# Supplement Chapter 1 Dioxins

## Section 1 Summary of dioxins

Dioxins are persistent organic pollutants that are nonintentionally produced via e.g. the course of combustion of waste, and defined by the Act on Special Measures against Dioxins (Law No. 105 of 1999) to include polychlorinated dibenzofurans, polychlorinated dibenzo-para-dioxins and coplanar polychlorinated biphenyls.

Dioxins taken into the living body bind to the intracellular aromatic hydrocarbon (Ah) receptor to cause various effects on DNA leading to carcinogenicity and reproductive toxicity; and additionally, they are supposed to cause endocrine disturbance at an extremely low dose.

Dioxins are apt to be accumulated in the living body because they are chemically stable and extremely lipid-soluble, and their tolerable daily intake (TDI) is designated as 4 pg-TEQ/kg/day, wherein TEQ is the toxic equivalents calculated as the sum of toxicity of dioxin compounds converted on the basis of 2,3,7,8-TeCDD, which needs to be quantitated for 29 compounds in total that are included in I to IV shown in Figure Sup. 1-1 in the analysis of dioxins.

While there is no standard value designated for dioxins in feeds in Japan, the maximum permissible levels etc. designated in the EU are shown in Table Sup. 1-1 for reference.

Table Sup. 1-1 Standard values for dioxins in feeds in the EU (2006)

(Unit: ng-WHO-TEQ/kg (adjusted to 12% water content))

| | Maximum level | | Action level (*3) | |
|---|---|---|---|---|
| | PCDDs+ PCDFs | PCDDs+ PCDFs+ Co-PCBs | PCDDs+ PCDFs | PCDDs+ PCDFs+ Co-PCBs |
| Feed materials of plant origin (except vegetable oils and vegetable oil byproducts) | 0.75 | 1.25 | 0.5 | 0.35 |
| Vegetable oils and vegetable oil byproducts | 0.75 | 1.5 | 0.5 | 0.5 |
| Feed materials of mineral origin | 1.0 | 1.5 | 0.5 | 0.35 |
| Animal fat (including milk fat and egg fat) | 2.0 | 3.0 | 1.0 | 0.75 |
| Other land animal products (including milk products and egg products) | 0.75 | 1.25 | 0.5 | 0.35 |
| Fish oil | 6.0 | 24.0 | 5.0 | 14.0 |
| Fish and aquatic animal products (*1) | 1.25 | 4.5 | 1.0 | 2.5 |
| Fish protein hydrolysis products containing ≥20% fat | 2.25 | 11.0 | 1.75 | 7.0 |
| Binders (*2) | 0.75 | 1.5 | 0.5 | 0.5 |
| Additives containing trace element compounds | 1.0 | 1.5 | 0.5 | 0.35 |
| Premix | 1.0 | 1.5 | 0.5 | 0.35 |
| Compound feedingstuffs (with the exception of feedingstuffs for fur animals, pet animals or fish) | 0.75 | 1.5 | 0.5 | 0.5 |
| Feedingstuffs for fish and pet animals | 2.25 | 7.0 | 1.75 | 3.5 |

*1 Except fish oil and fish protein hydrolysis products containing ≥20% fat

*2 Kaolinitic clay, calcium sulphate dihydrate, vermiculite, natrolite-phonolite, synthetic calcium aluminates and clinoptilolite of sedimentary origin

*3 The level as the indicator to take measures including identification of the source of contamination. (Action level)

The existing analysis methods for dioxins in Japan includes: “Measurement methods for dioxins and coplanar PCBs in gas emissions” and “Measurement methods for dioxins and coplanar PCBs in industrial water supply and industrial effluent” (September 1999) defined by JIS; “Provisional guidelines for the measurements of dioxins and coplanar PCBs in foods” (October 1999, hereinafter referred to as the “Food Guideline”) by the Ministry of Health, Labour and Welfare; and the “Soil survey and measurement manual relevant to dioxins” (January 2000) by the Ministry of the Environment.

These measurement methods are the same in principle in that isotope-labelled internal standards are added and microanalysis is conducted by high-resolution gas chromatography/ mass spectrometry (hereinafter referred to as “GC/MS” in this chapter), although there are differences in a part of pretreatment procedures depending on the subject sample type. They are also characterized in that they also define quality control in addition to analysis methods in order to guarantee accuracy.

During the course to establish the quantification method of dioxins in feeds, it was contemplated to apply some of pretreatment methods shown in the Food Guidelines to feed

analysis. However, considering the removal of contaminants specific to feeds as well as current findings relevant to dioxin analysis, a quantification method was developed by unique modification to a part of the Food Guideline and was confirmed to meet the analysis method requirements defined in the Guideline, and was established as the "Provisional guidelines for the measurements of dioxins in feeds."

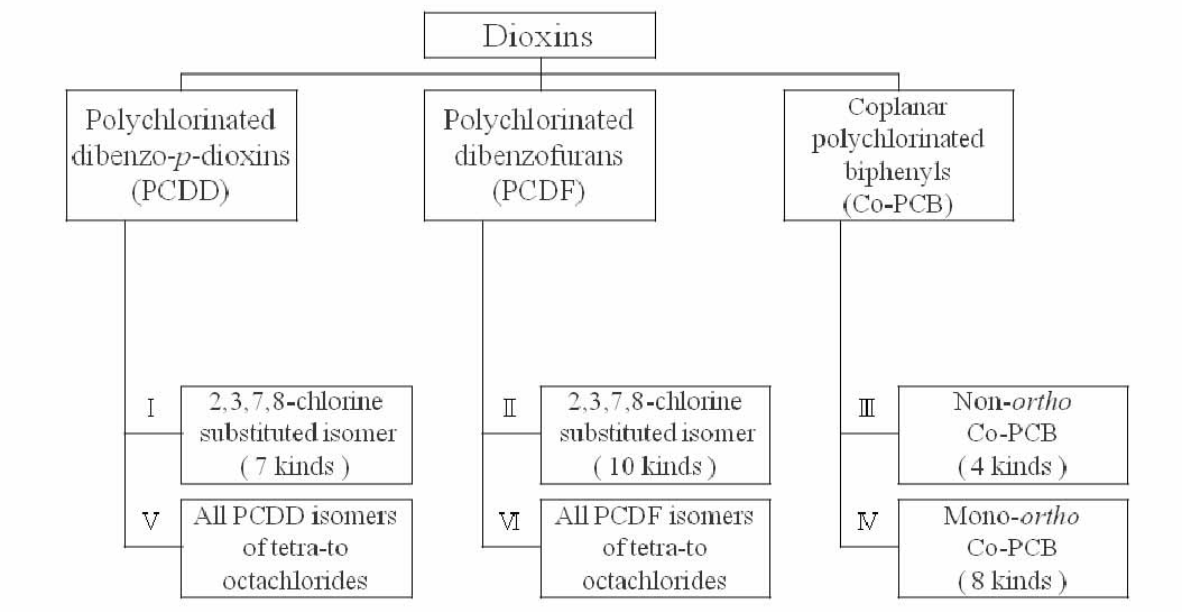


Figure Sup. 1-1 dioxins Analyte of dioxins (V and VI are analyzed by reference.)

History of guideline establishment

Established as: Notification No. 1725 dated June 26, 2003 of the Feed Division, Livestock Industry Department, Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries;
Full revision: Notification No. 5299 dated November 24, 2004 of the Animal Health and Animal Products Safety Division, Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries;
Addition of high-performance solvent extraction instrument: Notification No. 12544 dated March 24, 2006 of the Animal Products Safety Division, Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries;
TEQ revision: Notification No. 3750 dated July 1, 2008 of the Animal Products Safety Division, Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries.

## Section 2 Analysis methods for dioxins

This section is reconstitution of the Exhibit "Provisional guidelines for the quantification methods for dioxins in feeds" (hereinafter referred to as "the Guideline") in "Establishment of the provisional guidelines for the quantification methods for dioxins in feeds" (Notification No. 5299 dated November 24, 2004 of the Animal Health and Animal Products Safety Division, Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries) with notes that were indicated by "Note" in the text of the Notification to be shown as notes and precautions instead. However, notes and precautions labeled by a number with A are added by the developers as appropriate in addition to the Guideline.

**[Methods listed in the Guideline]**

### I Summary [Guideline Chapter 1]

**Introduction**

This Guideline shows general technical methods based on existing findings in order to guarantee the reliability of dioxin test relevant to feeds.
The Guideline may be revised in the future due to accumulation of scientific findings.

### 1 Analytes [Guideline Chapter 1-1]

In this Guideline, dioxins in feeds, specifically polychlorinated dibenzo-para-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) are analyzed. In particular, PCDDs (7 substances), PCDFs (10 substances) and Co-PCBs (12 substances) for which the toxicity equivalency factors are designated are hereinafter referred to as "analyte substances."

## 2 Definition of terms and abbreviations [Guideline Chapter 1-2]

| | |
|---|---|
| Dioxins: | Collective name for polychlorinated [A1] dibenzo-para-dioxins (PCDDs), polychlorinated [A1] dibenzofurans (PCDFs) and coplanar polychlorinated [A1] biphenyls (Co-PCBs). |
| Isomers: | Compounds in isomeric relationship. Isomers as used herein are compounds with the same chemical formula but chlorine substitutions in different positions. |
| Congeners: | A group of compounds that belong to the same homologous series with different numbers of substitutions or different substitution positions by chlorine; as used herein, congeners are tetra- to octa-chlorinated compounds. |
| PCDDs | Polychlorinated dibenzo-*p*-dioxins |
| PCDFs | Polychlorinated dibenzofurans |
| PCDD/DFs | Polychlorinated dibenzo-*p*-dioxins and Polychlorinated dibenzofurans |
| PCBs | Polychlorinated biphenyls |
| Co-PCBs: | Polychlorinated biphenyls with the coplanar structure. As used herein, the term indicates 12 compounds designated with toxicity equivalency factors, specifically 4 compounds without chlorine substitution in ortho positions (2, 2', 6 and 6') (non-*ortho* ) and 8 compounds with one chlorine substitution in an ortho position (mono-*ortho* ). |
| TeCDDs | Tetrachlorodibenzo-*p*-dioxins |
| PeCDDs | Pentachlorodibenzo-*p*-dioxins |
| HxCDDs | Hexachlorodibenzo-*p*-dioxins |
| HpCDDs | Heptachlorodibenzo-*p*-dioxins |
| OCDD | Octachlorodibenzo-*p*-dioxin |
| TeCDFs | Tetrachlorodibenzofurans |
| PeCDFs | Pentachlorodibenzofurans |
| HxCDFs | Hexachlorodibenzofurans |
| HpCDFs | Heptachlorodibenzofurans |
| OCDF | Octachlorodibenzofuran |
| TeCBs | Tetrachlorobiphenyls |
| PeCBs | Pentachlorobiphenyls |
| HxCBs | Hexachlorobiphenyls |
| HpCBs | Heptachlorobiphenyls |
| PFK | Perfluorokerosenes |
| TEF | 2,3,7,8-TeCDD Toxicity Equivalency Factor |
| TEQ | 2,3,7,8-TeCDD Toxicity Equivalent Quantity |
| HRGC | High Resolution Gas Chromatography or High Resolution Gas Chromatograph |
| HRMS | High Resolution Mass Spectrometry or High Resolution Mass Spectrometer |
| SIM | Selected Ion Monitoring |
| RRF | Relative Response Factor |

**<<Notes and precautions>>**

[A1] The chemically correct term is not “chloride” but “chlorinated”. However, this Guideline is prepared according to the description in the Act on Special Measures against Dioxins (Law No. 105 of July 16, 1999), and the original descriptions are used here.

## 3 Target lower limit of quantification [Guideline Chapter 1-3]

In this Guideline, the "target lower limit of quantification" is introduced as the criterion to judge the tolerance of the lower limit of quantification, operational blanks, etc. The target lower limit of quantification is set considering the analysis objectives, sample types and available sample amounts, etc., and are basically as shown in Table 1-1 in this Guideline.

Table 1-1 Target lower limit of quantification of dioxins

| PCDD/DFs | | | Co-PCBs | |
|---|---|---|---|---|
| Tetra-to pentachlorides | Hexa-to heptachlorides | Octachlorides | Non-*ortho* | Mono-*ortho* |
| 0.05 pg/g | 0.1 pg/g | 0.2 pg/g | 0.5 pg/g | 1 pg/g |

## 4 Analysis methods [Guideline Chapter 1-4]

### 4.1 Summary

Dioxin analysis in feeds includes processes of (1) extraction, (2) purification and fractionation, (3) identification and quantification.

While there are several ways for respective processes, this Guideline shows a method that has been confirmed to meet 4.2 Requirements for analysis methods. Analysts can use a method other than that shown in this Guideline if it is confirmed that the method meets 4.2 Requirements for analysis methods.

### 4.2 Requirements for analysis methods.

Those methods can be effectively used which are either newly developed or used in common although it is not employed in the Guideline, and are recognized by experimental proofs to have performance equivalent to or better than the one shown in the Guideline. In that case, the following matters need to be sufficiently examined so that the analytical accuracy shown in the Guideline are guaranteed

In addition, it is preferred to conduct validation studies in multiple institutions so that the method can be solid as an analysis method for dioxins.

I Pretreatment (extraction and degradation)
- a) The method should be stable in extraction efficiency depending on various feed types.

II Pretreatment (purification and fractionation )
- a) Blank values of reagents and apparatus should be low.
- b) Elution fractions of each chromatography should be stable.
- c) Interfering components that may be detected in actual samples should be steadily and effectively separated and removed.

