資料１

ダイオキシン類簡易測定法検討会の設置について

１．趣　旨

現行のダイオキシン類の測定に係る各種公定法は、異性体の１つ１つを測定する超微量かつ高度な分析法であり、高額で、分析に時間がかかることなどから、簡易な測定方法の開発・適用が求められている。

このため、環境管理局長の私的諮問機関として専門家からなる「ダイオキシン類簡易測定法検討会」を設置し、簡易測定法の適用可能性に係る技術的検討を行う。

２．委員構成

（50 音順、敬称略）

伊藤　裕康　国立環境研究所化学環境研究領域計測管理研究室 主任研究員
酒井　伸一　国立環境研究所循環型社会形成推進・廃棄物研究センター センター長
半野　勝正　千葉県環境研究センター廃棄物・化学物質部化学物質研究室 上席研究員
宮田　秀明　摂南大学薬学部 教授
森田　昌敏　国立環境研究所 統括研究官
渡邉　　肇　岡崎国立共同研究機構統合バイオサイエンスセンター助教授

３．検討事項

排出ガス、ばいじん及び燃え殻に含まれるダイオキシン類の簡易測定法に係る技術的適用可能性

４．検討方法

様々な手法が開発されている生物検定法について、測定法を公募し、分析試験、ヒアリングを実施し、その結果をもとに簡易測定法としての技術的適用可能性を検討・評価する。

併せて、これまで環境省で検討してきたその他の簡易測定法について技術的適用可能性を評価する。

５．検討スケジュール

４回程度検討会を開催し、平成 15 年度末を目途に検討結果を取りまとめる。

資料２

ダイオキシン類対策特別措置法に基づくダイオキシン類測定の現状

１．ダイオキシン法の特定施設、排出基準等

（１）排出ガス

（単位：ng-TEQ/m3N）

特定施設種類	施設規模（焼却能力）	新設施設基準	既設施設基準	
			H13.1.15-H14.11.30	H14.12.1-
廃棄物焼却炉（火床面積 0.5 ㎡以上、又は焼却能力が 50kg/h 以上）	４ t/h 以上	０．１	８０	１
	２ t/h ～４ t/h	１		５
	２ t/h 未満	５		１０
製鋼用電気炉		０．５	２０	５
鉄鋼業焼結施設		０．１	２	１
亜鉛回収施設		１	４０	１０
アルミニウム合金製造施設		１	２０	５

※ 既に大気汚染防止法において指定物質抑制基準が適用されている新設の廃棄物焼却炉（能力 200kg/h 以上）及び製鋼用電気炉については、上表の新設施設の排出基準が適用となる。

