

## INVESTIGATION ABOUT CROSS-CHECK RESULT OF DIOXIN ANALOGUES USING A FISH SAMPLE PREPARED BY CAMBRIDGE ISOTOPE LABORATORIES INC. (CIL)

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### Abstract

In this study, 11 analytical organization of our committee participated in the international cross-check of the dioxin analogues for the fish sample in 2006 planned by Cambridge Isotope Lab. Inc. (CIL). The analysis result was compared with the result of the international cross-check of CIL in the 2003 using the same fish sample as this time. As a result, as well as the result of CIL 2003 cross-check, the big error between organizations was accepted in some highly chlorinated congeners of PCDD/DFs. In addition, using on the basis of the result of CIL 2003 cross-check, Zscore to the result of 11 organizations was calculated. Consequently, it was revealed that the analysis method using alkaline decomposition-solvent extraction had few errors between organizations than one using Soxhlet extraction. Furthermore, about the two above methods, comparison examination of the analytical value of native and <sup>13</sup>C<sub>12</sub>-labeled congeners was carried out. As a result, it made clear that concerning 1,2,3,4,6,7,8-HpCDD, OCDD and OCDF, their <sup>13</sup>C<sub>12</sub>-labeled congeners were stable although the native ones were unstable in the process of alkaline decomposition.

### Introduction

Now, in Japan, the regulation value of dioxin analogues (abbreviated as "Dioxins") is set up about waste related substances (flue gas, fly ash, bottom ash) and environmental media (sediment, soil, atmosphere, water). However, since the regulation value about the food containing a fish has not been set up, there is no measurement duty of Dioxins. Therefore, as for the present condition, there are few track records of Dioxins analysis in most domestic analysis organizations. Therefore, "Accuracy Improvement Committee For Trace Chemical Substance Measurement" (23 affiliation companies, the chairman: Hideaki Miyata, Setsunan University) (abbreviated as "Committee") participated in the international cross-check of the dioxin analogues for the fish sample in 2006 planned by Cambridge Isotope Lab. Inc. (CIL). At the beginning, in this research, the result of CIL2006 international cross-check was due to be used as an international index of dioxin analysis. However, since the cross-check result had not been released yet, we used the result of CIL2003<sup>1)</sup> using the same fish sample as this time as an alternative international index.

### Materials and Methods

#### 1) Fish sample

The fish sample used this time produced by CIL as an international cross-check sample in 2003 and 2006

was used.

## 2) Analysis method

As shown in Table 1, the analysis method used in this study changed with participating organizations, and was divided roughly into three kinds of methods. Moreover, concerning the measurement conditions of HR-GC/HR-MS, since GC/MS equipment, a capillary column, a standard substance, an internal standard substance, etc. which was used in the participating organizations differed from each other, it was dependent on each analysis organization. The analysis flow outline was shown in Table 2.

Analysis method	No. of organization
The provisional manual for aquatic organism investigation concerning dioxins (Ministry of Environment; September, 1998)	5
The provisional guideline for the measuring method of dioxins and coplanar PCB in food (Ministry of Health, Labor and Welfare; October, 1999)	2
Other methods	4

Analysis flow	No. of organization
Method-A: Alkaline decomposition → Solvent extraction → Purification → Purified fraction → GC-MS measurement	7
Method-B: Soxhlet extraction → Purification → Purified fraction → GC-MS measurement	3
Method-C: Soxhlet extraction → Alkaline decomposition → Solvent extraction → Purification → Purified fraction → GC-MS measurement	1

## Results and Discussion

### 1) Contrast with the result of an international cross-check

In the case of CIL 2003 international cross-check<sup>1)</sup>, the number of participating organizations was 32 for the analysis of PCDD/DFs and, 24 for PCDD/DFs and Co-PCBs. Since the analysis method was not defined in the CIL cross-check, the participating organization analyzed by arbitrary methods. So, also in this CIL2006 international cross-check, 11 organizations, which participated from our committee, used the analysis method currently used in each organization.