III GC/MS analysis
- a) Isomer specific analysis of analyte substances can be conducted.
- b) Isomer resolution by a capillary column should be good.

c) The concentration range of a sample should match the quantifiable range (standard curve).
d) The lower limit of quantification should satisfy the target lower limit of quantification.
e) The sensitivity drift of instruments should be sufficiently low.
f) The resolution (M/⊿M, 10 % valley) of a high resolution mass spectrometer (HRMS) should be not less than 10,000.

IV Identification and quantification
a) The operation blank value should be sufficiently low.
b) The lower limit of quantification should be equivalent to the target lower limit of quantification.
c) There should be reproducibility for the same sample.

V Performance of the analysis method
a) The recovery of added internal standard for cleanup spike should be 40-120 %.
b) The standard sample [1] should be correctly analyzed.

**<<Notes and precautions>>**

[1] Currently there is no commercially available standard sample for dioxins in feeds. Therefore, the standard samples for foods etc. are substitutionally used, or samples for quality control check are used.

## II Detailed procedures [Guideline Chapter 2] Detailed procedures

### i Sampling [Guideline Chapter 2 Section 1 ]

**1 Sampling**

On sampling, the size of the subject to be tested, the number of samples or sampling sites, the sampling method for the primary samples and the riffle sampling method should be basically according to the Exhibit "Methods for sampling of feeds, etc." in "Establishment of test implementation guidelines for feeds, etc." (Notification No. 793 dated May 10, 1977 of the Bureau of Animal Industry). In addition, take care so that there is no dioxin contamination from outside (including sampling staffs) and sampling apparatus.

**2 Sample treatment**

Put the collected samples in containers that can be sealed and protected from light for storage and transportation to avoid dioxin contamination from outside as well as degradation.

Preparation methods for analysis samples are basically according to "Chapter 2 Preparation methods of analysis samples" in "Establishment of feed analysis standards" (Notification No. 14729 dated on April 1, 2008 of the Food Safety and Consumer Affairs Bureau). [2]

When the sample remained after analysis is to be stored for a long time, store in a freezer or a refrigerator as appropriate.

### 3 Sample amount to be used for analysis

In order to obtain the target lower limit of quantification as shown in Table 1-1 in Chapter 1, the sample amount to be used in analysis is generally 100 g when the lower limit of quantification of GC/MS is 0.1 pg as tetrachlorides, the final sample solution volume is 50 μL, and the injection volume to GC/MS is 1 μL (Table 2-1). The sample amount can be increased depending on the sample type when it is difficult to obtain the target lower limit of quantification with that sampling amount, or in order to obtain the additionally lower limit of quantification.

Table 2-1 General sample amount to be used for analysis

| Lower limit of quantification by GC/MS | Final sample solution volume | GC/MS injection volume | Sampling amount | Minimum quantifiable concentration in the sample |
|---|---|---|---|---|
| $A$ (pg) | $B$ (μL) | C (μL) | D (g) | E (pg/g) |
| 0.1 | 50 | 1 | 100 | 0.05 |

Calculate the required sample amount from the relationship $A \leq E \times D \times \frac{C}{B}$ .

**<<Notes and precautions>>**

[2] Crush and pass through a net sieve of 0.5-1 mm to mix well. During this procedure, take care so that there is no dioxin contamination from outside or between samples.

## ii Analysis methods [Guideline Chapter 2 Section 2 ]

### 1 Reagents and standard substances [Guideline Chapter 2 Section 2-1]

For reagents to be used in analysis, conduct a blank test (see Section 3 2.3) before use to make sure that no interfering component is contained that affect dioxin analysis.

1) Hexane, ethanol, acetone, toluene and dichloromethane
   Dioxin analysis grade [3], residual pesticide analysis grade or PCB analysis grade. When 1 μL of concentrate prepared according to the concentration factor in analysis is injected to a GC/MS, there should be no interference in the chromatograms of dioxin standards and internal standards.
2) Nonane, decane and isooctane
   Dioxin analysis grade [3], commercially available JIS Guaranteed Reagent, or of equivalent or higher purity. When 1 μL of concentrate prepared according to the concentration factor in analysis is injected to a GC/MS, there should be no interference to hinder the analysis in the chromatograms of dioxin standards and internal standards.
3) Hexane washed water
   Distilled water that is sufficiently washed with hexane and does not hinder the

analysis.

4) Sulfuric acid
Commercially available JIS Guaranteed Reagent, or of equivalent or higher purity which does not hinder the analysis.

5) Hexane washed dimethyl sulfoxide (hereinafter referred to as “DMSO”.)
Commercially available dimethyl sulfoxide of JIS Guaranteed Reagent grade, equivalent or higher purity that is sufficiently washed with hexane and does not hinder the analysis.

6) Sodium sulfate (anhydrous)
Dioxin analysis grade [3], residual pesticide analysis grade or PCB analysis grade which does not hinder the analysis.

7) Potassium hydroxide, silver nitrate and sodium chloride
Commercially available JIS Guaranteed Reagent grade, equivalent or higher purity which does not hinder the analysis.

8) Silica gel
Silica gel for column chromatography (PCB analysis grade, particle size 0.063-0.200 mm). [4] Activate according to the following as appropriate.
Pack the column with silica gel using methanol by the wet method, and flow twice as much weight of methanol. Then collect the content, fully evaporate methanol with a rotary evaporator. Next, transfer to a beaker so that the layer thickness is 10 mm or less, activate by drying at 130 °C for about 18 hours, and then let stand to cool in a desiccator for 30 minutes.

9) 2 % potassium hydroxide coated silica gel (hereinafter referred to as “2 % potassium hydroxide silica gel.”)
Add 40 mL of a potassium hydroxide solution (50 g/L) to 100 g of the methanol washed silica gel, and dehydrate under vacuum at about 50 °C in a rotary evaporator. After most of water is removed, continue dehydration at 80 °C for additional 1 hour to be a powder. [5] After preparation, put in a reagent bottle that can be sealed, and store in a desiccator.

10) 22 % sulfuric acid coated silica gel (hereinafter referred to as “22 % sulfuric acid silica gel.”)
Add 28.2 g of sulfuric acid to 100 g of the methanol washed silica gel, and shake sufficiently to be a powder. [5] After preparation, put in a reagent bottle that can be sealed, and store in a desiccator.

11) 44 % sulfuric acid coated silica gel (hereinafter referred to as “44 % sulfuric acid silica gel.”)
Add 78.6 g of sulfuric acid to 100 g of the methanol washed silica gel, and shake sufficiently to be a powder. [5] After preparation, put in a reagent bottle that can be sealed, and store in a desiccator.

12) 10 % silver nitrate coated silica gel (hereinafter referred to as “10 % silver nitrate silica gel.”)
Add 0.25 mL of 40 % silver nitrate solution per 1 g of unwashed silica gel, then mix well with a shaker, and activate by drying at 130 °C for about 3 hours immediately before use. [5] After preparation, put in an amber bottle that can be sealed, and store in a desiccator.

13) Activated charcoal silica gel
Commercially available dioxin analysis grade, or of equivalent or higher purity, which does not hinder the analysis by such as elution of interfering substances. Check the elution conditions of the column in advance. [6]

14) Alumina
Alumina for column chromatography (neutral or basic, activity level I, particle size 0.063-0.200 mm). Check the elution conditions of the column in advance. [7] Activate by the following method as appropriate.
Transfer to a plate or a beaker so that the thickness is 10 mm or less, dry at 130 °C for 18 hours or heat at 500 °C for about 8 hours, and then let stand to cool to room temperature in a desiccator.

15) Standard substances [8]
Standard substances used for the identification and quantification of dioxins are shown in Table 2-2 and Table 2-3.

16) Standard solutions
Prepare dilutions of a commercially available mixture solution [9] with nonane etc.[10] according to the concentration range of the standard curve.

17) Internal standards [8]
Use PCDDs, PCDFs and Co-PCBs with all the carbon atoms or chlorine atoms labeled with $^{13}C$ or $^{37}Cl$ for cleanup spike and syringe spike. [11] See Table Sup. 2-1 and Table Sup. 2-2.

18) Internal standard solution
Prepare dilutions of a commercially available mixture solution [9] with nonane etc.[10] according to amount to be spiked as the internal standard as well as the concentration range of the standard curve.

Table 2-2 Standard substance of PCDD/DFs

| | PCDDs | PCDFs |
|---|---|---|
| Tetrachlorides | 2,3,7,8-TeCDD | 2,3,7,8-TeCDF |
| Pentachlorides | 1,2,3,7,8-PeCDD | 1,2,3,7,8-PeCDF |
| | | 2,3,4,7,8-PeCDF |
| Hexachlorides | 1,2,3,4,7,8-HxCDD | 1,2,3,4,7,8-HxCDF |
| | 1,2,3,6,7,8-HxCDD | 1,2,3,6,7,8-HxCDF |
| | 1,2,3,7,8,9-HxCDD | 1,2,3,7,8,9-HxCDF |
| | | 2,3,4,6,7,8-HxCDF |
| Heptachlorides | 1,2,3,4,6,7,8-HpCDD | 1,2,3,4,6,7,8-HpCDF |
| | | 1,2,3,4,7,8,9-HpCDF |
| Octachlorides | OCDD | OCDF |

Table 2-3 Standard substance of Co-PCBs

| | Non-*ortho* Co-PCBs | Mono-*ortho* Co-PCBs |
|---|---|---|
| Tetrachlorides | 3,3',4,4'-TeCB (#77) | |
| | 3,4,4',5-TeCB (#81) | |
| Pentachlorides | 3,3'4,4',5-PeCB (#126) | 2,3,3',4,4'-PeCB (#105) |
| | | 2,3,4,4',5-PeCB (#114) |
| | | 2,3',4,4',5-PeCB (#118) |
| | | 2',3,4,4',5-PeCB (#123) |
| Hexachlorides | 3,3',4,4',5,5'-HxCB (#169) | 2,3,3',4,4',5-HxCB (#156) |
| | | 2,3,3',4,4',5'-HxCB (#157) |
| | | 2,3',4,4',5,5'-HxCB (#167) |
| Heptachlorides | | 2,3,3',4,4',5,5'-HpCB (#189) |

**<<Notes and precautions>>**

[3] Solvents and reagents of dioxin analysis grade include:
acetone, dichloromethane, ethanol, hexane, toluene, nonane (Kanto Chemical; Wako Pure Chemicals) and sodium sulfate (Wako Pure Chemicals)。

[4] Commercially available silica gel includes Wakogel S1, Wakogel DX (Wako Pure Chemicals), silica gel for column chromatography (Merck) and washed silica gel (SUPELCO).

[5] Commercially available, chemically modified silica gel includes that for dioxin analysis (Wako Pure Chemicals, SUPELCO).

[6] Commercially available activated charcoal silica gel includes activated charcoal dispersed silica gel for dioxin analysis (Kanto Chemical). Activated charcoal silica gel cartridges include the activated charcoal dispersed silica gel reverse column (Kanto Chemical).

[7] Commercially available alumina includes ICN Alumina B-Super I (ICN Pharmaceuticals).

[8] As analytical values are obtained via the comparison of analysis results of samples and standard substances, it is required to use standard substances with certified traceability as much as possible in order to ensure the reliability of analytical values.

[9] The standard solution and internal standard solution of dioxins are commercially available from Cambridge Isotope Laboratories (CIL) (Wako Pure Chemicals) and Wellington Laboratories (WL) (Kanto Chemical).

I PCDD/DFs

For example,

- Standard solution for standard curve preparation: DF-CVS-A10 (WL), EDF-4961 (CIL)
- Internal standard solution for cleanup spike: DF-LCS-A (WL), EDF-4974A (CIL)
- Internal standard solution for syringe spike: DF-IS-I (WL), EDF-4965A (CIL)

II Co-PCBs

PCB is designated as a Class I Specified Chemical Substance by the Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc. (Law No. 117 of October 16, 1973) and permission by the minister of economy, trade and industry is required for purchase, thus it takes 1-2 months to obtain it.

For example,

- Standard solution for standard curve preparation: DF-CVS-A10 (WL), EC-4962

(CIL),

- Internal standard solution for cleanup spike: PCB-LCS-A (WL), EC-4969A (CIL),
- Internal standard solution for syringe spike: PCB-IS-B (WL), EC-4970A (CIL)

Various products are commercially available in addition to these. One needs to be well-informed about isomers and their concentrations contained in respective standard solutions for the purchase.

[10] Decane or isooctane may be used.

[11] The internal standard that is added to the sample or the extract to confirm the result of pretreatment and to be the standard for the quantification of dioxins is referred to as the internal standard for cleanup spike. The internal standard that is added to the GC/MS sample solution for the confirmation of the injection volume and the calculation of the recovery of the internal standard for cleanup spike is referred to as the internal standard for syringe spike. Different isomers are used respectively for the internal standard for cleanup spike and the internal standard for syringe spike. Example use of internal standards is shown in Table Sup. 2-1 and Table Sup. 2-2.

