The Proficiency Testing of Determination of Dioxins in Food

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Introduction

Food intake is the main route of human dioxin exposure, making the determination of dioxins in food indispensable for risk assessment and risk management of dioxins. The uncertainty of analytical results, however, can be very great because of the low concentration of the analytes and complicated cleanup procedures. The risk assessment of dioxins based on analytical results also suffers from a similar degree of uncertainty. The Ministry of Health, Labor and Welfare of Japan has published "Guideline for the Determination of Dioxins in Food" to standardize the analytical procedures. The guideline contains the quality assurance procedures to obtain reliable analytical results and recommends participation in the relevant proficiency testing scheme. The proficiency testing provides the fair evaluation of the analytical results. The central science laboratory in England and the food and drug safety center in Japan offer the proficiency testing on food. The National Institute of Health Sciences of Japan (NIHS) also has carried out proficiency testing of dioxins in food since 1998 to assure the quality of analytical results for dioxins. In this presentation we will show the results of 5 rounds of proficiency testing.

Methods and Materials

Samples The samples used in the proficiency testing are listed in Table 1. Table 1 also shows the number of participants and the TEQ of each sample.

BCR CRM607 and BCR RM 534 were prepared by the European Commission's Institute for Reference Materials and Measurements. Eleven certified value for PCDDs and PCDFs were given to CRM607. Eleven values were assigned for PCDDs and PCDFs in RM534 although not certified. CARP-1 was prepared by the National Research Council of Canada. Eighteen concentrations are certified, including PCBs. The custom-prepared standard solutions containing native PCDDs,

PCDFs and PCBs were prepared by Wellington Laboratories (Canada).

Other samples (freeze-dried fish and freeze-dried spinach) were prepared by the Japan Food Research Laboratories. The homogeneity of the samples was verified by the Japan Food Research Laboratories and the NIHS.

Analytical methods All participants determined dioxins by HRGC/HRMS as stipulated in the "Guideline for the Determination of Dioxins in Food".

Statistical analysis The mean and the standard deviation (SD) of the concentrations reported for each compound from the participants were calculated. There was the possibility of outlyers, but the application of tests for outlyers such as the Grubbs test was not advisable due to the small number of participants. The robust mean and the robust SD were then calculated using algorithm A[1]. The RSDs of TEQ in Table 1 were calculated from the robust mean and the robust SD. Examples of the statistical results are shown in Table 2. One participant reported a very high concentration of OCDD. This outlying high value lead to the high mean (2.85 pg/g) and the large SD (6.33 pg/g). The robust mean and SD of the same data were 0.67 pg/g and 0.25 pg/g, respectively, after the effect of the outlyer was eliminated. The z-Score of each participant was calculated using the robust mean and robust SD. The techniques of participants who gave a z-score of more than 3 or less than -3 were regarded as unsatisfactory, and review of their analytical procedures was recommended.

Results and Discussion

Year 1998 A CRM was used to verify the trueness of the results. The participants used the same standard solution, provided by the NIHS. The mean values of the results reported for two isomers were out of the confidence intervals of the certified values. All the results reported by two participants fell within the 95% confidence interval of the certified value. The other 4 participants reported results outside the 95% confidence interval but *the number of the outlying results was only 1-3. Reproducibility calculated from the 6 participants was 2.8-48 % RSD for each isomer and 6.6 % RSD for total TEQ.

Year 1999 The same CRM was used to compare the results with those in 1998. Many reports suggested that fish is the main route of dioxin intake, making the reliability of analysis of dioxins in fish crucial[2]. CARP-1 was then included in the proficiency testing. One plausible reason for poor reproducibility was the difference

among the standard solutions used by the participants. Mixed standard solutions of PCDDs, PCDFs and PCBs were used to estimate the variation in standard solutions among the participants.

For 6 isomers, the mean of participants was outside the confidence intervals of the certified values. The reproducibility for CRM607 (TEQ) was 11% RSD and larger than that in 1998. The decline in analytical performance probably arose from the difference between standard solutions. In 1998, all participants performed the determinations using the same standard solution. In 1999, each participant used their own standard solution. The number of participants increased to 15 in 1999, and inexperienced laboratories were included. This explains the increase in RSD.

