

Translated English of Chinese Standard: GB5009.205-2024

www.ChineseStandard.net → Buy True-PDF → Auto-delivery.

Sales@ChineseStandard.net

GB

NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA

GB 5009.205-2024

**National food safety standard - Determination of toxic
equivalent of dioxins and their analogues in food**

食品安全国家标准 食品中二噁英及其类似物毒性当量的测定

Issued on: February 08, 2024

Implemented on: August 08, 2024

**Issued by: National Health Commission of the People's Republic of China;
State Administration for Market Regulation.**

Table of Contents

Foreword.....	3
1 Scope	4
2 Principle	4
3 Reagents and materials	4
4 Instruments and apparatuses.....	7
5 Analysis steps.....	8
6 Expression of analysis results.....	17
7 Precision	20
8 Detection-limit	20
9 Others.....	20
10 Principle	21
11 Reagents and materials	21
12 Instruments and apparatuses.....	21
13 Procedure	21
14 Expression of analysis results	25
15 Precision	27
16 Quantitation-limit of the method.....	27
17 Others.....	27
Appendix A Name, CAS number, IUPAC number, toxic equivalency factor (TEF) specified by WHO of 17 kinds of 2,3,7,8-substituted PCDD/Fs and 12 kinds of DL- PCBs.....	28
Appendix B Standard solutions	29
Appendix C Technical requirements for determination methods	34
Appendix D Separation and purification process of fully automatic sample purification system.....	41
Appendix E Standard solution chromatograms	44

National food safety standard - Determination of toxic equivalent of dioxins and their analogues in food

1 Scope

This Standard specifies the determination method of the contents of 17 kinds of 2,3,7,8-substituted polychlorodibenzo-p-dioxin, polychlorino-dibenzofura (PCDD/Fs) and 12 kinds of dioxin-like polychlorinated biphenyl (DL-PCBs) and their toxic equivalent (TEQ) in food (see Table A.1 in Appendix A).

Method I “Isotope dilution - gas chromatography - magnetic high-resolution mass spectrometry” applies to the determination of the content of 17 kinds of PCDD/Fs and 12 kinds of DLPCBs and their TEQ in food.

Method II “Isotope dilution - gas chromatography - triple quadrupole mass spectrometry” applies to the determination of the content of 17 kinds of PCDD/Fs and 12 kinds of DL-PCBs and its TEQ in meat and meat products, aquatic animals and their products, milk and dairy products, eggs and egg products, oils, and fats.

Method I – Isotope dilution - gas chromatography - magnetic high-resolution mass spectrometry

2 Principle

After the sample is extracted, purified and concentrated, measure by a gas chromatography - magnetic high-resolution mass spectrometer and quantify by the stable isotope dilution method; calculate the TEQ of PCDD/Fs and DL-PCBs in the sample by accumulating the toxic equivalency factor (TEF) of each target compound multiplied by the measured content.

3 Reagents and materials

3.1 Reagents

Unless otherwise specified, all the reagents in this method are analytical reagents, and the water is grade-1 water specified by GB/T 6682.

3.1.1 Acetone (C_3H_6O): pesticide residue grade.

it and place it on a shaker to shake until the silica gel is in a uniform flow state. Prepare it immediately before use.

3.2.3 Alkalized silica gel (33%, mass fraction): Weigh 100.0 g of active silica gel into a 250 mL stoppered ground-rotatory flask; add 49.0 g of 1 mol/L sodium hydroxide solution; seal it and place it on a shaker to shake until the silica gel is in a uniform flow state. Prepare it immediately before use.

3.2.4 Silver nitrate silica gel: Weigh 10.0 g of silver nitrate into a 250 mL stoppered ground-rotatory flask; add 40 mL of water to dissolve it; then, slowly add 90.0 g of active silica gel; seal it and place it on a shaker to shake until the silica gel is in a uniform flow state. Prepare it immediately before use.