Table Sup. 2-1 Example of internal standards（PCDD/DFs）

| Internal standard | example 1 | | example 2 | |
|---|---|---|---|---|
| | CS[a)] | SS[b)] | CS[a)] | SS[b)] |
| $^{13}C_{12}$-2,3,7,8-TeCDD | ○ | | ○ | |
| $^{13}C_{12}$-1,2,3,7,8-PeCDD | ○ | | ○ | |
| $^{13}C_{12}$-1,2,3,4,7,8-HxCDD | ○ | | | |
| $^{13}C_{12}$-1,2,3,6,7,8-HxCDD | ○ | | ○ | |
| $^{13}C_{12}$-1,2,3,7,8,9-HxCDD | ○ | | | |
| $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD | ○ | | ○ | |
| $^{13}C_{12}$-OCDD | ○ | | ○ | |
| $^{13}C_{12}$-2,3,7,8-TeCDF | ○ | | ○ | |
| $^{13}C_{12}$-1,2,3,7,8-PeCDF | ○ | | ○ | |
| $^{13}C_{12}$-2,3,4,7,8-PeCDF | ○ | | | |
| $^{13}C_{12}$-1,2,3,4,7,8-HxCDF | ○ | | ○ | |
| $^{13}C_{12}$-1,2,3,6,7,8-HxCDF | ○ | | | |
| $^{13}C_{12}$-1,2,3,7,8,9-HxCDF | ○ | | | |
| $^{13}C_{12}$-2,3,4,6,7,8-HxCDF | ○ | | | |
| $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF | ○ | | ○ | |
| $^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF | ○ | | | ○ |
| $^{13}C_{12}$-OCDF | ○ | | ○ | |
| $^{13}C_{12}$-1,3,6,8-TeCDD | ○ | | | |
| $^{13}C_{12}$-1,3,6,8-TeCDF | ○ | | | |
| $^{13}C_{12}$-1,2,3,4-TeCDD | | | | ○ |
| $^{13}C_{12}$-1,2,7,8-TeCDF | | ○ | | |
| $^{13}C_{12}$-1,2,3,4,6,9-HxCDF | | ○ | | |
| $^{13}C_{12}$-1,2,3,4,6,8,9-HpCDF | | ○ | | |

a) Internal standard for cleanup spike
b) Internal standard for syringe spike

Table Sup. 2-2 Example of internal standards（Co-PCBs）

| Internal standard | example 1 | | example 2 | |
|---|---|---|---|---|
| | CS[a)] | SS[b)] | CS[a)] | SS[b)] |
| $^{13}C_{12}$-3,4,4',5-TeCB (#81) | ○ | | ○ | |
| $^{13}C_{12}$-3,3',4,4'-TeCB (#77) | ○ | | ○ | |
| $^{13}C_{12}$-3,3'4,4',5-PeCB (#126) | ○ | | ○ | |
| $^{13}C_{12}$-3,3',4,4',5,5'-HxCB (#169) | ○ | | ○ | |
| $^{13}C_{12}$-2',3,4,4',5-PeCB (#123) | ○ | | | |
| $^{13}C_{12}$-2,3',4,4',5-PeCB (#118) | ○ | | ○ | |
| $^{13}C_{12}$-2,3,4,4',5-PeCB (#114) | ○ | | | |
| $^{13}C_{12}$-2,3,3',4,4'-PeCB (#105) | ○ | | | |
| $^{13}C_{12}$-2,3,3',4,4',5-HxCB (#156) | ○ | | | |
| $^{13}C_{12}$-2,3,3',4,4',5'-HxCB (#157) | ○ | | | |
| $^{13}C_{12}$-2,3',4,4',5,5'-HxCB (#167) | ○ | | ○ | |
| $^{13}C_{12}$-2,3,3',4,4',5,5'-HpCB (#189) | ○ | | ○ | |
| $^{13}C_{12}$-3,3',4,5'-TeCB (#79) | | ○ | | |
| $^{13}C_{12}$-2,3',4',5-TeCB (#70) | | ○ | | ○ |
| $^{13}C_{12}$-2,3,3',5,5'-PeCB (#111) | | ○ | | |
| $^{13}C_{12}$-2,2',3,4,4',5'-HxCB (#138) | | ○ | | |
| $^{13}C_{12}$-2,2',3,3',5,5',6-HpCB (#178) | | ○ | | |

a) Internal standard for cleanup spike
b) Internal standard for syringe spike
#Number indicates IUPAC No.

## 2 Apparatus and instruments [Guideline Chapter 2 Section 2.2]

Apparatus used in the analysis (including containers for sample storage and transportation) should be for exclusive use, and should be washed well with acetone and hexane, etc. In particular, when there is concern of contamination from the previous sample, conduct a blank test (see Section 3 2.3) before use to make sure that no interfering component is contained that may affect the analysis of dioxins.

### 2.1 Apparatus for pretreatment

Do not grease ground joints. [12]。

1) General analysis apparatus
   Separatory funnel, Soxhlet extraction apparatus, etc.
2) Concentrator
   Use a Kuderna-Danish (KD) concentrator or a rotary evaporator.

### 2.2 Gas chromatograph/ mass spectrometer (GC/MS)

High resolution gas chromatograph mass spectrometer (HRGC/HRMS) using a double-focusing mass spectrometer

1) Column oven
   With a temperature control range of 50-350 °C and programmable to have temperature rise to obtain the temperature of the optimal separation conditions

for the analyte substance.

2) Capillary column
Of 0.25-0.32 mm in inner diameter, 25-60 m in length, made of fused silica, and its inner surface is coated with the liquid phase. [13]

3) Detector (MS)
Double-focusing detector that can at least analyze with high resolution of 10,000 resolution (M/⊿M, 10 % valley).
The ion source should be able to maintain the temperature at 160-350 °C, to perform electron ionization (hereinafter referred to as the "EI method."), and with electron energy around 25~70 eV.
It should be possible to employ SIM quantification as the detection method, to set the cycle in the SIM method to be up to 1 second, and to use the lock mass method.

4) Sample injector
One that can introduce the whole amount of the sample with good reproducibility (splitless or on-column type).
One that can be adjusted to 250-280 °C.

5) Carrier gas
Highly purified helium (≥ 99.999 % purity) 。

**<<Notes and precautions>>**

[12] It is preferred to use apparatus with transparent ground joints to reduce dioxins adhesion. Additionally, use apparatus without damage in the inner wall for the same reason.

[13] Use a column with lower column bleed.

I For the analysis of PCDD/DFs, basically a column that gives good separation of 2,3,7,8-chlorine-substituted isomers and with the known elution order of all isomers on a chromatogram should be used. Considering various factors, it is preferred to use simultaneously two or more types of capillary columns of different polarity. There are SP-2331 (Supelco), HP-5ms (Agilent), DB-5ms (J&W Scientific), and DB-17ms (J&W Scientific), etc.

II For the analysis of Co-PCBs, basically a column that gives good separation of all the 12 isomers and with the known elution order of the isomers on a chromatogram should be used. There are DB-5ms (J&W Scientific), and HT-8 (SGE), etc.

## 3 Sample pretreatment [Guideline Chapter 2 Section 2. 3]

Methods for dioxin extraction are broadly classified into two categories by sample types in the pretreatment for the quantification of dioxins in feeds (Figure 2).

For samples that consist mainly of materials of plant origin with comparatively low fat content such as formula feeds and hay, dioxins are extracted by Soxhlet extraction, and organic matters are degraded by sulfuric acid treatment (3.1.1 Soxhlet extraction, 3.2.1 Sulfuric acid treatment).

For feed materials of animal origin such as fish meal, lipids are degraded by alkaline

degradation, then dioxins are extracted with hexane (3.1.2 Alkaline degradation /hexane extraction).

The sample solution after extraction and degradation is purified by multi-layer silica gel column chromatography (3.2.2), and further purified and fractionated by activated charcoal silica gel column chromatography (3.2.3), and then the sample solution is concentrated (3.3) to be subjected to GC/MS measurement.

When extraction or purification is insufficient by the pretreatment shown in this Guideline, investigate extraction method as appropriate or add a purification process needed.

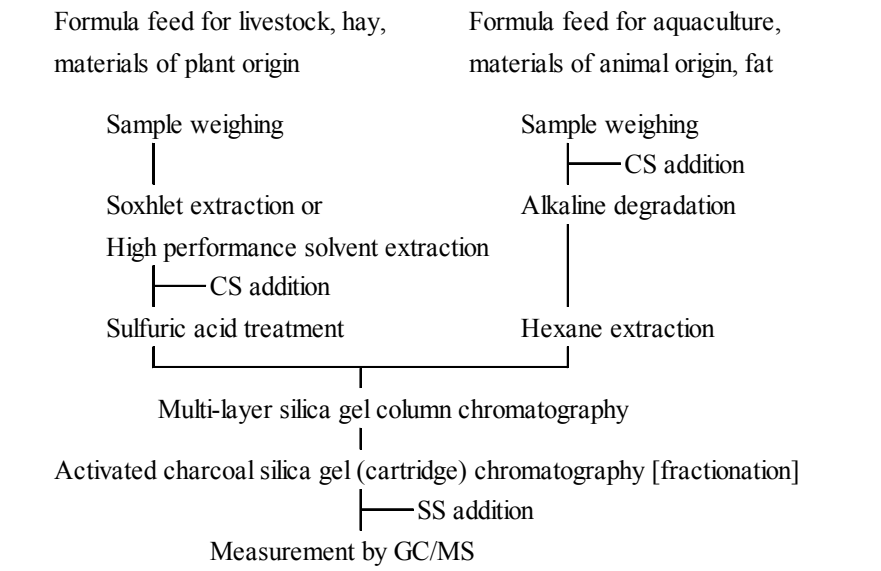


Figure 2 Summary of pretreatment method for the measurement of dioxins in feeds

## 3.1 Extraction and degradation [Guideline Chapter 2 Section 2 3.1]

### 3.1.1 Soxhlet extraction

#### 3.1.1.1 Soxhlet extraction

Put 50-100 g of a sample [14] in a filter paper thimble [15] and load it into a Soxhlet extraction tube. Connect the extraction tube, a Dean-Stark adapter [16] and a condenser sequentially to the top of a Soxhlet flask, and extract for 16 hours or more. If the sample volume is large, divide the sample into several Soxhlet extraction tubes to conduct extraction.

#### 3.1.1.2 Addition of the internal standard for cleanup spike

Add the following substance to the extract as internal standards: for PCDD/DFs, at least one 2,3,7,8-chlorine-substituted isomer labeled with $^{13}C$ or $^{37}Cl$ for each number of chlorines; and for Co-PCBs, 4 non-*ortho* Co-PCBs and at least one chlorine-substituted mono-*ortho* Co-PCBs labeled with $^{13}C$ for each number of chlorines. The amount of the internal standards to be added should be such that the concentration in the GC/MS sample

solution will be similar to that in the standard solution for the preparation of the standard curve. Generally, the amount should be such that dioxins in the GC/MS sample solution will be 1-20 ng/mL. Concentrate this extract under vacuum, and pool if the sample has been divided, and further concentrate under vacuum to be almost dried up. Dissolve the residue in about 100 mL of hexane, and transfer to a 500-mL separatory funnel. [17]

**3.1.2 Alkaline degradation /hexane extraction**

**3.1.2.1 Addition of the internal standard for cleanup spike**

Weigh 25-50 g (10-15 g for fat) of a sample in a 1-L tall beaker, add 50 mL of ethanol to this, stir using a high-speed homogenizer [18] if necessary, and then add the internal standard for cleanup spike to the tall beaker in the same way as 3.1.1.2.

**3.1.2.2 Alkaline degradation**

Add 250 mL of ethanol and 50 mL of 10 mol/L potassium hydroxide solution gradually to a tall beaker, and stir mildly using a magnetic stirrer for 2 hours. [19]

**3.1.2.3 Hexane extraction**

Transfer the alkaline degradation solution to a 1-L separatory funnel. Add 100 mL of hexane washed water and 100 mL of hexane, and extract by shaking. After leaving at rest, collect the hexane layer, add 100 mL of hexane to the water layer, and further repeat the same procedure twice or three times.
Pool the hexane extract, add 150 mL of 2 % sodium chloride solution in water, mildly shake rotationally, leave at rest, remove the water layer, and further repeat the same procedure twice or three times. [20]
Dehydrate the hexane layer with sodium sulfate (anhydrous).

**3.1.3 High performance solvent extraction [21]**

**3.1.3.1 High performance solvent extraction**

Put 50 g of a sample [14] into an extraction container, [22] load it to a high performance solvent extraction instrument [23] to conduct extraction. If the sample volume is large, divide the sample into several extraction containers [24] to conduct extraction.
Example of extraction conditions

| | |
|---|---|
| Extraction solvent: | Hexane- acetone (1:1) |
| Temperature: | 100 °C |
| Heating time: | 5 minutes |
| Pressure: | 10.3 MPa[25] |
| Retention time: | 5 minutes |
| Number of retention cycles [26]: | 3 |
| Total solvent substitution [27] : | 60% of extraction container |
| Nitrogen gas purge time: | 120-150 seconds |

**3.1.3.2 Addition of the internal standard for cleanup spike**

Add the internal standard for cleanup spike to extract in the same way as 3.1.1.2. [28] Transfer this extract to a 1-L separatory funnel. If the sample has been divided, pool the extracts to the said separatory funnel. Wash a sampling bottle with a small amount of hexane several times, and add the washing to the said separatory funnel. Add hexane so that the amount of hexane in the separatory funnel will be about 200 mL, and then add acetone so that the amount of acetone will be around 150 mL. [29] Add 150 mL of 2 % sodium chloride solution in water, mix by shaking, [29] leave at rest and then remove the water layer. Further add 150 mL of 2 % sodium chloride solution in water, mildly shake rotationally, [30] leave at rest, and remove the water layer.

**<<Notes and precautions>>**

[14] In the case of a wet sample, the sampling amount is different because it shall be dried in advance according to the Feed Analysis Standards.

[15] Made of glass or silica fiber. Wash in advance with acetone and toluene before use.

[16] A connector to remove water derived from sample that is condensed after evaporation out of the extraction system by utilizing the difference in specific gravity with toluene. In the case of feeds, about 5-20 mL of water is trapped per 100g of a sample.

[17] Add 100 mL of hexane in several aliquots to the flask, wash the flask wall, and transfer to the separatory funnel.

[18] Usually it is not necessary if the sample is ground and passed through a sieve of 0.5-1 mm.