<ダイオキシン類対策特別措置法施行令別表第一、規則別表第一・附則別表第一、二>

（２）ばいじん・燃え殻

廃棄物焼却炉に係るばいじん等の処理基準：３ ng-TEQ/g（既設施設は H14.12.1- 適用）

<「廃棄物焼却炉に係るばいじん等に含まれるﾀﾞｲｵｷｼﾝ類の量の基準及び測定の方法に関する省令」（平成 12 年厚生省令第１号））>

２．ダイオキシン法における測定方法（排出ガス・ばいじん・燃え殻）

ダイオキシン類対策特別措置法

第２８条（設置者による測定）

第１項　排出ガス自主測定義務：毎年１回以上で政令で定める回数、政令で定める方法で測定

第２項　排出ガス測定時に併せてばいじん及び焼却灰その他燃え殻の自主測定義務：
政令で定める方法で測定

第３項　都道府県への報告義務

第４項　都道府県による測定結果の公表

↓

ダイオキシン類対策特別措置法施行令

第４条（設置者による測定）

→ 第１項 **《排出ガス》**：毎年１回以上、環境省令で定める方法により行う

ダイオキシン類対策特別措置法施行規則
第２条（測定方法）
第１号 排出ガス → ＪＩＳ　K0311（高分解能質量分析計使用）

→ 第２項 **《ばいじん・燃え殻》**：環境省令で定める方法により行う

廃棄物焼却炉に係るばいじん等に含まれるダイオキシン類の量の基準及び測定の方法に関する省令

↓

第２条（ダイオキシン類の量の測定の方法）
環境大臣が定めるところによる

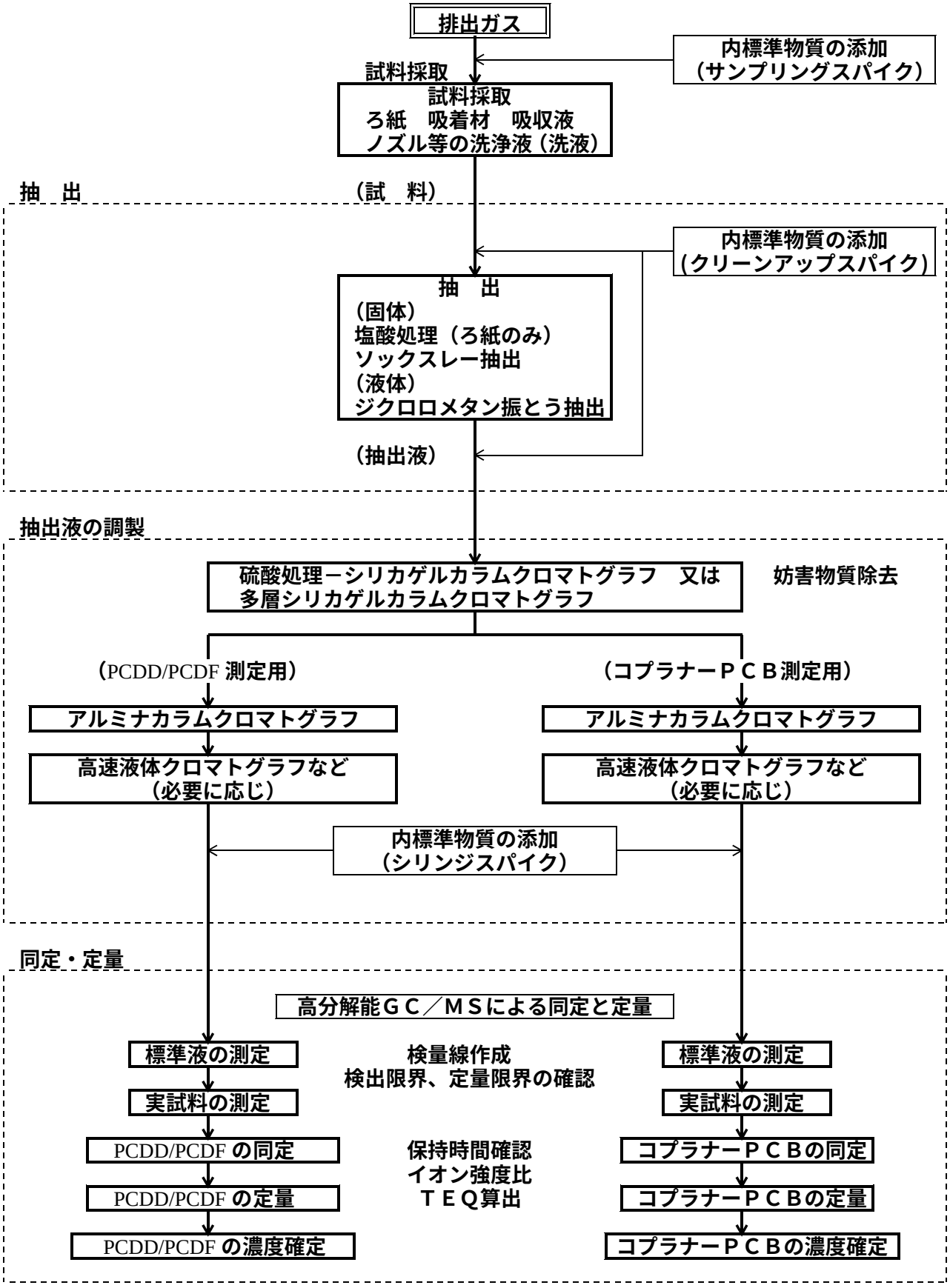
↓

廃棄物焼却炉に係るばいじん等に含まれるダイオキシン類の量の基準及び測定の方法に関する省令第１条第２項及び第２条の規定に基づき環境大臣が定める方法

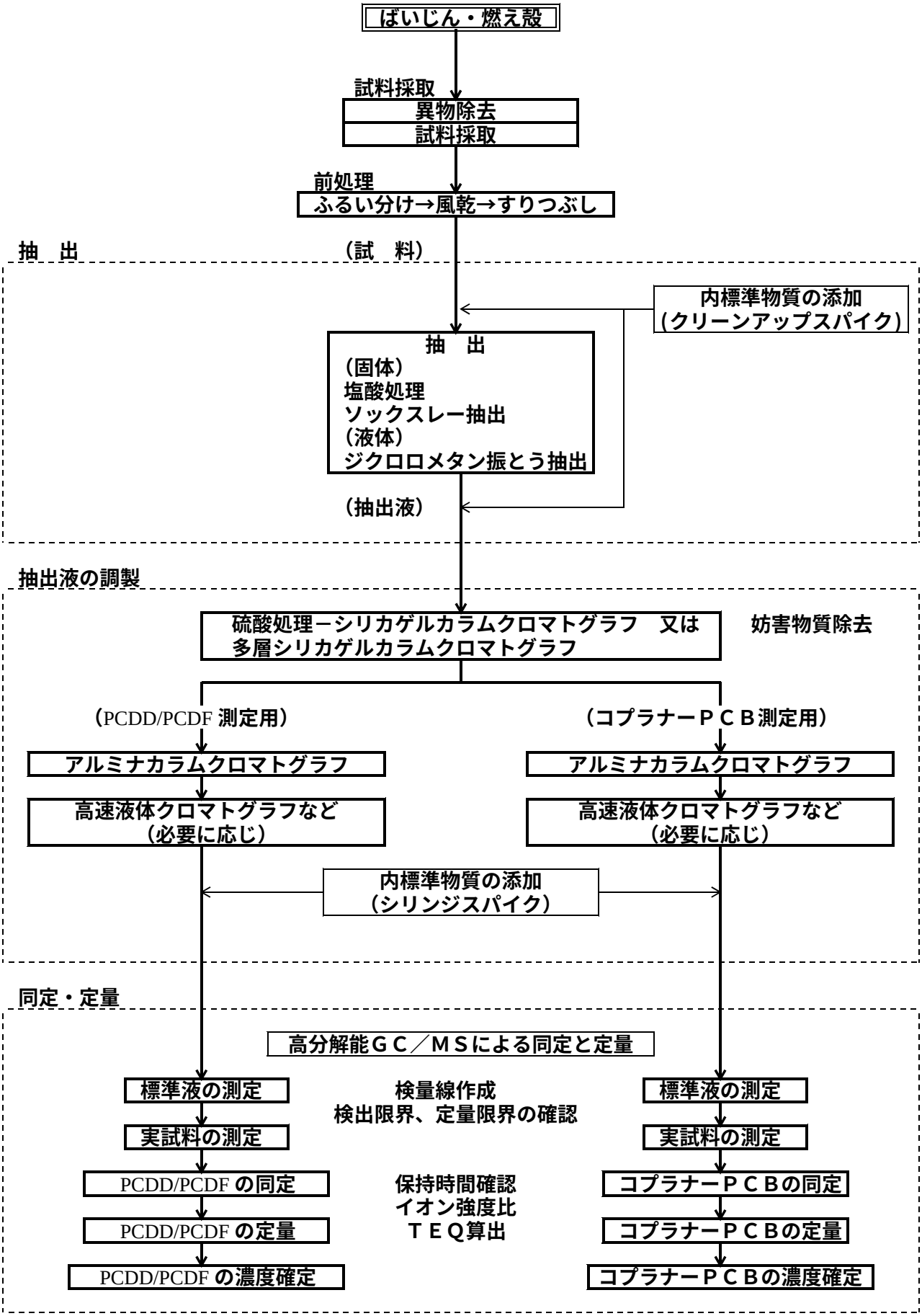
本　則
特別管理一般廃棄物及び特別管理産業廃棄物に係る基準の検定方法
別表第一に定める方法
→　ＪＩＳ　K0311（高分解能質量分析計使用）