The difference in the mean of the reported value for the mixed standard solution sample and the stated concentration was below 10%. The reproducibility of the standard solution sample was 8-15 RSD %. Bavel reported the RSDs of reported values of participants in proficiency testing in which a standard solution was used[3]. The RSDs after removing the outlyers were, with one exception, 10-17%. These results are similar to ours. The analysis of the solution required no cleanup procedure and the results were expected to represent the variability of the standard solutions of participants. According to the manufacturer's statement, the range of standard solution concentration is ± 5%, corresponding to an RSD of 2.9%. The higher reproducibility suggested other causes, such as the change in the concentration of the internal standards due to unsuitable storage conditions.

The mean of the reported values for CARP-1 was within the confidence interval of the certified value. The reproducibility of TEQ was 8.0% RSD. The TEQ of CARP-1 was 79 pg/g and was fairly large compared with the CRM607 (3.3). The large TEQ of CARP-1 led to its small reproducibility RSD.

Year 2000 Another RM and a standard solution with different isomer concentrations were used. The mean of the reported value for the RM was lower than the reference value for all compounds with reference values. The reproducibility of RM534 (TEQ) was 18% RSD. The reason for this poor reproducibility was not clear. The bias and reproducibility of the mixed standard solution sample were comparable to those in 1999. Differences in the standard solution used by the participants could not explain the large negative bias or large RSD.

Year 2001 As mentioned above, dioxin intake from marine fish is of great concern, and the use was requested of samples from wild polluted marine fish. The TEQ of CARP-1 is higher than that of wild fish, so it did not seem appropriate for proficiency testing aiming at the assurance of quality for analysis of common foods. Because no appropriate samples made of marine fish were available, we attempted the preparation of our own samples. Since 1998, no vegetable samples had been used in the proficiency testing, in spite of public concern about the contamination of leaf vegetables by dioxins.[4] For assurance of the performance of the vegetable analysis, a sample made of spinach was also prepared. Both samples were confirmed to be homogeneous and were thus suitable for proficiency testing. The reproducibilities of TEQ for the fish sample and spinach sample were 10% and 30%, respectively. The TEQ of the spinach sample was quite low (0.34 pg/g) at 1/20 of that of the fish sample. The large RSD was not extraordinary taking the low TEQ into consideration.

Year 2002 Another marine fish sample was prepared from grey mullet. Grey mullet contain more fat than sea bass and require further cleanup procedures. The results are likely to represent the actual analytical performance. The reproducibility was 7.1% RSD and comparable to the result of CARP-1.

The results of 5 rounds of proficiency testing revealed several problems with the determination of dioxins in foods. The variability of the standard solution is of major importance. Periodical confirmation of the validity of the standard by the use of CRM or by participation in proficiency testing is strongly recommended.

Although the TEQ of sea bass or grey mullet samples was about 1/10 of that of CARP-1, the reproducibility RSDs were comparable. These results show that repeated participation in proficiency testing improves the analytical skills of the laboratories. It is clear that for proficiency testing, the use of samples representing actual foods is preferable. Our attempted production of samples led to sufficiently homogeneous samples of fish and vegetables that could be prepared by freeze-drying. This technique opens the possibility of preparing samples from a variety of foods, leading to enhanced the effectiveness of proficiency testing.

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Table 1 Samples used in the proficiency testing and their reproducibility

Year	No of Participants	Samples		TEQ (pg/g)	Reproducibility RSD%
1998	6	BCR CRM607	spray-dried milk	3.3	6.6
1999	15	BCR CRM607	spray-dried milk	3.6	11
		CARP-1	homogenized fish	79	8.0
		Nonane solution of standards		23	8.7
2000	10	BCR RM534	spray-dried milk	4.6	18
		Nonane solution of standards		16	9.0
2001	9	Sea bass	freeze-dried	6.1	11
		Spinach	freeze-dried	0.32	31
2002	8	Grey mullet	freeze-dried	7.3	7.1

Table 2 Results of proficiency testing in 2001 Sample: Sea bass