[19] If it is left overnight after stirring, a phenomenon is observed in which OCDF in particular is degraded and recovery is reduced. For that reason, it is preferred to conduct both procedures of alkaline degradation and hexane extraction in the same day.

[20] If necessary, sulfuric acid treatment shown in 3.1.1.3 can be conducted in this stage.

[21] It is also known as high-pressure liquid extraction, high-pressure fluid extraction, or pressurized fluid extraction.

[22] If there is a room in the extraction container after sampling, diatomite or glass beads may be added. Diatomite includes Chemtube hydromatrix (VARIAN) and ASE Prep D.E. (DIONEX).

[23] High performance solvent extraction instruments include ASE-100, ASE-200, and ASE-300 (DIONEX).

[24] A 33-mL extraction container can contain about 10 g of hay or about 20 g of grain. On the other hand, a 99-mL extraction container can contain about 30 g of hay or about 60 g of grain.

[25] 1,500 psi

[26] Number of cycles.

[27] Injected in aliquots depending on the number of retention cycles. For example, in the case of “Number of retention cycles: 3” and “Total solvent substitution: 60% of extraction container,” the high performance solvent extraction instrument is operated as follows:
Firstly, inject the extraction solvent until the pressure in the extraction container will be 10.3 MPa, and heat for 5 minutes to elevate the temperature in the extraction container. Then retain for 5 minutes, inject 20% volume of the extraction container of the extraction solvent to the container, collect eluted extract into the sampling bottle, and repeat the procedure of retention for 5 minutes and injection of 20 % volume solvent twice. After the third injection of the extraction solvent, immediately purge nitrogen gas, and collect eluted extract into the sampling bottle.

[28] If the sample has been divided, the internal standard for cleanup spike can be added to one of the extracts.
[29] Emulsion is more likely to be produced if the percentage of solvent volume is significantly different.
[30] Emulsion is produced if it is mixed by shaking.

## 3.2 Purification and fractionation [31] [Guideline Chapter 2 Section 2 3.2]

The sample solution that has been obtained by extraction and degradation is purified by sulfuric acid treatment (3.2.1) and multi-layer silica gel column chromatography (3.2.2), and is fractionated by activated charcoal silica gel column chromatography (3.2.3) into the mono-*ortho* Co-PCBs fraction, the non-*ortho* Co-PCBs fraction and the PCDD/DFs fraction. In the case in which the sample solution has been obtained by alkaline degradation /hexane extraction, sulfuric acid treatment can usually be skipped.
If purification is not sufficient by the procedure described above, purification methods shown from 3.2.3 can be added as appropriate.

### 3.2.1 Sulfuric acid treatment

Add a suitable amount of concentrated sulfuric acid to the separatory funnel containing the sample solution, shake mildly, leave at rest, and then remove the sulfuric acid layer. Repeat this procedure until there is almost no color in the sulfuric acid layer. [32] Wash the hexane layer with 50-100 mL of hexane washed water 3-5 times, and dehydrate with sodium sulfate (anhydrous).

### 3.2.2 Multi-layer silica gel column chromatography

Pack a column of 15 mm in inner diameter and 300 mm in length sequentially by the dry pack method with 0.9 g silica gel, 3.0 g 2 % potassium hydroxide silica gel, 0.9 g silica gel, 4.5 g 44 % sulfuric acid silica gel, 6.0 g 22 % sulfuric acid silica gel, 0.9 g silica gel, 3.0 g 10 % silver nitrate silica gel, 2.0 g silica gel [33] and 6.0 g sodium sulfate (anhydrous). [34]
Wash the column sufficiently with hexane, elute until the liquid level reaches to the upper end of the column, and then load the sample solution that is evaporated under vacuum to be about 5 mL. Wash the inner side several times with a small amount of hexane, elute with 150-200 mL of hexane at a flow rate about 2.5 mL/min (approximately one drop per second) to elute dioxins. [35]

### 3.2.3 Activated charcoal silica gel column chromatography

Pack a column (7-10 mm in inner diameter, -300 mm in length) sequentially by the dry pack method with 3.0 g sodium sulfate (anhydrous), 1.0 g activated charcoal silica gel and 3.0 g sodium sulfate (anhydrous).
Load the sample solution that is evaporated under vacuum to be about 0.5 mL. Wash the container with about 0.5 mL of hexane, [36] and add the washing to the activated charcoal silica gel column. Repeat this procedure once more. After leaving at rest for 15 minutes, elute with 50 mL of hexane at a flow rate about 2.5 mL/min (approximately one drop per second) until the liquid level reaches to the upper end of the column to wash the column.
Then elute with 60 mL of hexane- dichloromethane (3:1) at a flow rate about 2.5 mL/min (approximately one drop per second) until the liquid level reaches to the upper end of the

column, to obtain the mono-*ortho* Co-PCBs fraction.
Then elute with 25 mL of hexane- toluene (3:1) at a flow rate about 2.5 mL/min (approximately one drop per second) until the liquid level reaches to the upper end of the column, to obtain the non-*ortho* Co-PCB fraction.
Then elute with 300 mL of toluene at a flow rate about 2.5 mL/min (approximately one drop per second), to obtain the PCDD/DFs fraction.

**3.2.4 Silica gel column chromatography [37]**

Pack a column (7-15 mm in inner diameter, -300 mm in length) with 3 g of silica gel using hexane, and add on it a layer of about 10 mm of sodium sulfate (anhydrous).
Wash the column sufficiently with hexane, elute until the liquid level reaches to the upper end of the column, and then load the sample solution that is concentrated under vacuum to be about 5 mL. Wash the inner side several times with a small amount of hexane, elute until the liquid level reaches to the upper end of the column, elute with 150 mL of hexane at a flow rate about 2.5 mL/min (approximately one drop per second) to elute dioxins.

**3.2.5 Silver nitrate silica gel column chromatography [38]**

Pack a column (7-15 mm in inner diameter, -300 mm in length) sequentially with 1 g of silica gel and 1 g of 10 % silver nitrate silica gel, and add on it a layer of about 10 mm of sodium sulfate (anhydrous).
Wash the column sufficiently with hexane, elute until the liquid level reaches to the upper end of the column, and then load the sample solution that is concentrated under vacuum to be about 5 mL. Wash the inner side several times with a small amount of hexane, elute with 200 mL of hexane at a flow rate about 2.5 mL/min (approximately one drop per second) to elute dioxins.

**3.2.6 DMSO extraction /hexane reverse extraction [39]**

**3.2.6.1 DMSO extraction**

Transfer the sample solution that is evaporated under vacuum to be about 5 mL to a 300- to 500-mL separatory funnel, wash the container several times with about 1 mL of hexane, and add the washing to the separatory funnel. Add 25 mL of hexane-washed DMSO, extract by shaking, and collect the DMSO layer. Further repeat the same procedure three times, pool the DMSO layer and transfer to a 300-mL separatory funnel, and then add 100 mL of hexane washed water.

**3.2.6.2 Hexane reverse extraction**

Add 75 mL of hexane to the DMSO-water layer, extract by shaking, collect the hexane layer and transfer to a 300- to 500-mL separatory funnel. Repeat this procedure two or three times, and wash the hexane layer several times with 25 mL of hexane washed water. Dehydrate the hexane layer with sodium sulfate (anhydrous).

**3.2.7 Alumina column chromatography [40]**

Pack a column (10-15 mm in inner diameter, 300 mm in length) with 15 g of alumina, and

add on it a layer of about 10 mm of sodium sulfate (anhydrous).
Wash the column sufficiently with hexane, elute until the liquid level reaches to the upper end of the column, and then load the sample solution that is concentrated under vacuum to be about 5 mL. Wash the inner side several times with a small amount of hexane, and elute with 150 mL of hexane at a flow rate about 2.5 mL/min (approximately one drop per second).
Then elute with 200 mL of hexane- dichloromethane (49:1) at a flow rate about 2.5 mL/min (approximately one drop per second). Mono-*ortho* Co-PCBs are eluted in this fraction.
Then elute with 200 mL of dichloromethane - hexane (3:2) at a flow rate about 2.5 mL/min (approximately one drop per second). Non-*ortho* Co-PCBs and PCDD/DFs are eluted in this fraction.

**3.2.8 Activated charcoal column high performance liquid chromatography (HPLC)**

Install an activated charcoal column (e.g., 4.6 mm in inner diameter, 100 mm in length) [41] to an HPLC, wash the column sufficiently with toluene in advance, and then substitute with a sufficient amount of hexane. Inject the prepared sample solution into the HPLC column, and elute with 8 mL of hexane as the mobile phase.
Then elute with 40 mL of hexane- dichloromethane (1:1) to obtain the mono-*ortho* Co-PCBs fraction.
Then elute with 40 mL of hexane- toluene (7:3) 40 mL to obtain the non-*ortho* Co-PCBs fraction.
Then heat the column oven to be 50 °C, reverse the flow direction of the mobile phase, and elute with 30 mL of toluene to obtain the PCDD/DFs fraction.

**3.2.9 Activated charcoal silica gel cartridge column chromatography**

Load the sample solution that is concentrated under vacuum to be about 0.5 mL on the activated charcoal silica gel cartridge. [6] Wash the container with about 0.5 mL of hexane, [36] and add the washing to the activated charcoal silica gel cartridge. Repeat this procedure once more. After leaving at rest for 15 minutes, add 60 mL of hexane to the cartridge, and elute until the liquid level reaches to the upper end of the column to wash the column.
Then add 50 mL of hexane- dichloromethane (3:1) to the cartridge, elute until the liquid level reaches to the upper end of the column, to obtain the mono-*ortho* Co-PCBs fraction.
Then reverse the cartridge, and add 50 mL of toluene to the cartridge, to obtain the non-*ortho* Co-PCB and PCDD/DFs fraction.

**<<Notes and precautions>>**

[31] When packing etc. to be used in column chromatography is significantly changed, it is needed to conduct a fractionation test using the standard solution etc. to check the type and amount etc. of packing and solvent. Conduct the similar procedure for the sample solution for blank measurement.

[32] Conduct sulfuric acid addition with enough care as bumping of solvent may occur due to the heat produced by the reaction of sulfuric acid and organic matters. Depending on the type of feeds, the extract of 100 g sample may be significantly colored and thus the interface between the hexane layer and the sulfuric acid layer is often unclear by the addition of a small amount of sulfuric acid. For that reason, an effective method is to

add by dropping about 40-50 mL of sulfuric acid first, leave at rest for about one night to clarify the hexane layer, and pour away the tarry substance present in the sulfuric acid layer. Then add about 10-15 mL of sulfuric acid and shake, and repeat this until the sulfuric acid layer becomes clear.

[33] When the sample solution obtained by sulfuric acid treatment is subjected to the multi-layer silica gel column, it may not be needed to pack 2.0 g of silica gel over 3.0 g of 10 % silver nitrate silica gel.

[34] Commercially available prepacked multi-layer silica gel columns include glass multi-layer silica gel column (SUPELCO) and Presep multi-layer silica gel (Wako Pure Chemicals).

[35] When respective packings are significantly colored to the lower end, a new multi-layer silica gel column can be prepared to repeat the same procedure. In that case, it is not necessary to pack all of the layers.

[36] If the volume of wash solvent is excessive, 2,3,4,4',5-PeCB (#114) is eluted and its recovery is specifically reduced. When $^{13}C_{12}$-2,3,4,4',5-PeCB is not used as the internal standard for cleanup spike, the quantification value will be underestimated; therefore wash with the smallest possible amount of hexane.

[37] It is used for simple removal of polar substances. For some of sample types, it can be used after Soxhlet extraction / sulfuric acid treatment and instead of multi-layer silica gel column chromatography. Also, it is effective for the removal of precipitate during concentration of the sample solution when DMSO extraction /hexane reverse extraction is conducted after alkaline degradation.

[38] It is used to remove sulfur.

[39] It is effective for the removal of lipids etc. After this procedure, it is required to conduct multi-layer silica gel column chromatography or silver nitrate silica gel column chromatography.

[40] The activity of alumina varies depending on the production lot, storage period and method after opening the package. When alumina with reduced activity is used, 1,3,6,8-TeCDD and 1,3,6,8-TeCDF etc. are eluted in the first fraction. On the other hand, when a lot with high activity is used, octachlorides may not be fully eluted in the second fraction. These should be checked in a fractionation test. In addition, when the separation between mono-*ortho* Co-PCBs and non-*ortho* Co-PCBs•PCDD/DFs is not sufficient, it is required to divide in the step of extraction or sulfuric acid treatment and operate using two alumina columns.

[41] Activated charcoal columns for HPLC include Shandon Hypercarb S (porous graphitized carbon).

### 3.3 Preparation of the sample solution and addition of the internal standard for syringe spike [Guideline Chapter 2 Section 2 3.3]

After purification and fractionation of dioxins, concentrate under vacuum respective fractions in which dioxins are eluted to be about 1 mL. Transfer the concentrate to a Kuderna-Danish (KD) concentrator, wash the container several times with 1-2 mL of hexane, add the washing to the KD concentrator, and concentrate under nitrogen flow to be about 0.1 mL or less. [42]

Then add the internal standard for syringe spike so that it will be similar concentration as the internal standard for cleanup spike, wash the wall of the KD concentrator with a small amount of hexane, and further concentrate under nitrogen flow until approximately minimal

amount of solvent is left. Then add 0.05-0.1 mL of nonane, and further concentrate under nitrogen flow to be the final solution volume [42][43] to be the GC/MS sample solution. [44]

**<<Notes and precautions>>**

[42] During concentration procedure under nitrogen flow, adjust nitrogen flow so that the movement of the solution surface can just be seen to avoid scattering of the solution, and at the same time take care not to dry it up. There may be loss of dioxins if nitrogen flow is as strong as to make a swirl in the solution or if the solution is dried up.