3.2.5 Alkaline alumina: Take alkaline alumina and activate it at 600 °C for 24 hours. Prepare it immediately before use.

3.2.6 Hydrous florisol (1%, mass fraction): Put an appropriate amount of florisol into a Soxhlet extractor; use n-hexane: dichloromethane (1:1, volume ratio) to extract it for 24 hours; after evaporating, weigh 99.0 g; add 1.0 mL of water; mix evenly in a closed container (such as a stoppered glass flask or a stoppered glass conical flask); prepare immediately before use.

3.2.7 Mixed activated carbon: Weigh 9.0 g of activated carbon and 41.0 g of diatomaceous earth for purification; mix evenly in a closed container (such as a stoppered glass flask or a stoppered glass conical flask); then, activate at 130 °C for 6 hours. Prepare immediately before use.

3.2.8 Anhydrous sodium sulfate: Take anhydrous sodium sulfate and burn it at 660 °C for 6 hours. Prepare immediately before use.

3.3 Standard solution

3.3.1 Standard solution for separation degree check: solution containing natural PCDD/Fs. For specific compounds and concentrations, see Table B.1 in Appendix B.

3.3.2 Standard solution for PCDD/Fs isotope labeled quantitative internal standard: solution containing 15 kinds of $^{13}\text{C}_{12}$ -PCDD/Fs. See Table B.2 for specific compounds and concentrations.

3.3.3 Standard solution for PCDD/Fs isotope labeled recovery rate internal standard: solution containing $^{13}\text{C}_{12}$ -1,2,3,4-TCDD and $^{13}\text{C}_{12}$ -1,2,3,7,8,9-HxCDD. See Table B.3 for specific concentrations.

3.3.4 Standard solution for PCDD/Fs calibration: standard solution containing natural and isotope-labeled PCDD/Fs series calibration curve. See Table B.4 for specific compounds and concentrations.

3.3.5 Standard solution for DL-PCBs isotope labeled quantitative internal standard: solution containing 12 kinds of $^{13}\text{C}_{12}$ -DL-PCBs. See Table B.5 for specific compounds and concentrations.

3.3.6 Standard solution for DL-PCBs isotope labeled recovery rate internal standard: solution containing $^{13}\text{C}_{12}$ -PCB 70, $^{13}\text{C}_{12}$ -PCB 111 and $^{13}\text{C}_{12}$ -PCB 170. See Table B.6 for specific concentrations.

3.3.7 Standard solution for DL-PCBs calibration: standard solution containing natural and isotope-labeled DL-PCBs series calibration curve. See Table B.7 for specific compounds and concentrations.

3.3.8 Standard solution for sensitivity check: Standard solution containing natural and isotope-labeled PCDD/Fs series. See Table B.8 for specific compounds and concentrations.

Note: Store the standard solution at room temperature in the dark, where it has a shelf life of 6 years.

4 Instruments and apparatuses

4.1 Gas chromatography - magnetic high-resolution mass spectrometer (GC-HRMS)

4.2 Balance: The sensitivity is 0.1 g and 0.001 g.

4.3 Tissue homogenizer.

4.4 Pulverizer.

4.5 Freeze dryer.

4.6 Rotary evaporator.

4.7 Nitrogen concentrator.

4.8 Ultrasonic cleaner.

4.9 Shaker.

4.10 Soxhlet extractor, equipped with extraction sleeve.

4.11 Accelerated solvent extraction instrument (optional).

4.12 Muffle furnace: able to maintain constant temperature ($\pm 10\text{ }^{\circ}\text{C}$) within the range of $200\text{ }^{\circ}\text{C} \sim 700\text{ }^{\circ}\text{C}$.

4.13 Oven: able to maintain constant temperature ($\pm 5\text{ }^{\circ}\text{C}$) within the range of $105\text{ }^{\circ}\text{C} \sim 250\text{ }^{\circ}\text{C}$.

4.14 Glass chromatography column: equipped with polytetrafluoroethylene plunger, 150 mm (length) $\times$ 8 mm (inner diameter), 300 mm (length) $\times$ 15 mm (inner diameter).

4.15 Fully automatic sample purification system: equipped with acid-base composite silica gel column, alkaline alumina column and activated carbon purification column.

4.16 Gel permeation chromatography column (GPC): glass column (inner diameter 15 mm $\sim$ 20 mm), containing 50 g of S-X3 gel, or equivalent fully automatic GPC.