As how to deal with an analytical value, ND was carried out the outside of an object and average value was computed about the value which obtained by performing Grubbs rejection (5%) for each 2,3,7,8-chlorine substituted congener. As well as the result of the CIL2003 cross-check, in this result, the error between organizations was a large tendency at the highly chlorinated PCDD/DFs congeners composing of OCDD, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF and OCDF. The average value and permissible error range ( $\pm 2S.D.$ ) in the results of this research and CIL2003 cross-check were shown in Fig. 1. The analytical value of all the target

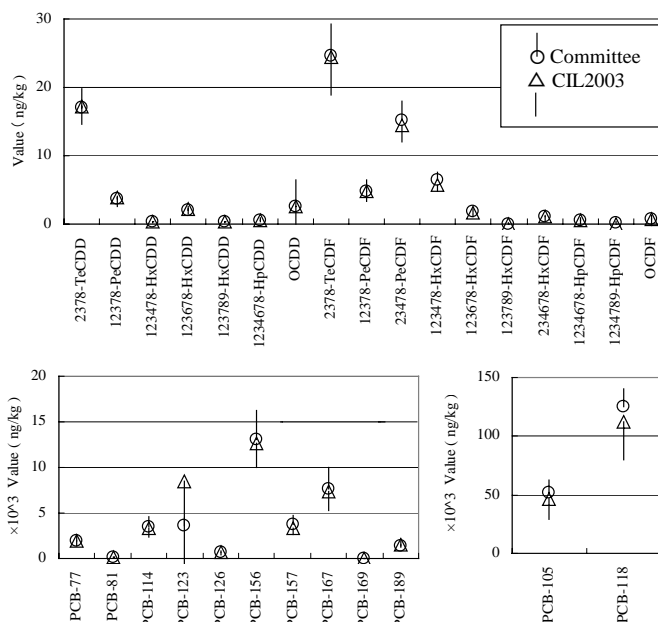


Fig. 1 Comparison of analytical values of PCDDs, PCDFs and Co-PCBs by our committee and CIL2003

compounds at this research was within the permissible error range at the CIL2003 cross-check. Therefore, it was judged that the analysis result of 11 organizations was well in agreement with that of CIL2003 cross-check.

## 2) Comparison examination by the Zscore technique

As shown in Table 2, the analysis method of 11 organizations was classified into two ways consisting of alkaline decomposition-solvent extraction and Soxhlet extraction by an extraction method. Then, an examination and consideration were performed about the analysis result of the fish sample in the classification of an extraction method.

In addition, as for the analysis method, organizations of A – G, I – K and H used respectively Method-A, Method -B and Method-C of Table 2. Although Method-C was using a combination of Soxhlet extraction and alkaline decomposition-solvent extraction, it was taken as "Soxhlet extraction" as

Taget compound	Evaluating poin	
	Method-1* (Organizations of A - G)	Method-2** (Organizations of H - G)
PCDDs, PCDFs, Co-PCBs	7.0	12.5
PCDDs, PCDFs	2.5	11.5
Co-PCBs	4.5	1.0
Zscore  < 2 ... 0 point    2 ≤  Zscore  < 3 ... 0.5 point    3 ≤  Zscore  ... 1 point		
*: using alkaline dcomposition-solvent extraction    **: using Soxhlet extraction		

classification of an extraction method. Using on the basis of the result of an international cross-check (CIL2003), Zscore to the result of each organization was computed, a suitable mark was prepared for the Zscore, it was totaled for each congener of PCDDs, PCDFs and Co-PCBs, and the method of comparing the magnitude of the figure was used. Here, the mark of |Zscore| made |Zscore| < 2 -- 0 point,  $2 \leq |Zscore| < 3$  -- 0.5 point and  $3 \leq |Zscore|$  -- 1 point. The total of the mark based on Zscore was also shown in Table 3. Method-1 using an alkaline decomposition-solvent extraction method had mark lower than Method-2 using the Soxhlet extraction, as a whole. From this result, it was revealed that the former method had few errors between organizations than did the latter method.