[43] Another way is to concentrate to be a prescribed minimal amount (about 20 μL) and add nonane to be the final solution volume. Or otherwise, concentrate until approximately minimal amount of solvent is left, and fill up with the internal standard solution for syringe spike.

[44] It is preferred that the sample solution is sufficiently purified to be the state without coloring and with no involatile component by visual inspection. Conduct re-purification if necessary. If coloring or residual material is observed, it may cause reduction in resolution of components by a capillary column, reduction in accuracy due to sensitivity variance resulting from contamination in the ion source of the instrument, and interference in accurate tuning using reference substances such as PFK.

## 4 Identification and quantification [Guideline Chapter 2 Section 2 4]

Identification and quantification of dioxins are conducted by a gas chromatograph (GC) using a capillary column gas chromatography mass spectrometry (GC/MS) using a double-focusing mass spectrometer (MS).

### 4.1 Setup of GC/MS analysis conditions [Guideline Chapter 2 Section 2 4.1]

#### 4.1.1 Operation conditions of a gas chromatograph (GC)

Set conditions including column oven temperature, injector temperature, and carrier gas flow so that the peak of analyte substance is well separated from those of other isomers, its retention time is in an appropriate range, and stable response is obtained. Examples of GC setup conditions are shown in the following.

(1) PCDD/DFs, Example 1

1) Analytes: TeCDD/DFs and PeCDFs
Column used: SP-2331 (0.32 mm in inner diameter, 60 m in length, 0.2 μm in membrane thickness)
Column temperature: 100 °C (retained for 1.5 min) → (elevated by 20 °C/min) →180 °C→ (elevated by 3 °C/min) →260 °C (retained for 25 min)
Sample introduction: Splitless (90 s)
Injector temperature: 260 °C

2) Analytes: PeCDDs and HxCDD/DFs
Column used: SP-2331 (0.32 mm in inner diameter, 60 m in length, 0.2 μm in

membrane thickness)
Column temperature: 100 °C (retained for 1.5 min) → (elevated by 20 °C/min) →210 °C→ (elevated by 3 °C/min) →260 °C (retained for 25 min)
Sample introduction: Splitless (90 s)
Injector temperature: 280 °C

3) Analytes: HpCDD/DFs and OCDD/DF
Column used: DB-17 (0.32 mm in inner diameter, 30 m in length, 0.15 μm in membrane thickness)
Column temperature: 100 °C (retained for 1.5 min) → (elevated by 20 °C/min) →200 °C→ (elevated by 10 °C/min) →280 °C (retained for 5 min)
Sample introduction: Splitless (90 s)
Injector temperature: 280 °C

(2) PCDD/DFs, Example 2

1) Analytes: TeCD/DFs、PeCDD/DFs and HxCDD/DFs
Column used: SP-2331 (0.25 mm in inner diameter, 60 m in length, 0.2 μm in membrane thickness)
Column temperature: 100 °C (retained for 1 min) → (elevated by 20 °C/min) →200 °C→ (elevated by 2 °C/min) →260 °C (retained for 25 min)
Sample introduction: Splitless (60 s)
Injector temperature: 260 °C

2) Analytes: HpCDD/DFs and OCDD/DF
Column used: HP-5 (0.20 mm in inner diameter, 25 m in length, 0.33 μm in membrane thickness)
Column temperature: 100 °C (retained for 1 min) → (elevated by 20 °C/min) →200 °C→ (elevated by 5 °C/min) →300 °C (retained for 15 min)
Sample introduction: Splitless (60 s)
Injector temperature: 260 °C

(3) PCDD/DFs Example 3

1) Analytes: TeCDD/DFs~OCDD/DF
Column used: SP-2331 (0.32 mm in inner diameter, 60 m in length, 0.2 μm in membrane thickness)
Column temperature: 180 °C (retained for 3 min) → (elevated by 3 °C/min) →230 °C (retained for 3 min) → (elevated by 3 °C/min) →260 °C (retained for 30 min)
Sample introduction: on-column
Injector temperature: 170 °C → (elevated by 100 °C/min) →300 °C

2) Analytes: TeCDD/DFs~HxDD/DFs
Column used: DB-17 (0.32 mm in inner diameter, 30 m in length, 0.25 μm in membrane thickness)
Column temperature: 150 °C (retained for 3 min) → (elevated by 20 °C/min) →200 °C → (elevated by 3 °C/min) →280 °C (retained for 10 min)
Sample introduction: on-column
Injector temperature: 150 °C → (elevated by 100 °C/min) →300 °C

(4) Co-PCBs Example 1

1) Analytes: Co-PCBs
Column used: HT-18 (0.22 mm in inner diameter, 50 m in length, 0.25 μm in membrane thickness)
Column temperature: 130 °C (retained for 1 min) → (elevated by 20 °C/min)

→220 °C → (elevated by 5 °C/min) →320 °C (retained for 3 min)
Sample introduction: Splitless (90 s)
Injector temperature: 280 °C

(5) Co-PCBs Example 2

1) Analytes: Co-PCBs
Column used: DB-5MS (0.32 mm in inner diameter, 60 m in length, 0.25 μm in membrane thickness)
Column temperature: 150 °C (retained for 1 min) → (elevated by 20 °C/min) →185 °C (retained for 3 min) → (elevated by 2 °C/min) →245 °C (retained for 3 min) → (elevated by 6 °C/min) →290 °C (retained for 10 min)
Sample introduction: on-column
Injector temperature: 170 °C → (elevated by 100 °C/min) →300 °C

**4.1.2 Operation conditions of a mass spectrometer (MS)**

To conduct analysis by selected ion monitoring (SIM) by the lock mass method using the standard substance for mass calibration (PFK), set up resolution, electron energy, ionization current, ion source temperature, monitor ion (2 or more monitor ions for respective chlorine numbers of analyte substance, 1 or more monitor ion(s) for the internal standard, and monitor ion for PFK) and SIM cycle. [45]

Examples of MS setup conditions are shown in the following.

Resolution (M/⊿M, 10 % valley) : ≥ 10,000
Electron energy: 25-70 eV
Ionization current: 300-1,000 μA
Ion source temperature: 260-290 °C
Monitor ion mass number: See Table 2-4 and Table 2-5

Table 2-4 Example of mass number of PCDD/Fs for monitoring ion

| | | $M^+$ | $(M+2)^+$ | $(M+4)^+$ |
|---|---|---|---|---|
| Analyte substance | TeCDDs | 319.8965 | 321.8936 | |
| | PeCDDs | 353.8576 | 355.8546 | 357.8516 |
| | HxCDDs | | 389.8157 | 391.8127 |
| | HpCDDs | | 423.7766 | 425.7737 |
| | OCDD | | 457.7377 | 459.7348 |
| | TeCDFs | 303.9016 | 305.8987 | |
| | PeCDFs | | 339.8597 | 341.8567 |
| | HxCDFs | | 373.8208 | 375.8178 |
| | HpCDFs | | 407.7818 | 409.7789 |
| | OCDF | | 441.7428 | 443.7399 |
| Internal standard | $^{13}C_{12}$-TeCDDs | 331.9368 | 333.9338 | |
| | $^{13}C_{12}$-PeCDDs | | 367.8949 | 369.8919 |
| | $^{13}C_{12}$-HxCDDs | | 401.8559 | 403.8530 |
| | $^{13}C_{12}$-HpCDDs | | 435.8169 | 437.8140 |
| | $^{13}C_{12}$-OCDD | | 469.7780 | 471.7750 |
| | $^{13}C_{12}$-TeCDFs | 315.9419 | 317.9389 | |
| | $^{13}C_{12}$-PeCDFs | | 351.9000 | 353.8970 |
| | $^{13}C_{12}$-HxCDFs | | 385.8610 | 387.8580 |
| | $^{13}C_{12}$-HpCDFs | | 419.8220 | 421.8191 |
| | $^{13}C_{12}$-OCDF | | 453.7830 | 455.7801 |
| Standard substance for mass calibration (PFK) | | 330.9792 | | |
| | | 380.9760 | | |
| | | 430.9728 | | |
| | | 442.9728 | | |

Table 2-5 Example of mass number of Co-PCBs for monitoring ion

| | | $M^+$ | $(M+2)^+$ | $(M+4)^+$ |
|---|---|---|---|---|
| Analyte substance | TeCBs | 289.9224 | 291.9194 | |
| | PeCBs | | 325.8804 | 327.8776 |
| | HxCBs | | 359.8415 | 361.8385 |
| | HpCBs | | 393.8025 | 395.7995 |
| Internal standard | $^{13}C_{12}$-TeCBs | 301.9626 | 303.9597 | |
| | $^{13}C_{12}$-PeCBs | | 337.9207 | 339.9178 |
| | $^{13}C_{12}$-HxCBs | | 371.8817 | 373.8788 |
| | $^{13}C_{12}$-HpCBs | | 405.8428 | 407.8398 |
| Standard substance for mass calibration (PFK) | | 304.9824 | | |
| | | 330.9792 | | |
| | | 380.9760 | | |

**<<Notes and precautions>>**

[45] While the peak width obtained by a capillary column is about 5-10 seconds, the cycle in SIM has to be 1 second or less in order to secure data points that comprise a peak. The number of monitor channels that can be configured in one analysis is related to the required sensitivity, and thus has to be selected based on adequate investigation.
Analysis can also be conducted by time-resolved grouping considering retention time of respective peaks on a chromatogram; in this case, conditions have to be set so that a suitable internal standard peak appears in each group.

## 4.2 Adjustment of GC/MS [Guideline Chapter 2 Section 2 4.2]

Set analysis conditions depending on measurement objectives and adjust so that a sample can be analyzed. Then make sure that sufficiently reliable analysis can be conducted, for example the presence or absence or the size of interference that causes analytical error and the method to correct that in addition to sensitivity, linearity and stability etc.

### 4.2.1 Adjustment of a gas chromatograph (GC)

Make sure that the response is stable, the retention time of respective chlorine substitutes is in an appropriate range, and peaks are sufficiently separated. Adjust the splitless time and purge gas flow etc. to suitable values.
If separation between analyte substances and other substances is not sufficient due to deterioration of the capillary column, exchange it with a new one. However, exchange is not required when there is no problem in the separation between analyte substances and other substances by cutting off the capillary column by about 300 mm (at both ends or at one end).

### 4.2.2 Adjustment of a mass spectrometer (MS)

Introduce PFK to a MS to calibrate the mass pattern and resolution ($M/\Delta M \geq 10{,}000$, 10 % valley) etc. within the measurement mass range as well as to check basic points such as sensitivity of the instrument.

### 4.2.3 Confirmation of GC/MS sensitivity

Inject respective analyte substances and obtain the lower limit of quantification of GC/MS from the *SN* ratio ($S/N$=10). [46] Confirm that the lower limit of quantification in a sample calculated from the lower limit of quantification of GC/MS does not exceed the target lower limit of quantification (see Section 3 3.2.3). Similarly, obtain the lower limit of detection of GC/MS from the *SN* ratio ($S/N$=3) and the lower limit of detection in a sample.

**<<Notes and precautions>>**

[46] The noise width (N) and the signal height (S) are generally calculated as follows. Firstly, measure noises in the proximity of a signal (in a range about 10-fold of the half width of the signal) to obtain the noise width (N) as twofold of their standard deviation, or otherwise, the width between the maximum ($E_1$) and the minimum ($E_2$) of noises is

empirically about 5-fold of the standard deviation and thus the noise width (N) is obtained as 2/5 of that width. On the other hand, the signal height (S) is obtained as the width between the median of noises (C) as the baseline and the signal top (D) determined based on the baseline noises (see the figure below).

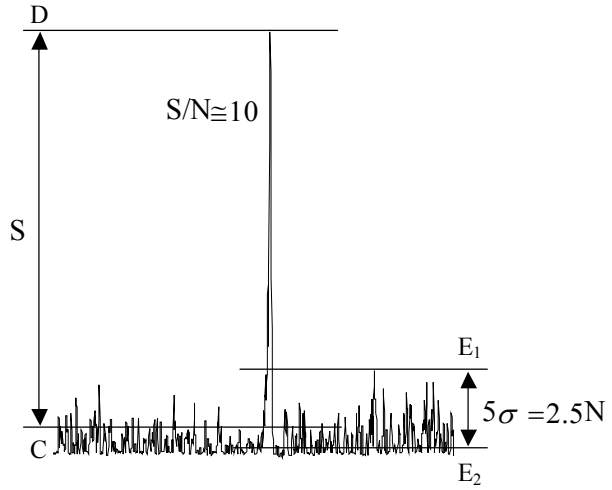


Figure Approximation of the *SN* ratio in a chromatogram

### 4.3 Preparation of the standard curve (calculation of RRF and $RRF_{SS}$) [Guideline Chapter 2 Section 2 4.3]

Prepare a new standard curve and obtain RRF (relative response factor of analyte substances and corresponding internal standards for cleanup spike) and $RRF_{SS}$ (relative response factor of internal standards for cleanup spike and corresponding internal standards for syringe spike) when a new GC/MS instrument is introduced and when the standard solution is exchanged.