5 Analysis steps

5.1 Sample preparation

5.1.1 General food samples: After dry food is crushed evenly, weigh 10 g $\sim$ 20 g (accurate to 0.01 g) for later use; for milk powder, weigh 15 g (accurate to 0.01 g) for later use; after other samples are homogenized, weigh 50 g $\sim$ 200 g (accurate to 0.01 g); freeze-dried for later use.

5.1.2 Grease sample: directly weigh 5 g of sample (accurate to 0.01 g) into an eggplant-shaped bottle; add 10 μ L each of standard solutions for PCDD/Fs and DL-PCBs isotope labeled quantitative internal standard; wait for purification.

5.2 Extraction

5.2.1 Soxhlet extraction

Before extraction, put an empty extraction sleeve into the Soxhlet extractor (4.10); use n-hexane: methylene chloride (1:1, volume ratio) as the extraction solvent; pre-extract at a reflux speed of 3 times/h $\sim$ 4 times/h for 8 hours; then, take it out to dry.

Place the sample (5.1.1) in a ceramic mortar; add anhydrous sodium sulfate and grind it to make a free-flowing powder. Transfer to a pre-cleaned sleeve; respectively add 10 μ L each of standard solutions for PCDD/Fs and DL-PCBs isotope labeled quantitative internal standard; use glass wool to cover the sample; balance for 30 minutes and put it into a Soxhlet extractor; use n-hexane: dichloromethane (1:1, volume ratio) as the extraction solvent; carry out extraction for 18 h $\sim$ 24 h at a reflux rate of 3 times/h $\sim$ 4 times/h.

5.2.2 Accelerated solvent extraction

Place the sample (5.1.1) in a ceramic mortar; grind it and mix it evenly with an appropriate amount of diatomaceous earth; transfer it to the extraction tank; add 10 μ L each of standard solutions for PCDD/Fs and DL-PCBs isotope labeled quantitative internal standard. After sealing, place it on an accelerated solvent extraction instrument for extraction. The extraction reference conditions are: extraction solvent: n-hexane: dichloromethane (1:1, volume ratio); pressure: 10.3 MPa; temperature: 150 $^{\circ}$ C; static extraction time: 10 min; cycle: 1 time.

Depending on laboratory conditions, choose to use acidified silica gel for fat removal and/or gel permeation chromatography for fat removal.

5.4 Purification and separation

5.4.1 Packed column for purification

5.4.1.1 Composite silica column for purification

Take a glass chromatography column with an inner diameter of 15 mm; fill the bottom with an appropriate amount of glass wool; sequentially add 2 g of active silica gel, 5 g of alkalized silica gel, 2 g of active silica gel, 10 g of acidified silica gel, 2 g of active silica gel, 5 g of silver nitrate silica gel, 2 g of active silica gel and 2 g of anhydrous sodium sulfate; pack by the dry method; tap the chromatography column to make it evenly distributed.

First use 150 mL of n-hexane to pre-elute. When the liquid level drops to about 2 mm above the anhydrous sodium sulfate layer, close the column valve and discard the eluent. Check the chromatography column and repack it if channeling occurs. Add the degreased and concentrated extract; use 5 mL of n-hexane to wash the eggplant-shaped bottle (5.3) twice; add them to the column together; open the column valve; when the liquid level drops to the anhydrous sodium sulfate layer, add 400 mL of n-hexane; elute at a flow rate of 1 drop/s ~ 2 drops/s; use the eggplant-shaped bottle to collect the eluate; use a rotary evaporator to concentrate to 3 mL ~ 5 mL, for the next step of purification. Avoid bumping during concentration.