## 3) Comparison of the alkali tolerance of analytical target compounds

Concerning the result of the organization of A-K, the average value for each target compounds in two analysis methods using alkaline decomposition-solvent extraction and Soxhlet extraction was calculated and compared. The comparison was performed by computing and contrasting the ratio of the average level of native congeners and the recovery of internal standards in two methods of Method-1 and Method-2 (Table 4). As shown in Table 4, the recovery of 29  $^{13}\text{C}_{12}$ -labeled internal congeners was almost the same in Method-1 and Method-2, showing the recovery ratio of (Method-1)/(Method-2) to be in the range of 1.01 - 1.18 for PCDDs, 1.02 – 1.15 for PCDFs and 0.89 – 1.12 for Co-PCBs, respectively. However, in the case of native compounds, there was a great difference in the analytical value of 1,2,3,4,6,7,8-HpCDD, OCDD and OCDF between Method-1 and Method-2. The ratio of (Mehod-1)/(Method-2) was 0.58 for 1,2,3,4,6,7,8-HpCDD, 0.29 for OCDD and 0.42 for OCDF, respectively.

From this result, it became clear that alkali tolerance completely differed in native type and  $^{13}\text{C}_{12}$ -labeled type of these three compounds, that is, the former type was easily decomposed in the process of alkaline decomposition, whereas the latter type was stable. This result emphasizes the necessity for the check of a recovery about both native type and  $^{13}\text{C}_{12}$ -label type in an analysis method for Dioxins

## References

1. Cambridge Isotope Laboratories Cerilliant Corporation: 2003 International Interlaboratory Study on Sediment, Soil and Fish Tissue Reference Materials 2004

Table 4. Comparison of level of native compounds and the recovery of internal standards in two analysis methods

Compounds	Method-1* (Organizations of A - G)		Method-2** (Organizations of H - K)		Ratio of (Method 1)/(Method 2)	
	Average level of native comp. (ng/kg sample)	Average recovery of internal std. (%)	Average level of native comp. (ng/kg sample)	Average recovery of internal std. (%)	Average level of native comp.	Average recovery of internal std.
2378-TeCDD	14.4	85.0	13.2	76.5	1.09	1.11
12378-PeCDD	3.22	90.0	3.35	89.5	0.96	1.01
123478-HxCDD	0.285	94.0	0.424	82.3	0.67	1.14
123678-HxCDD	1.84	91.3	1.90	85.3	0.97	1.07
123789-HxCDD	0.264	92.2	0.265	88.8	1.00	1.04
1234678-HpCDD	0.486	92.2	0.845	79.5	0.58	1.16
OCDD	1.21	78.5	4.14	66.5	0.29	1.18
2378-TeCDF	20.6	87.2	20.0	78.0	1.03	1.12
12378-PeCDF	4.25	87.2	4.22	83.5	1.01	1.04
23478-PeCDF	12.7	85.3	15.2	80.0	0.84	1.07
123478-HxCDF	5.42	88.8	6.94	86.8	0.78	1.02
123678-HxCDF	1.56	90.7	1.57	86.5	0.99	1.05
123789-HxCDF	0.0776	91.5	0.0819	88.3	0.95	1.04
234678-HxCDF	0.883	92.0	0.968	84.3	0.91	1.09
1234678-HpCDF	0.475	89.5	0.528	77.8	0.90	1.15
1234789-HpCDF	0.186	86.3	0.174	83.3	1.07	1.04
OCDF	0.544	75.0	1.31	71.0	0.42	1.06
PCB-77	1650	91.7	1660	83.3	0.99	1.10
PCB-81	130	86.8	145	79.8	0.90	1.09
PCB-105	49500	93.7	44200	84.5	1.12	1.11
PCB-114	2820	78.5	2910	88.3	0.97	0.89
PCB-118	111000	90.0	101000	82.5	1.10	1.09
PCB-123	3630	83.5	2990	81.8	1.21	1.02
PCB-126	613	97.2	574	86.5	1.07	1.12
PCB-156	12600	93.5	11500	90.0	1.10	1.04
PCB-157	3260	87.7	3370	87.5	0.97	1.00
PCB-167	6770	89.2	6870	90.0	0.99	0.99
PCB-169	49.1	94.0	45.4	90.0	1.08	1.04
PCB-189	1310	91.7	1410	94.5	0.93	0.97
*: using alkaline decomposition-solvent extraction		**: using Soxhlet extraction				