For respective analyte substances, prepare standard solutions of about five steps in the concentration range of 0.1 ng/mL-500 ng/mL. [47] Add the same internal standard as used in cleanup spike and syringe spike to these standard solutions in advance before filling up to the volume to be 1-20 ng/mL as dioxins. Inject 1 μL of the standard solution to a GC/MS to record chromatograms of respective analyte substances. Obtain the intensity ratio of peak areas of two monitor ions of respective analyte substances to confirm that it almost matches to the natural abundance ratio (Table 2-6). [48] Prepare a standard curve using the peak area ratio of respective analyte substances to corresponding internal standards for cleanup spike as well as the concentration ratio of respective analyte substances in the injected standard solution and the internal standard for cleanup spike, and calculate the relative response factor (RRF).

$$RRF_k = \frac{C_{CS,k}}{C_{s,k}} \times \frac{A_{s,k}}{A_{CS,k}}$$

RRFk : Relative response factor of the analyte substance k (hereinafter the same shall apply)
CCS : Concentration of the internal standard for cleanup spike in the standard solution
$C_s$ : Concentration of the analyte substance in the standard solution
$A_s$ : Peak area of the analyte substance in the standard solution
$A_{CS}$ : Peak area of the internal standard for cleanup spike in the standard solution

For the preparation of the standard curve, conduct analysis at least three times repeatedly for one concentration, and obtain 15 or more data in total in the whole concentration range, to obtain the mean value as RRF. The coefficient of variation here has to be within 20 %. [49]

On the other hand, RRF can be obtained as the slope of the linear regression line calculated from the data of the standard curve by the least square method. In that case, linearity should be sufficient and the intercept of the regression formula should infinitely approach zero.

Similarly, calculate the relative response factor ($RRF_{SS}$) of respective internal standards for cleanup spike to internal standards for syringe spike is calculated by the following formula:

$$RRF_{SS,k} = \frac{C_{SS}}{C_{CS,k}} \times \frac{A_{CS,k}}{A_{SS}}$$

RRFSS, k : Relative response factor of the internal standard for cleanup spike k hereinafter the same shall apply)
CSS : Concentration of the internal standard for syringe spike in the standard solution
CCS : Concentration of the internal standard for cleanup spike in the standard solution
$A_{CS}$ : Peak area of the internal standard for cleanup spike in the standard solution
$A_{SS}$ : Peak area of the internal standard for syringe spike in the standard solution

Table 2-6 Natural abundance ratio of isotope peaks of dioxins by the number of chlorine atoms

(%)

| Congener | Analyte substance (native) | | | | |
|---|---|---|---|---|---|
| | $M^+$ | $(M+2)^+$ | $(M+4)^+$ | $(M+6)^+$ | $(M+8)^+$ |
| TeCDDs | 77.43 | 100.00 | 48.74 | 10.72 | 0.94 |
| PeCDDs | 62.06 | 100.00 | 64.69 | 21.08 | 3.50 |
| HxCDDs | 51.79 | 100.00 | 80.66 | 34.85 | 8.54 |
| HpCDDs | 44.43 | 100.00 | 96.64 | 52.03 | 16.89 |
| OCDD | 34.54 | 88.80 | 100.00 | 64.48 | 26.07 |
| TeCDFs | 77.55 | 100.00 | 48.61 | 10.64 | 0.92 |
| PeCDFs | 62.14 | 100.00 | 64.57 | 20.98 | 3.46 |
| HxCDFs | 51.84 | 100.00 | 80.54 | 34.72 | 8.48 |
| HpCDFs | 44.47 | 100.00 | 96.52 | 51.88 | 16.80 |
| OCDF | 34.61 | 88.89 | 100.00 | 64.39 | 25.98 |
| TeCBs | 77.68 | 100.00 | 48.48 | 10.56 | 0.90 |
| PeCBs | 62.22 | 100.00 | 64.45 | 20.87 | 3.42 |
| HxCBs | 51.90 | 100.00 | 80.43 | 34.60 | 8.42 |
| HpCBs | 44.51 | 100.00 | 96.40 | 51.73 | 16.71 |

**<<Notes and precautions>>**

[47] The concentration range must include low concentrations near the lower limit of quantification and must be within the dynamic range of the GC/MS.

[48] Conduct quantification using the mean of peak areas of two ions or the areas of major peaks.

[49] The specification is within 5% in quantification methods relevant to environmental measurements including JIS; in this Guideline, however, the acceptable range is wider because the concentration of the standard solution is low and the standard curve is prepared for each measurement. The value does not exceed 10% under normal conditions when the GC/MS is appropriately maintained.

## 4.4 Sample analysis [Guideline Chapter 2 Section 2 4.4]

### 4.4.1 Checking of the standard curve

At the start of measurement, measure one or more standard solutions for the standard curve, calculate relative response factor (RRF) of respective analyte substances and corresponding internal standards for cleanup spike as well as relative response factor ($RRF_{SS}$) of the internal standard for cleanup spike and corresponding internal standard for syringe spike (see 4.3), compare these to RRF and $RRF_{SS}$ at the time of the preparation of the standard curve, and confirm that the variance is within 20 %. When RRF and $RRF_{SS}$ vary out of the range, eliminate the cause and measure the solution for the standard curve again. In addition, as for the retention time, when it varies in a relatively short period (variance in retention time ≥ ±1 %, or in the relative retention ratio to the internal standard ≥ ±0.5 % per day under normal conditions), eliminate the cause and measure the solution for the standard curve again.

After starting measurement, measure the standard solution for the standard curve of similar concentration as the estimated concentration in the sample solution at regular intervals to check RRF and retention time in the same way as shown above. If RRF and retention time vary out of the range indicated above, eliminate the cause and analyze the samples before that again.

### 4.4.2 Identification and quantification of a sample

Inject 1-2 μL of the operation blank and the sample solution to a GC/MS for analysis. Record chromatograms of analyte substances set in 4.1.2. After the end of measurement, calculate the ratio of peak areas of two monitor ions for each sample. [50] Check the monitor channel of lock mass for each sample. [51] Calculate the ratio of peak areas of respective analyte substances and corresponding internal standards for cleanup spike, and calculate the amount of respective analyte substances in the sample extract by the following formula using corresponding relative response factor (RRF) obtained in advance in 4.3. [48][52]

$$Q_{s,k} = \frac{A_{s,k}}{A_{CS,k}} \times \frac{Q_{CS,k}}{RRF_k}$$

$Q_{s,k}$ : Amount of the analyte substance *k* (hereinafter the same shall apply) (pg) in the sample extract

$A_s$ : Peak area of the analyte substance in the sample solution

$A_{CS}$ : Peak area of the internal standard for cleanup spike in the sample solution
$Q_{CS}$ : Amount of the internal standard for cleanup spike (pg) added to the sample extract
RRF : Relative response factor of the analyte substance to the internal standard for cleanup spike calculated at the time of preparation of the standard curve (see 4.3)

**4.4.3 Calculation of recovery**

Recovery is calculated by the following formula using the ratio of the peak area of the internal standard for cleanup spike and the peak area of the internal standard for syringe spike, and corresponding relative response factor ($RRF_{SS}$, see 4.3), to check the recovery (Rc) of extraction and cleanup. [53]

$$Rc_k = \frac{A_{CS,k}}{A_{SS}} \times \frac{Q_{SS}}{Q_{CS,k}} \times \frac{1}{RRF_{SS,k}} \times 100$$

$Rc_k$ : Recovery (%) of the internal standard for cleanup spike *k* (hereinafter the same shall apply)
$A_{CS}$ : Peak area of the internal standard for cleanup spike in the sample solution
$A_{SS}$ : Peak area of the internal standard for syringe spike in the sample solution
$Q_{SS}$ : Amount (pg) of the internal standard for syringe spike added in the sample solution
$Q_{CS}$ : Amount (pg) of the internal standard for cleanup spike added in the sample solution
$RRF_{SS}$ : Relative response factor of the internal standard for cleanup spike to the internal standard for syringe spike calculated at the time of preparation of the standard curve

**<<Notes and precautions>>**

[50] Identify 2,3,7,8-chlorine-substituted isomer by good separation of the peak on the obtained SIM chromatogram, and by the fact that the retention time is almost the same as the standard substance, and that the relative retention time to corresponding internal standard also matches to the standard substance. Identify isomers without the standard substance with reference to literature.
Quantify when the peak area ratio of two monitor ions on the SIM chromatogram is almost the same as that of the standard substance, and is within ±15 % (it can be ±25 % for concentration near the lower limit of detection) of the natural abundance ratio of isotopes (Table 2-6). For deviation from the natural abundance ratio, quantify a peak that is positively an analyte substance using one of the two monitor ions only when the other monitor ion is clearly affected by noises using the other monitor ion.

[51] When there is variance such as rise and fall in the SIM chromatogram of the lock mass channel, and in particular when the phenomenon is observed in the location where the analyte substance appears, the component must not be quantified because the peak may not be accurately captured and the accuracy is significantly reduced. A possible major reason is insufficient purification of the sample solution; therefore it is needed to re-purify or re-analyze the GC/MS sample solution to minimize the variance of the lock mass.

[52] Quantify 2,3,7,8-chlorine-substituted isomer using the corresponding standard substance. When the other isomers need to be quantified, calculate on the assumption that the sensitivity is the same as the 2,3,7,8-chlorine-substituted isomer for each chlorine number.

[53] When the recovery of the cleanup spike is outside the range of 40 % or more up to 120 % or less, analyze again basically from pretreatment.

## 5 Use of numerical values [Guideline Chapter 2 Section 2 5]

### 5.1 Expression of concentration

#### 5.1.1 Calculation of concentration

Calculate the concentration of dioxins in feeds from the results obtained in 4.4.2 using the following formula. [54] Round off the concentration at the third digit in principle to be expressed in two significant digits. However, the digits below those expressed in the lower limit of detection in the sample obtained in 4.2.3 shall not be displayed.

$$C_k = \frac{Q_{s,k} - Q_{B,k}}{W}$$

$C_k$ : Concentration (pg/g) of the analyte substance *k* (hereinafter the same shall apply) in the sample

$Q_s$ : Amount (pg) of the analyte substance in the sample extract

$Q_B$ : (When there is the operation blank value) amount (pg) of the analyte substance in the sample solution in the blank test (see Section 3)

W : Sampling amount (g)

#### 5.1.2 Conversion into toxicity equivalency quantity

To convert the concentration of dioxins into toxicity equivalency quantity, multiply the concentrations of respective analyte substances (pg/g) calculated in 5.1.1 by the toxicity equivalency factor (TEF) shown in Table 2-7 and Table 2-8, and obtain their total to be the toxicity equivalency quantity (TEQ: pg-TEQ/g). Do not round off the toxicity equivalency quantity of each isomer, but round off the third digit of the sum, to be expressed in two significant digits. When the measured value is either below the lower limit of quantification or below the lower limit of detection, convert using one of calculation methods shown in Table 2-9, and indicate which calculation method is used. [55]

Table 2-7 Toxicity equivalency factor of PCDD/DFs

| | Analyte substance | TEF(2005) |
|---|---|---|
| PCDDs | 2,3,7,8-TeCDD | 1 |
| | 1,2,3,7,8-PeCDD | 1 |
| | 1,2,3,4,7,8-HxCDD | 0.1 |
| | 1,2,3,6,7,8-HxCDD | 0.1 |
| | 1,2,3,7,8,9-HxCDD | 0.1 |
| | 1,2,3,4,6,7,8-HpCDD | 0.01 |
| | OCDD | 0.0003 |
| PCDFs | 2,3,7,8-TeCDF | 0.1 |
| | 1,2,3,7,8-PeCDF | 0.03 |
| | 2,3,4,7,8-PeCDF | 0.3 |
| | 1,2,3,4,7,8-HxCDF | 0.1 |
| | 1,2,3,6,7,8-HxCDF | 0.1 |
| | 1,2,3,7,8,9-HxCDF | 0.1 |
| | 2,3,4,6,7,8-HxCDF | 0.1 |
| | 1,2,3,4,6,7,8-HpCDF | 0.01 |
| | 1,2,3,4,7,8,9-HpCDF | 0.01 |
| | OCDF | 0.0003 |

Table 2-8 Toxicity equivalency factor of Co-PCBs

| | Analyte substance（IUPAC No.） | TEF(2005) |
|---|---|---|
| Non-*ortho* Co-PCBs | 3,3',4,4'-TeCB (#77) | 0.0001 |
| | 3,4,4',5-TeCB (#81) | 0.0003 |
| | 3,3',4,4',5-PeCB (#126) | 0.1 |
| | 3,3',4,4',5,5'-HxCB (#169) | 0.03 |
| Mono-*ortho* Co-PCBs | 2,3,3',4,4'-PeCB (#105) | 0.00003 |
| | 2,3,4,4',5-PeCB (#114) | 0.00003 |
| | 2,3',4,4',5-PeCB (#118) | 0.00003 |
| | 2',3,4,4',5-PeCB (#123) | 0.00003 |
| | 2,3,3',4,4',5-HxCB (#156) | 0.00003 |
| | 2,3,3',4,4',5'-HxCB (#157) | 0.00003 |
| | 2,3',4,4',5,5'-HxCB (#167) | 0.00003 |
| | 2,3,3',4,4',5,5'-HpCB (#189) | 0.00003 |

#Number indicates IUPAC No.

Table 2-9 Use of quantification values in calculation methods for toxicity equivalency quantity

| Calculation method | Size of quantification value | | |
|---|---|---|---|
| | Not less than the lower limit of quantification | Below the lower limit of quantification and not less than the lower limit of detection | Below the lower limit of detection |
| 1 | Use the quantification value | Use 0 | Use 0 |
| 2 | Use the quantification value | Use the quantification value | Use the lower limit of detection |
| 3 | Use the quantification value | Use the quantification value | Use 1/2 value of the lower limit of detection |

**<<Notes and precautions>>**

[54] When a specific peak is interfered, that data cannot be used in principle. However, the data can be used if the operation blank value is 30% or less of the analytical value of the peak.