5.4.1.2 Alkaline alumina column for separation

Take a glass chromatography column with an inner diameter of 15 mm; fill the bottom with an appropriate amount of glass wool; then add 25 g of alkaline alumina and 10 g of anhydrous sodium sulfate in sequence. Pack by the dry method; tap the column lightly to distribute it evenly. First use 150 mL of n-hexane to pre-elute. When the liquid level drops to about 2 mm above the alumina, close the column valve. Discard the eluent. Check the chromatography column and refill the column if channeling occurs. Add the extract purified by the mixed silica gel column; use 5 mL of n-hexane to wash the eggplant-shaped bottle (5.4.1.1) twice; add them to the column together. Use 60 mL of n-hexane to elute the alumina column; discard the eluent. Use 90 mL of toluene to elute at a flow rate of 1 mL/min ~ 2 mL/min; use an eggplant-shaped bottle to collect the eluate for DL-PCBs analysis. Then, use 200 mL of n-hexane: methylene chloride (1:1, volume ratio) to elute at a flow rate of 1 drop/s ~ 2 drops/s; use an eggplant-shaped bottle to collect the eluent for PCDD/Fs analysis. For each eluent, concentrate to 1 mL ~ 2 mL using a rotary evaporator; then, add 50 mL of n-hexane; concentrate to 1 mL ~ 2 mL. Avoid bumping during concentration.

5.4.1.3 Alkaline alumina column for purification

Further purification of the eluent containing PCDD/Fs components: Take a glass chromatography column with an inner diameter of 8 mm; fill the bottom with an appropriate amount of glass wool; then add 2.5 g of alkaline alumina and 2 g of anhydrous sodium sulfate in sequence. Pack by the dry method; tap the column lightly to distribute it evenly. Use 20 mL of n-hexane to pre-elute; discard the eluent. Add the concentrated eluate. Use 40 mL of n-hexane: methylene chloride (98:2, volume ratio) to eluent; discard the eluent. Use 30 mL of n-hexane: dichloromethane (1:1, volume ratio) to elute at a flow rate of 1 drop/s ~ 2 drops/s; use an eggplant-shaped bottle to collect the eluate; use a rotary evaporator to concentrate to 1 mL ~ 2 mL. Avoid bumping during concentration.

Further purification of the eluent containing DL-PCBs components: Take a glass chromatography column with an inner diameter of 8 mm; fill the bottom with an appropriate amount of glass wool; then add 2.5 g of alkaline alumina and 2 g of anhydrous sodium sulfate in sequence. Pack by the dry method; tap the column lightly to distribute it evenly. Use 30 mL of n-hexane: methylene chloride (99:1, volume ratio) to pre-eluent; discard the eluent. Add the concentrated eluent; use 15 mL of n-hexane: dichloromethane (1:1, volume ratio) to elute at a flow rate of 1 drop/s ~ 2 drops/s; use an eggplant-shaped bottle to collect the eluate; use a rotary evaporator to concentrate to 1 mL ~ 2 mL. Avoid bumping during concentration.

5.4.2 Fully automatic sample purification system for purification

Connect the acid-base composite silica gel column, alkaline alumina column and activated carbon purification column to the fully automatic sample purification system in sequence; prepare each elution solution according to the program and connect the pipelines (see Figure D.1). Transfer the degreased and concentrated extract to the injection tube of the fully automatic sample purification system. Elute sequentially according to the elution program (see Table D.1); purify and separate the sample; use the eggplant-shaped bottle to collect the eluate containing PCDD/Fs and DL-PCBs components respectively; use a rotary evaporator to concentrate to 1 mL ~ 2 mL; then, add 50 mL of n-hexane and concentrate to 1 mL ~ 2 mL. Avoid bumping during concentration.

According to laboratory conditions, choose to use packed column for purification or fully automatic sample purification system for purification. If the PCDD/Fs purification effect cannot meet the measurement requirements, choose activated carbon column and/or florisil column for further purification.

5.4.3 Supplementary purification methods

5.4.3.1 Activated carbon column for purification

Take a 10 mL disposable glass pipette; cut off both ends; make a glass tube about 10 cm long; put in an appropriate amount of glass wool and plug it tightly; then, add 0.55 g of mixed activated carbon; then, put in an appropriate amount of glass wool; compact

5.7.1.1 Gas chromatography reference conditions

The gas chromatography reference conditions are as follows:

- a) Chromatographic column: 5% diphenyl-95% dimethylpolysiloxane column, 60 m $\times$ 0.25 mm $\times$ 0.25 μ m, or equivalent;
- b) Temperature of the sample injector: 280 °C;
- c) Injection mode: splitless injection, constant flow mode;
- d) Injection volume: 2 μ L;
- e) Transmission line temperature: 310 °C;
- f) Column temperature: 120 °C (maintain for 1 min); rise to 220 °C at 43 °C/min (maintain for 15 min); rise to 250 °C at 2.3 °C/min, to 260 °C at 0.9 °C/min, and to 310 °C at 20 °C/min (maintain for 9 min);
- g) Carrier gas: high purity helium (>99.999%), 0.8 mL/min.

