Evaluation of toxic equivalent quantity of dioxins in human milk using different toxicity equivalence factors

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Introduction

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"[1] took effect in Japan in 1999, the number of substances to be sampled and measured has been increasing. Up to now, we have supported making the Japanese official analytical method of human milk, blood and food for dioxin measurement. In addition, we have participated in a collaborative study of dioxin in food as well as human milk and blood in order to validate the method, and have contributed to improving public health. However, there are some problems in the assessment of dioxin exposure in human milk, blood and so on. For instance, three toxicity equivalence factors (TEFs), namely, I-TEF, WHO-TEF (1993) and WHO-TEF (1998), are mainly used for calculating the toxic equivalent quantity (TEQ) that is widely used for the toxicity evaluation of dioxins. 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

Chemicals:

All of the dioxin standards were from Wellington Laboratories (Canada) and were diluted with decane to the appropriate concentrations. Most of the organic solvents, such as hexane, acetone, dichloromethane, toluene, diethyl ether, ethanol and methanol (MeOH), were of dioxin analysis grade and were from Kanto Kagaku (Tokyo, Japan) or Wako Pure Chemicals (Osaka, Japan). Decane was of special grade and was redistilled prior to use. All other chemicals were of PCB analysis grade or special 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.

Analysis of dioxins:

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[2]. A stable isotope of each congener of the PCDD/Fs and 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 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[3]), 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 25% dichloromethane/hexane 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 fraction.

GC/MS measurement:

The dioxins were subjected to HR-GC/MS with a JEOL JMS-700 mass spectrometer 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 the 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 with an ion source temperature of 260 °C. The PCDD/Fs, non-ortho PCBs and mono-ortho PCBs were quantified using a molecular ion (M), M+2 ion or M+4 ion. 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. The toxic equivalent quantity (TEQ) was calculated using WHO-TEF (1998)[4].

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 and by WHO/IPCS-TEF (1993) [5]

(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

Because the di-ortho PCBs are not considered to be measurement objects in the currently used official analytical method, the fractionation behavior of the PCBs by activated carbon/silica gel column chromatography was examined. As a result, we found that the first 10 mL of hexane was able to eliminate most of the non-planar PCBs, and 8 mono-ortho PCBs and 2 di-ortho PCBs were fractionated into the 40 mL eluate of 25% dichloromethane/hexane, and 17 PCDD/Fs and 4 non-ortho PCBs were fractionated into the 100 mL toluene eluate.

Human milk samples were analyzed by using the proposed method, and data analyses were carried out according to the above protocol. As predicted initially, TEQ was confirmed to increase in the order of I-TEF < WHO-TEF (1998) < WHO-TEF (1993). The I-TEF of 20 congeners are available (including 3 non-ortho PCBs by WHO/IPCS-TEF (1993)), whereas the WHO-TEF (1998) and 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 of WHO-TEF(1998) for 1,2,3,7,8-PeCDD, which is frequently detected in human milk at a comparatively high level, is the twice as large as that of WHO-TEF(1993) and I-TEF.

Moreover, we confirmed that there were significant correlations among the TEQs (Figure 1). As regards the regression line, a first-order straight line was obtained by the combination of any two of the three TEQs. Therefore, we surmised 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.

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 differed.

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.

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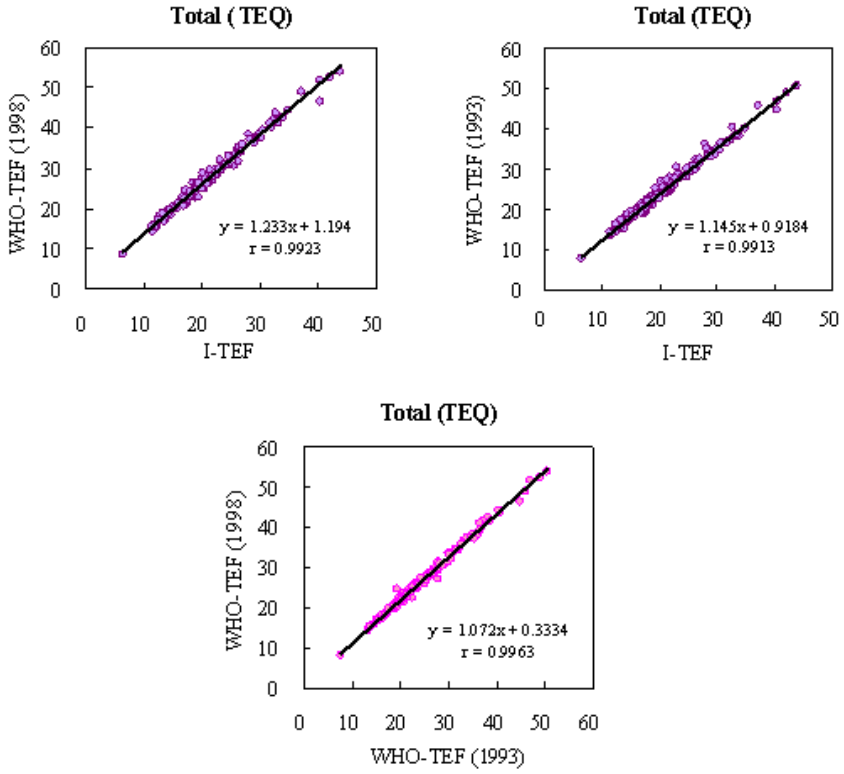

Figure 1. Correlation among the dioxin TEQs (of human milk samples) obtained by using different TEFs.

Table 1. TEQ correction factor 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