[55] Generally in the analysis conducted to comprehend the actual state of persistence, isomers detected above the lower limit of quantification are converted into toxicity equivalency quantity, and their sum is calculated as the toxicity equivalency quantity of detected dioxins. For risk (intake amount) evaluation, there is a method to estimate the toxicity equivalency quantity as the sum of: isomers detected above the lower limit of detection converted into the toxicity equivalency quantity; and the lower limit of detection multiplied by factors such as 1/2 for isomers that are not detected considering the possibility that the sample may contain isomers below the lower limit of detection.

## 6 Expression [Guideline Chapter 2 Section 2 6]

Measurement results for dioxins are expressed as follows:

a) PCDD/DFs

Measurement results for PCDD/DFs concentration are expressed according either to Table 2-10 for 2,3,7,8-chlorine-substituted isomers shown in Table 2-10, or to Table 2-11 as the total of congeners for the analysis of the other tetra- to hepta-chlorides (TeCDDs-HpCDDs and TeCDFs-HpCDFs).

As for the concentration of respective analyte substances, express values not less than the lower limit of quantification as is, and values below the lower limit of quantification and not less than the lower limit of detection in parenthesis. In addition, values below the lower limit of detection shall be described so that it is clear that it was not detected.

The lower limit of quantification to be shown shall be the lower limit of quantification

measured for the respective samples. Or otherwise, the target lower limit of quantification can be shown if the measured lower limit of quantification was lower than the target lower limit of quantification.

b) Co-PCBs

Measurement results for Co-PCBs concentration are expressed according to Table 2-10 in the same way as PCDD/DFs.

Table 2-10 Expression of measurement results for dioxins (example)

| Dioxins | | TEF (WHO, 2005) | Concentration (pg/g) | Toxicity equivalency quantity (TEQ) (pg-TEQ/g) | lower limit of quantification (pg/g) | lower limit of detection (pg/g) |
|---|---|---|---|---|---|---|
| PCDDs | 2,3,7,8-TeCDD | 1 | | | | |
| | 1,2,3,7,8-PeCDD | 1 | | | | |
| | 1,2,3,4,7,8-HxCDD | 0.1 | | | | |
| | 1,2,3,6,7,8-HxCDD | 0.1 | | | | |
| | 1,2,3,7,8,9-HxCDD | 0.1 | | | | |
| | 1,2,3,4,6,7,8-HpCDD | 0.01 | | | | |
| | OCDD | 0.0003 | | | | |
| PCDFs | 2,3,7,8-TeCDF | 0.1 | | | | |
| | 1,2,3,7,8-PeCDF | 0.03 | | | | |
| | 2,3,4,7,8-PeCDF | 0.3 | | | | |
| | 1,2,3,4,7,8-HxCDF | 0.1 | | | | |
| | 1,2,3,6,7,8-HxCDF | 0.1 | | | | |
| | 1,2,3,7,8,9-HxCDF | 0.1 | | | | |
| | 2,3,4,6,7,8-HxCDF | 0.1 | | | | |
| | 1,2,3,4,6,7,8-HpCDF | 0.01 | | | | |
| | 1,2,3,4,7,8,9-HpCDF | 0.01 | | | | |
| | OCDF | 0.0003 | | | | |
| Total PCDD/DFs | | — | — | | — | — |
| Non-*ortho* Co-PCBs | 3,3',4,4'-TeCB (#77) | 0.0001 | | | | |
| | 3,4,4',5-TeCB (#81) | 0.0003 | | | | |
| | 3,3',4,4',5-PeCB (#126) | 0.1 | | | | |
| | 3,3',4,4',5,5'-HxCB (#169) | 0.03 | | | | |
| Mono-*ortho* Co-PCBs | 2,3,3',4,4'-PeCB (#105) | 0.00003 | | | | |
| | 2,3,4,4',5-PeCB (#114) | 0.00003 | | | | |
| | 2,3',4,4',5-PeCB (#118) | 0.00003 | | | | |
| | 2',3,4,4',5-PeCB (#123) | 0.00003 | | | | |
| | 2,3,3',4,4',5-HxCB (#156) | 0.00003 | | | | |
| | 2,3,3',4,4',5'-HxCB (#157) | 0.00003 | | | | |
| | 2,3',4,4',5,5'-HxCB (#167) | 0.00003 | | | | |
| | 2,3,3',4,4',5,5'-HpCB (#189) | 0.00003 | | | | |
| Total Co-PCBs | | — | — | | — | — |
| Total | | — | — | | — | — |

A value in parenthesis in the Concentration column indicates that the concentration is not less than the lower limit of detection and is below the lower limit of quantification.
"N. D." in the Concentration column means it is below the lower limit of detection.
Conversion into the toxicity equivalent quantity was conducted assuming values below the

lower limit of quantification as 0.
#Number indicates IUPAC No.

Table 2-11 Expression of total dioxin congeners (example)

| | Congener | Concentration (pg/g) | lower limit of quantificaication (pg/g) |
|---|---|---|---|
| PCDDs | TeCDDs | | |
| | PeCDDs | | |
| | HxCDDs | | |
| | HpCDDs | | |
| | OCDD | | |
| PCDFs | TeCDFs | | |
| | PeCDFs | | |
| | HxCDFs | | |
| | HpCDFs | | |
| | OCDF | | |

### iii Quality control of measurement data [Guideline Chapter 2 Section 3 ]

In the analysis of dioxins, sufficient accuracy control is needed not only because hypersensitive analysis is required but because extreme accuracy is needed for the separation and identification of many isomers of chlorine-substituted congeners. See Figure 2-3.

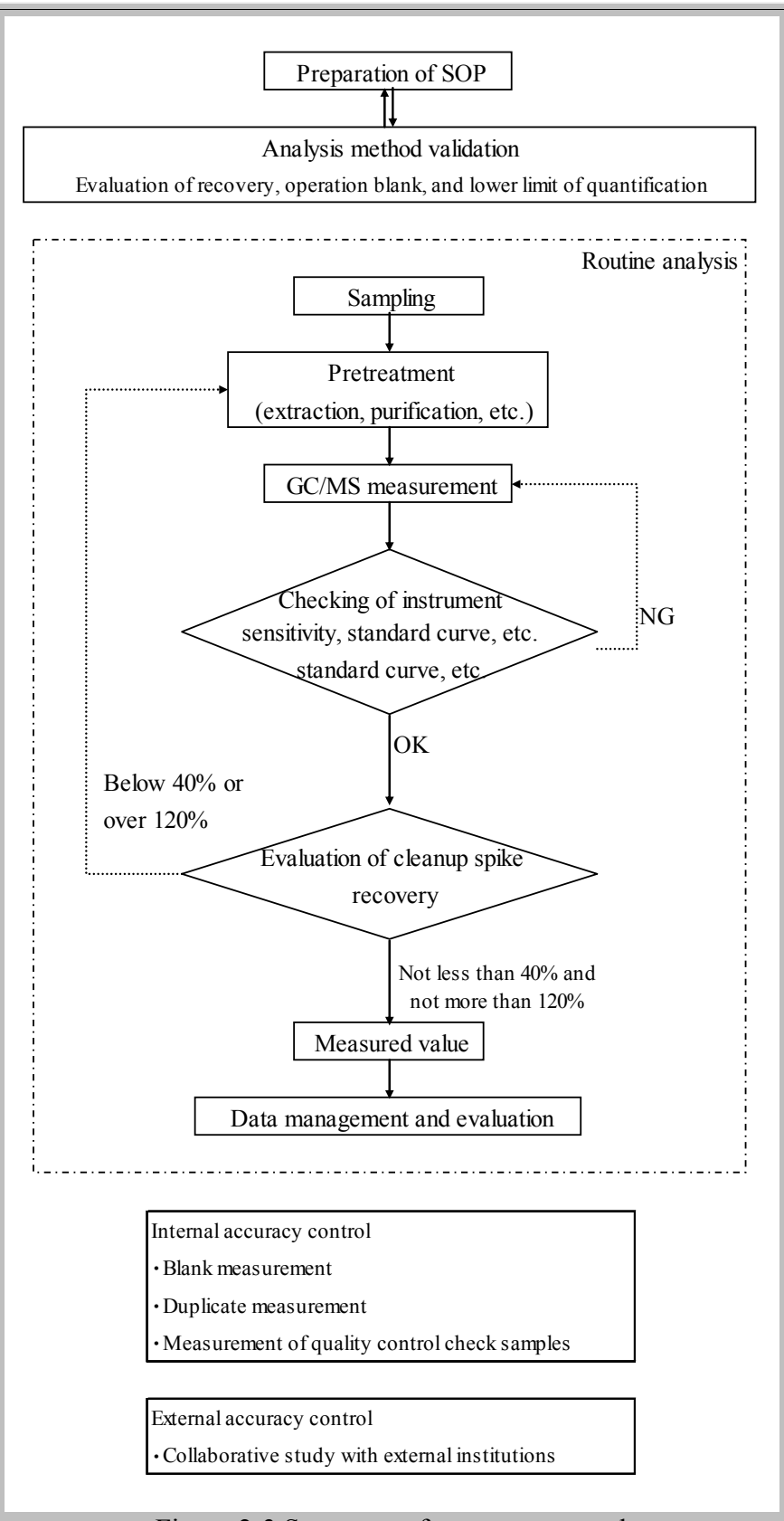


Figure 2-3 Summary of accuracy control

## 1 Preparation of the standard operation procedures (SOP) [Guideline Chapter 2 Section 3 1]

Test institutions should set up operation procedures for the following items etc. The operation procedures need to be specific, easy to understand, and made well known to persons involved.

1) Methods for preparation, maintenance, storage and handling of apparatus for sampling, etc.
2) Methods for preparation, purification, storage and handling of reagents for pretreatment.
3) Methods for preparation of reagents for analysis and standard substances, etc., and for preparation, storage and handling of standard solutions.
4) Setup, adjustment, operation procedure and maintenance of analytical instruments.
5) Records of all the processes of the analysis method (including computer hardware and software used.).

## 2 Analysis method validation [Guideline Chapter 2 Section 3 2]

Conduct validation and record the results when a new analysis method is introduced and when the analysis method is modified.

### 2.1 Evaluation of cleanup procedure

Because fractionation conditions differ in column chromatography depending on the type and activity of packing used, or on the type and amount of solvent, conduct a fractionation test using the standard solution that contains all the analyte substances to set up conditions in advance.

### 2.2 Evaluation of recovery

Measure the recovery of the internal standard for cleanup spike. Confirm that the recovery of the internal standard for cleanup spike is not less than 40 % and not more than 120 % (see Section 2 4.4.3).

### 2.3 Evaluation of blank value [56]

Conduct blank tests five times or more repeatedly to obtain the mean and the standard deviation of the blank value.

### 2.4 Confirmation of accuracy by the analysis of the standard sample

Analyze a suitable standard sample [1] to confirm that the obtained value is within the range of the certified value.

### 2.5 Evaluation of the lower limit of quantification

For compounds that have the blank value in 2.3, obtain the amount equivalent to 10-fold of the standard deviation of the blank value. On the other hand, calculate the amount equivalent to *S*/*N*=10 from noises on the chromatogram and the signal height obtained from the standard solution. [46] The larger value of these two values shall be the lower limit of quantification.

**<<Notes and precautions>>**

[56] A blank test is conducted simultaneously with sample analysis in order to check contamination due to sample solution preparation, introduction to the GC/MS, etc. to set up analysis environment with no hindrance to sample analysis to secure the reliability of analytical values.
When the blank value converted into the concentration in feeds is over the target lower limit of quantification, wash the apparatus again and adjust the instruments to reduce the blank value as much as possible.

## 3 Confirmation of reliability during analysis [Guideline Chapter 2 Section 3 3]

Conduct procedures listed below each time analysis is conducted, and record the results. In addition, conduct re-analysis in principle when the prescribed standard is not confirmed.

### 3.1 Reliability of instruments

#### 3.1.1 Confirmation of instrument sensitivity

Set up the operation procedure of the GC/MS, adjust as appropriate, and then inject the standard solution to obtain the lower limit of quantification of the GC/MS from the *SN* ratio. Confirm that the lower limit of quantification in a sample calculated from the lower limit of quantification of the GC/MS does not exceed the target lower limit of quantification, and record it (see Section 2 4.2.3).

#### 3.1.2 Confirmation of the standard curve (RRF)

Analyze the standard solution for the standard curve at regular intervals to confirm that the relative sensitivity of respective chlorine-substituted dioxins and internal standards is not fluctuated (see Section 2 4.3).

#### 3.1.3 Confirmation of injection volume

For every sample solution measured by a GC/MS, confirm that the liquid volume as configured is injected to the GC/MS based on peak areas of the internal standard for syringe spike, etc.

### 3.2 Reliability of measured values

#### 3.2.1 Evaluation of the blank value

Confirm that the blank value is similar to the value obtained in analysis method validation (2.3). However, it is not needed to conduct this each time if the blank value is controlled to be low enough so that it does not affect the analytical value.

**3.2.2 Evaluation of recovery**

Obtain recovery from the area ratio of syringe spike and cleanup spike in the sample solution (see Section 2 4.4.3). Confirm that the recovery is in the range not less than 40 % and not more than 120 %.

**3.2.3 Evaluation of the lower limit of quantification**

For analyte substances that have the blank value in 2.3, obtain the amount equivalent to 10-fold of the standard deviation of the blank value. On the other hand, calculate the amount equivalent to $S/N$=10 from noises on the chromatogram and the signal height obtained from the standard solution. The larger value of these two values shall be the lower limit of quantification. For analyte substances with no blank value, the latter shall be the lower limit of quantification. Confirm that the lower limit of quantification does not exceed the target lower limit of quantification.
When the lower limit of quantification of dioxins is higher than the target lower limit of quantification, check instruments and apparatus etc. to adjust so that the target lower limit of quantification is not exceeded. When the value is not reached, state as such in the test report.