5.7.1.2 Mass spectrometry reference conditions

The mass spectrometry reference conditions are as follows:

- a) Ionization mode: electron impact source (EI);
- b) Electron energy: 45 eV;
- c) Reference gas: perfluorotributylamine (FC-43) or perfluorinated kerosene (PFK);
- d) Resolution: $\geq 10\,000$, reference ion: 313.983 3 (FC-43) or 342.978 7 (PFK);
- e) Ion source temperature: 270 °C;
- f) Ion monitoring mode: multiple ion monitoring (MID);
- g) Monitoring ions: See Table C.1 for the accurate mass of monitoring ions for each compound.

5.7.2 DL-PCBs analysis conditions

5.7.2.1 Gas chromatographic conditions

The gas chromatographic conditions are shown as below:

- a) Chromatographic column: Same as a) in 5.7.1.1;
- b) Temperature of the sample injector: 290 °C;

Under given conditions, separately inject the CS1 standard solution for PCDD/Fs calibration standard solution and DL-PCBs calibration standard solution. The relative retention time of each target compound shall comply with the provisions of Table C.2.

5.8.4 Ion abundance ratio

Under given conditions, separately inject the CS1 standard solution for PCDD/Fs calibration standard solution and DL-PCBs calibration standard solution. The ion abundance ratio of each target compound shall comply with the provisions of Table C.3.

5.9 Preparation of the standard curve

5.9.1 Relative response factor

Inject the PCDD/Fs calibration standard solution and DL-PCBs calibration standard solution into GC-HRMS in order of concentration from low to high, and obtain the peak area. Calculate the relative response factor (RRF) of each target compound according to Formula (3). Formula (3) is applicable to other PCDD/Fs and DL-PCBs than 1,2,3,7,8,9-HxCDD and OCDF.

$$\text{RRF} = \frac{(A_{n1} + A_{n2}) \times c_1}{(A_{11} + A_{12}) \times c_n} \dots\dots\dots (3)$$

Where:

A_{n1} and A_{n2} – peak areas of the first and second mass ions of the target compound;

c_1 – concentration of quantitative internal standard, in nanograms per milliliter (ng/mL);

A_{11} and A_{12} – peak areas of the first and second mass ions of the quantitative internal standard;

c_n – concentration of the target compound, in nanograms per milliliter (ng/mL).

Within the concentration range of the calibration standard solution, the relative standard deviation of the RRF of each compound is $\leq 20\%$.

5.9.2 Response factor

5.9.2.1 Response factors of 1,2,3,7,8,9-HxCDD and OCDF

Inject the PCDD/Fs calibration standard solution into the GC-HRMS in order from low to high concentration to obtain the peak area. Calculate the response factors (RF) of 1,2,3,7,8,9-HxCDD and OCDF according to Formula (4) (for 1,2,3,7,8,9-HxCDD, use $^{13}\text{C}_{12}$ -1,2,3, 6,7,8-Hx-CDD as the quantitative internal standard; for OCDF, use $^{13}\text{C}_{12}$ -OCDD as the quantitative internal standard).

Report the results in terms of fat mass and convert according to the fat content (5.2.3) in the sample.

7 Precision

Intra-laboratory repeatability, calculated as TEQ_{total} , $RSD \leq 15\%$.

8 Detection-limit

Based on a sampling volume of 50 g, 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and 2,3,7,8-tetrachloro-dibenzofura (2,3,7,8-TCDF) are 0.04 ng/kg, octachlorodibenzo-p-dioxin (OCDD) and octachloro-dibenzofuran (OCDF) are 0.40 ng/kg, the remaining PCDD/Fs are 0.20 ng/kg, and DL-PCB is 1.00 ng/kg.