３．公定法の測定フロー

（１）排出ガス



（２）ばいじん・燃え殻


ばいじん・燃え殻
試料採取
異物除去
試料採取
前処理
ふるい分け→風乾→すりつぶし
抽　出
（試　料）
内標準物質の添加
(クリーンアップスパイク)
抽　出
（固体）
塩酸処理
ソックスレー抽出
（液体）
ジクロロメタン振とう抽出
（抽出液）
抽出液の調製
硫酸処理－シリカゲルカラムクロマトグラフ　又は
多層シリカゲルカラムクロマトグラフ
妨害物質除去
（PCDD/PCDF 測定用）
（コプラナーＰＣＢ測定用）
アルミナカラムクロマトグラフ
アルミナカラムクロマトグラフ
高速液体クロマトグラフなど
（必要に応じ）
高速液体クロマトグラフなど
（必要に応じ）
内標準物質の添加
（シリンジスパイク）
同定・定量
高分解能ＧＣ／ＭＳによる同定と定量
標準液の測定
検量線作成
検出限界、定量限界の確認
標準液の測定
実試料の測定
実試料の測定
PCDD/PCDF の同定
保持時間確認
イオン強度比
ＴＥＱ算出
コプラナーＰＣＢの同定
PCDD/PCDF の定量
コプラナーＰＣＢの定量
PCDD/PCDF の濃度確定
コプラナーＰＣＢの濃度確定