## 4 Data management and evaluation [Guideline Chapter 2 Section 3 4]

**4.1 Handling of outliers and missing values**

When there is large variance in the sensitivity of analytical instruments, it is needed to conduct re-analysis or re-sampling as missing values because there is a problem in the reliability of analytical values. If such a problem emerges, it not only takes significant labor, time and cost but affect the evaluation of analytical results in total when there are many outliers and missing values; therefore care should be taken so that outliers or missing values will not be observed by, for example, sufficient checking in advance. In addition, it is important to fully examine the background of outliers and missing values and record it to utilize it to prevent similar troubles in the future.

**4.2 Records relevant to reliability of analysis**

Record, organize and store the following data and submit it if a report on reliability control of measured data is requested.
(1) Methods for sampling and sample storage
(2) Sample status
(3) Methods for sample pretreatment
(4) Manufacturers, product numbers, and lot numbers of standard substances, etc.
(5) Operation conditions and calibration results of analytical instruments.
(6) RRF of the standard curve, sensitivity, operation blank values, recovery, and the lower limit of quantification.

## 5 Internal accuracy control [Guideline Chapter 2 Section 3 5]

Conduct a test at regular intervals in order to confirm general reliability of the analysis environment and analysis procedures.

**5.1 Blank measurement**

In the cases to which any of the following apply, conduct a blank test using the same amount of the same reagents used for sample pretreatment, and confirm that the result is at a similar level to the blank value obtained in 2.3.

(1) When 10 samples are pretreated in series.
(2) When there is a significant change in the pretreatment procedure such as employment of new reagents or instruments or repaired instruments.
(3) When a sample of high concentration is measured for which there may be cross contamination.

**5.2 Duplicate measurement**

Conduct duplicate measurements of the same sample under the same conditions at a frequency of about 10 % of the sample number, to calculate the mean of measured values not less than the lower limit of quantification, and confirm that the difference between quantification values and the mean are within 30%, respectively.

**5.3 Measurement of quality control check samples**

Analyze a suitable sample of known concentration once or more a year, and record the result.

## 6 External accuracy control [Guideline Chapter 2 Section 3 6]

It is preferred to participate in collaborative studies involving external institutions and evaluate the reliability of the quantification value.

## iv Safety control [Guideline Chapter 2 Section 4]

### 1 Facility

There should be equipment to prevent emission of dioxins to outside, for example, all the handling of the standard substances and analytical procedures should be conducted in the controlled area, and the area should have negative pressure.

1) Laboratory

I A laboratory should be for exclusive use.

II It is preferred, if possible, to compart a laboratory into 2-3 areas. The role of each area in that case is as described below:

a) An area for sample degradation, extraction, purification and concentration. [57]

b) An area for the analysis by a gas chromatograph mass spectrometer (GC/MS).

III When a GC/MS for common use is used, the instrument should be for exclusive use for dioxin analysis for a certain period, and at the same time, conduct experiments not to contaminate the instrument and its surroundings.

**2 Entry restriction to laboratories etc.**

1) Restrict entry into the laboratory to persons involved.
2) The laboratory door should have signs so that there shall be no unauthorized entry.

**3 Ventilation system**

1) The laboratory should be ventilated by a draft chamber.
2) Air emission should be treated by treatment instruments such as activated charcoal filters to prevent pollution of the external environment.
3) Air supply should be controlled using filters etc. to avoid entrance of dioxins adsorbed to particulate matter.
4) Install filters etc. in the pump of the mass spectrometer and in the emission part of the gas chromatograph to avoid pollution in the room.

**4 Other equipment**

1) Glove box
   Conduct operations in a glove box when a large amount of dioxins is handled.
2) Ultraviolet lamp
   An ultraviolet lamp is used to radiate the area contaminated by dioxins.
3) Air emission
   All the air emission from pumps attached to the GC/MS should be emitted via treatment instruments such as activated charcoal filters in the same way as the duct of the draft chamber.

**5 Operation in the laboratory**

1) Wear exclusive laboratory cloths and shoes in the laboratory.
2) Wear gloves and safety glasses during operation.
3) Use a pipette aid etc. to use a transfer pipette or a measuring pipette, etc., and do not suck with the mouth.

**6 Handling of the standard substances**

1) Prepare the list of all the standard solutions.
2) Store all the standard solutions in e.g. double-capped sample bottles in a refrigerator.

A large amount of standard substances and standard solutions should be stored in a locked cabinet.

## 7 Sample handling

1) Store concentrated extracts in e.g. sealable mini sample bottles in a refrigerator.
2) When long-term storage is expected, seal in amber ampoules, protect from damage, and store in a refrigerator.
3) When samples containing dioxins are transported, carry in sealable plastic containers to avoid leakage to outside even if bottles are damaged.

## 8 Measures against accidents during experiment

While it is unlikely that there is a risk in particular in the analysis for dioxins in feeds because the amount to be handled is minute, in case of accident during experiment, contact persons who use the laboratory and take the following measures:

1) In case of skin contact, for example, when a person is wet by splash of extract containing dioxins during experiment, wash immediately the contact area with soap.
2) In case of spill in the laboratory, wipe the contaminated area with a paper towel wet with water, then wipe the area off with an organic solvent such as alcohol or toluene.

## 9 Waste storage and disposal etc.

1) Store harmful solid waste (gloves, masks, paper towel, and activated charcoal filters etc. that are suspected to be contaminated) in an exclusive plastic bucket.
2) Store harmful liquid waste (contaminated solvent waste produced in the analysis processes and in the washing of apparatus, and oil waste from the gas chromatograph mass spectrometer, etc.) in an exclusive airtight container.
3) Treat waste water suitably with activated charcoal, etc. before discharge.

## 10 Work record

1) Record persons that enter into the laboratory.
2) Prepare daily work reports and record work time etc. of analysis staffs.
3) Record the name of substance, number, concentration, source, and acceptor, as well as the state of use of standard solutions.
4) Record storage status and disposal status of waste.
5) Record other relevant matters.

## 11 Medical examination

As organic solvents etc. are used in the analysis shown in the Guideline, conduct periodic medical examination relevant to specified chemical substances as defined in the Industrial Safety and Health Act. Serum triglycerides and cholesterols, etc. are indicators of the effects of dioxins.

**<<Notes and precautions>>**

[57] In a laboratory where contaminated samples of higher concentrations such as exhaust and fly ash are analyzed, it is preferred to further compart the area to handle higher concentration samples and the area to handle lower concentration samples such as feeds. When a laboratory for common use is used, ensure the reduction in the blank value during the period of analysis of feeds, etc. by avoiding bringing contaminated samples of higher concentrations such as exhaust and fly ash into the laboratory.

Reference Yamata, T., Kashima, S., Shirai, Y. Research Report of Animal Feed, 29, 84 (2004).
Shirai, Y., Yamata, T., Akimoto, K. Research Report of Animal Feed. 29, 114 (2004).
Shirai, Y., Morito, K., Yamata, T. Research Report of Animal Feed. 29, 130 (2004).
Shirai, Y., Morito, K. Research Report of Animal Feed. 29, 150 (2004).

《Analysis method validation》

・Results of a collaborative study

（Each isomer）

| isomer | Sample type | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Rice straw | | | | | Fish meal | | | | |
| | The number of laboratories | mean value (ng/kg) | $RSD_r$ (%) | $RSD_R$ (%) | HorRat | The number of laboratories | mean value (ng/kg) | $RSD_r$ (%) | $RSD_R$ (%) | HorRat |
| (PCDD) | | | | | | | | | | |
| 2,3,7,8-TeCDD | 9 | 0.04 | 12.9 | 23.4 | 1.1 | – | – | – | – | – |
| 1,2,3,7,8-PeCDD | 12 | 0.20 | 7.1 | 21.9 | 1.0 | 8 | 0.05 | 16.4 | 15.9 | 0.7 |
| 1,2,3,4,7,8-HxCDD | 11 | 0.24 | 6.6 | 22.3 | 1.0 | – | – | – | – | – |
| 1,2,3,6,7,8-HxCDD | 12 | 0.53 | 6.2 | 15.5 | 0.7 | – | – | – | – | – |
| 1,2,3,7,8,9-HxCDD | 11 | 0.46 | 7.4 | 17.3 | 0.8 | – | – | – | – | – |
| 1,2,3,4,6,7,8-HpCDD | 12 | 12.1 | 6.2 | 10.3 | 0.5 | 8 | 0.10 | 21.3 | 21.4 | 1.0 |
| OCDD | 11 | 227 | 4.4 | 9.6 | 0.5 | 9 | 0.78 | 10.5 | 11.9 | 0.5 |
| (PCDF) | | | | | | | | | | |
| 2,3,7,8-TeCDF | 11 | 0.22 | 6.1 | 13.6 | 0.6 | 10 | 0.47 | 2.9 | 10.0 | 0.5 |
| 1,2,3,7,8-PeCDF | 11 | 0.36 | 5.4 | 17.3 | 0.8 | 8 | 0.05 | 17.7 | 18.7 | 0.9 |
| 2,3,4,7,8-PeCDF | 12 | 0.29 | 4.7 | 13.9 | 0.6 | 10 | 0.15 | 8.7 | 9.2 | 0.4 |
| 1,2,3,4,7,8-HxCDF | 10 | 0.39 | 6.6 | 8.2 | 0.4 | – | – | – | – | – |
| 1,2,3,6,7,8-HxCDF | 10 | 0.44 | 3.5 | 7.5 | 0.3 | – | – | – | – | – |
| 1,2,3,7,8,9-HxCDF | – | – | – | – | – | – | – | – | – | – |
| 2,3,4,6,7,8-HxCDF | 11 | 0.58 | 3.8 | 12.5 | 0.6 | – | – | – | – | – |
| 1,2,3,4,6,7,8-HpCDF | 12 | 2.08 | 7.9 | 9.5 | 0.4 | 7 | 0.05 | 23.0 | 27.7 | 1.3 |
| 1,2,3,4,7,8,9-HpCDF | 11 | 0.19 | 11.9 | 13.6 | 0.6 | – | – | – | – | – |
| OCDF | 11 | 2.39 | 6.7 | 9.9 | 0.5 | – | – | – | – | – |
| (Non-*ortho* Co-PCB) | | | | | | | | | | |
| 3,3',4,4'-TeCB (#77) | 10 | 24.9 | 4.1 | 6.1 | 0.3 | 11 | 36.2 | 14.5 | 23.9 | 1.1 |
| 3,4,4',5-TeCB (#81) | 11 | 1.26 | 4.6 | 8.8 | 0.4 | 9 | 1.55 | 20.9 | 20.9 | 1.0 |
| 3,3',4,4',5-PeCB (#126) | 11 | 1.74 | 4.0 | 8.2 | 0.4 | 9 | 4.81 | 3.4 | 6.0 | 0.3 |
| 3,3',4,4',5,5'-HxCB (#169) | 11 | 0.27 | 7.9 | 10.6 | 0.5 | 10 | 0.88 | 3.8 | 12.1 | 0.6 |
| (Mono-*ortho* Co-PCB) | | | | | | | | | | |
| 2,3,3',4,4'-PeCB (#105) | 12 | 54.8 | 4.3 | 6.0 | 0.3 | 11 | 192 | 7.4 | 10.5 | 0.5 |
| 2,3,4,4',5-PeCB (#114) | 10 | 4.52 | 6.2 | 9.6 | 0.4 | 11 | 12.5 | 13.5 | 15.9 | 0.7 |
| 2,3',4,4',5-PeCB (#118) | 10 | 119 | 4.0 | 7.2 | 0.3 | 9 | 574 | 3.5 | 8.7 | 0.5 |
| 2',3,4,4',5-PeCB (#123) | 10 | 3.58 | 6.5 | 6.5 | 0.3 | 9 | 9.48 | 14.2 | 17.7 | 0.8 |
| 2,3,3',4,4',5-HxCB (#156) | 11 | 7.48 | 3.4 | 7.0 | 0.3 | 11 | 71.2 | 3.2 | 9.0 | 0.4 |
| 2,3,3',4,4',5'-HxCB (#157) | 11 | 2.25 | 4.5 | 6.8 | 0.3 | 11 | 19.6 | 3.2 | 8.2 | 0.4 |
| 2,3',4,4',5,5'-HxCB (#167) | 11 | 3.41 | 2.8 | 5.6 | 0.3 | 10 | 53.9 | 3.7 | 8.5 | 0.4 |
| 2,3,3',4,4',5,5'-HpCB (#189) | 9 | 0.79 | 4.8 | 6.8 | 0.3 | 9 | 9.56 | 2.7 | 5.4 | 0.2 |

$RSD_r$ : Relative standard deviations of repeatability within laboratory

$RSD_R$ : Relative standard deviations of reproducibility between laboratories

（TEQ）

| TEQ | Sample type | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Rice straw | | | | Fish meal | | | |
| | The number of laboratories | mean value (ng-TEQ/kg) | $RSD_r$ (%) | $RSD_R$ (%) | The number of laboratories | mean value (ng-TEQ/kg) | $RSD_r$ $RSD_r$(%) | $RSD_R$ $RSD_R$(%) |
| PCDDs + PCDFs | 12 | 0.834 | 4.6 | 12.4 | 11 | 0.141 | 19.2 | 19.4 |
| Co-PCB | 11 | 0.205 | 4.3 | 7.8 | 10 | 0.632 | 3.0 | 7.2 |
| Total | 12 | 1.035 | 3.4 | 10.2 | 11 | 0.759 | 3.6 | 7.6 |

$RSD_r$ : Relative standard deviations of repeatability within laboratory

$RSD_R$ : Relative standard deviations of reproducibility between laboratories