9 Others

9.1 After the organic reagents used for analysis are concentrated 10 000 times, PCDD/Fs and DL-PCBs with $S/N > 3$ shall not be detected.

9.2 The S/N of 2,3,7,8-TCDD in the blank test shall not be greater than 3.

9.3 The laboratory used to detect dioxins and their analogs in food shall be set up separately; the laboratory shall not be shared with other testing items, especially the detection of dioxins and their analogs in environmental samples. During the detection process, standard solutions, sample extraction solutions and sample extraction residues added with stable isotopes to replace the standards shall be used and stored with caution to avoid laboratory contamination. During the detection process, experimenters shall wear gloves, adsorbent masks, safety glasses, and lab coats to avoid unnecessary personal contact and exposure. The laboratory shall be equipped with a dedicated independent laboratory ventilation system with good performance; the fume hood shall meet the requirements for negative pressure operation. An activated carbon adsorption device shall be installed at the end of its exhaust outlet, and the adsorption material shall be replaced regularly to ensure that exhaust gas emissions meet environmental protection requirements. Liquid and solid waste generated during sample extraction, purification, concentration and analysis in laboratories of dioxins and their analogues shall be collected and disposed of in accordance with national and local waste management regulations.

Method II – Isotope dilution - gas chromatography - triple quadrupole mass spectrometry

13.7 Apparatus reference conditions

13.7.1 PCDD/Fs analysis conditions

13.7.1.1 Gas chromatography reference conditions

Same as 5.7.1.1.

13.7.1.2 Mass spectrometry reference conditions

The mass spectrometry reference conditions are as follows:

- a) Resolution: The resolution of the quadrupole shall be better than or equal to the unit mass resolution.
- b) Ionization mode: electron impact source (EI), energy 70 eV; or atmospheric pressure chemical ionization source (APCI), corona needle current 3 μ A.
- c) Ion source temperature: 280 °C for electron impact source (EI); or 150 °C for atmospheric pressure chemical ionization source (APCI).
- d) Data acquisition mode: Multiple reaction ion monitoring mode (MRM), which monitors two specific parent ions and one product ion generated by each parent ion for all compounds. The specific information on the ion pairs of each compound is shown in Table C.4.

13.7.2 DL-PCBs analysis conditions

13.7.2.1 Gas chromatography reference conditions

Chromatographic column: Same as 5.7.2.1.

13.7.2.2 Mass spectrometry reference conditions

Same as 13.7.1.2.

13.8 Instrument performance requirements

13.8.1 Resolution

Same as 5.8.1.

13.8.2 Sensitivity

Inject the sensitivity check standard solution, the deviation between the RRF (RF) of the target compound and the average RRF (RF) of the target compound calculated by the calibration standard curve is less than 30%.

13.8.3 Ion abundance ratio

This is an excerpt of the PDF (Some pages are marked off intentionally)

Full-copy PDF can be purchased from 1 of 2 websites:

1. <https://www.ChineseStandard.us>

- SEARCH the standard ID, such as GB 4943.1-2022.
- Select your country (currency), for example: USA (USD); Germany (Euro).
- Full-copy of PDF (text-editable, true-PDF) can be downloaded in 9 seconds.
- Tax invoice can be downloaded in 9 seconds.
- Receiving emails in 9 seconds (with download links).

2. <https://www.ChineseStandard.net>

- SEARCH the standard ID, such as GB 4943.1-2022.
- Add to cart. Only accept USD (other currencies - <https://www.ChineseStandard.us>).
- Full-copy of PDF (text-editable, true-PDF) can be downloaded in 9 seconds.
- Receiving emails in 9 seconds (with PDFs attached, invoice and download links).

Translated by: Field Test Asia Pte. Ltd. (Incorporated & taxed in Singapore. Tax ID: 201302277C)

About Us (Goodwill, Policies, Fair Trading...): <https://www.chinesestandard.net/AboutUs.aspx>

Contact: Wayne Zheng, Sales@ChineseStandard.net

Linkin: <https://www.linkedin.com/in/waynezhengwenrui/>

----- The End -----