmed that there were significant correlations among the total TEQs (PCDD/Fs + Co-PCBs) calculated from respective TEFs (Figure 1). As regards the regression line, a first-order straight line was obtained by the combination of any two of the three TEQs. It is assumed that it was possible to treat the slopes of the regression line as the correction factor between the TEQs. The correction factors are shown in Table 1. Each correction factor was expressed as the ratio of the TEQ obtained from I-TEF (Ahlborg's TEF was used for non-ortho PCBs) to that obtained from WHO-TEF (1993) or WHO-TEF (1998).

As expected, total TEQ (PCDD/Fs + Co-PCBs) was confirmed to increase in the order of I-TEF < WHO-TEF (1993) < WHO-TEF (1998). The ratio was 1:1.14:1.23. The I-TEF of 20 congeners is available (including 3 non-ortho PCBs from Ahlborg's TEF), whereas the WHO-TEF (1998) and the WHO-TEF (1993) of 29 and 30 congeners, respectively, are available. It seemed that the TEQ calculated by using WHO-TEF (1998) showed the highest value, because the TEF value (TEF = 1) for 1,2,3,7,8-PeCDD in WHO-TEF(1998), which is frequently detected in human milk at a comparatively high level, is twice as large as that (TEF = 0.5) of WHO-TEF(1993) and I-TEF.

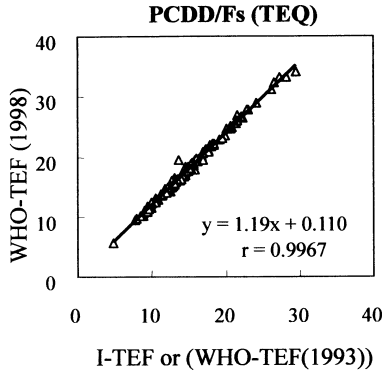

Figure 2. Correlation among the TEQs of PCDD/Fs in human milk samples obtained by using 3 kinds of TEFs (I-TEF, WHO-TEF (1993), and WHO-TEF (1998)).

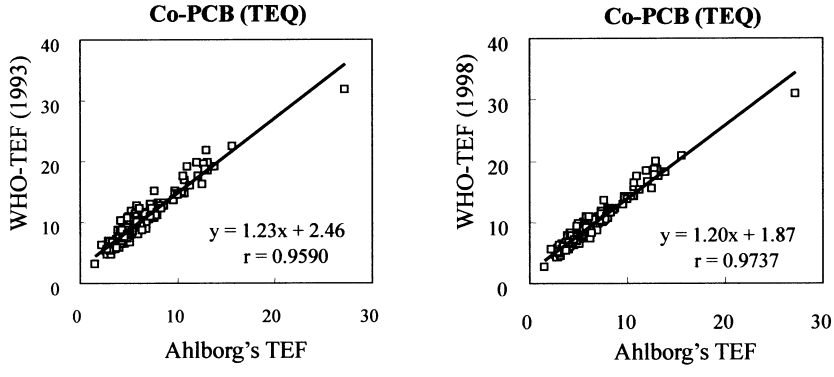

Figure 3. Correlation among the TEQs of Co-PCBs in human milk samples obtained by using 3 kinds of TEFs (Ahlborg's TEF, WHO-TEF (1993), and WHO-TEF (1998)).

Similar data were obtained for PCDD/Fs, i.e., there were significant correlations among the TEQs of PCDD/Fs that were calculated from respective TEFs, as shown in Figure 2. As for the PCDD/Fs in both I-TEF and WHO-TEF(1993), because the kinds of congeners and the TEF values mutually coincided, the correction factor of WHO-TEF(1993) was 1. There were also significant correlations among the TEQs of Co-PCBs that were calculated from respective TEFs, as shown in Figure 3. However, in Co-PCBs, the TEQ was increased in the order of I-TEF (Ahlborg's TEF) < WHO-TEF(1998) < WHO-TEF(1993). The ratio was 1:1.20:1.23. The reason for this are as follows; 1) two kinds of di-ortho

PCBs such as #170 and #180, were measured although one non-ortho PCB (#81) was not measured in WHO-TEF(1993) compared with WHO-TEF(1998). 2) The di-orhto PCBs were present at high levels compared with other PCBs while the residual level of PCB #81 was low. 3) Because the TEQs of the di-ortho PCBs made up approximately 10% of the TEQs of all the Co-PCBs, the TEQ obtained from WHO-TEF (1993) indicated a value that was higher than that obtained from WHO-TEF (1998).

On the basis of these results, the conversion from a certain TEQ into another TEQ has become possible, even if the TEFs of the dioxin congeners to be measured are different. Moreover, even if neither mono-ortho PCBs nor di-ortho PCBs were actually measured in the past, their TEQs could be calculated by using the correction factor in Table 1. Although the proposed procedure seems to be applicable to documented data of dioxin analysis in human milk in Japan in the 1990s, the above-mentioned findings might not be applicable to other countries because the exposure situation to dioxins and PCBs is different depending on the country.

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