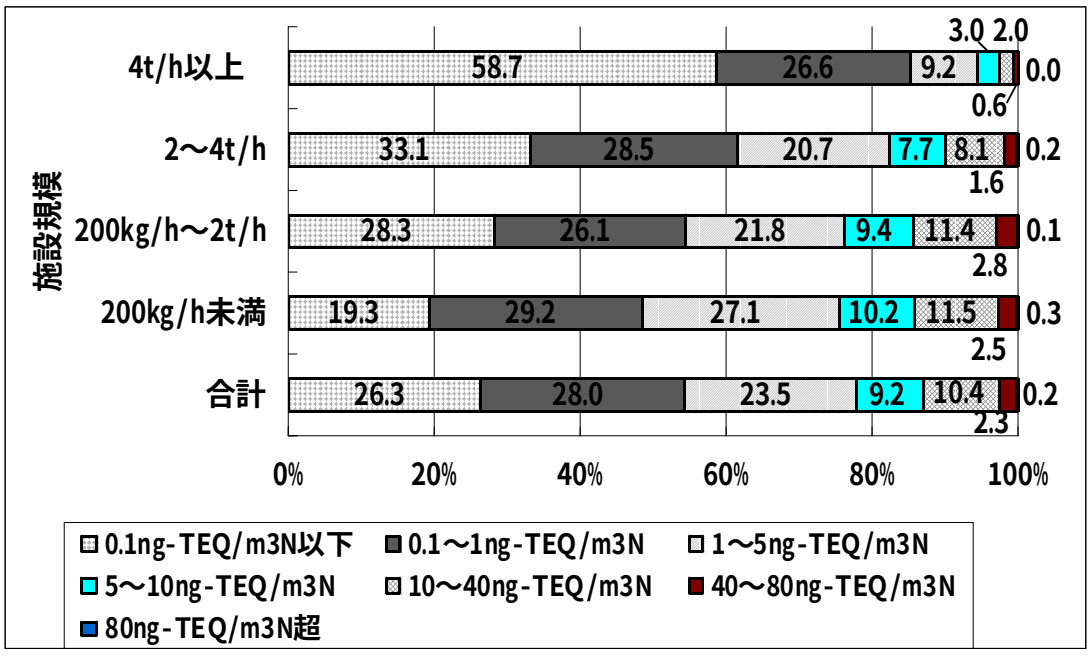
４．大気基準適用施設の届出等施設数

（平成１４年３月３１日現在）

大気基準適用施設		特定事業場数 （注１）	届出施設数 （注１，２）
焼結鉱の製造の用に供する焼結炉		１５（ １５）	３１（ ３１）
製鋼用電気炉		７２（ ７２）	１２３（１２３）
亜鉛回収施設（焙焼炉、焼結炉、溶鉱炉、溶解炉、乾燥炉）		８（ ７）	１８（ １５）
アルミニウム合金製造施設（焙焼炉、溶解炉、乾燥炉）		２３７（２３７）	７８６（７８６）
廃棄物焼却炉	４ t/h 以上	－	１，１０５（ １，１０１）
	２ t/h 以上 ～４ t/h 未満	－	１，７１４（ １，７１４）
	２ t/h 未満 （注３）	－	１４，５３８（１４、５１５）
	小 計	１３，８７３（１３，８５６）	１７，３５７（１７，３３０）
合 計		１４，２０５（１４，１８７）	１８，３１５ （１８，２８５）

（注１）鉱山保安法等関係法令施設及び事業場を含めた数である。うち、法に基づく届出がなされた施設及び事業場の数を（ ）に再掲した。
（注２）１つの特定事業場に複数の特定施設を有する場合、最もその事業場を代表する特定施設の欄に計上した。なお、法に基づき届出された施設と鉱山保安法等関係法令施設とを有する事業場とが重複する場合には、よりその事業場を代表する施設に計上した。
（注３）焼却能力５０ kg/h 以上又は火床面積０．５m^2以上のもの。

５．廃棄物焼却炉における排出ガス中のダイオキシン類の濃度分布



（注）平成 13 年度中に報告があった施設(平成 13 年度中稼働していない施設を除く)の 12,152 炉のデータをもとに作成。

資料３

ダイオキシン類簡易測定法に係る検討の進め方について（案）

１．検討方法

○生物検定法

測定技術を公募し、書類審査、分析試験（一次、二次）、ヒアリングを行い、それらの結果をもとに検討会において検討・評価する。

○その他簡易測定法（低分解能ＧＣ/ＭＳ法など）

これまでの環境省請負調査における検討結果を踏まえ、検討会において検証する。

２．生物検定法の公募方法

（１）趣旨

・近年、民間企業等において、ダイオキシン類の簡易な測定方法の開発等が進められているが、特に様々な手法が開発されている生物検定法（バイオアッセイ法）について、先般設置された「ダイオキシン類簡易測定法検討会」において、それらの適用可能性に係る技術的検証を行うため、検討の対象とする測定技術を広く公募する。

・なお、今回の検討は、個別機関の方法についての認定に係るものではなく、各種生物検定法についての技術的検証をもって、ダイオキシン類の測定方法に係る制度的検討の基礎資料とするものである。

（２）応募対象技術の要件

・排出ガス、ばいじん、燃え殻中のダイオキシン類を簡易に測定することができる生物検定法（小型の生物、細胞、あるいはその構成物質等を用いて、物質の活性あるいは量を測定する方法）による測定技術。

・実用化されていること。

・公定法に比べ、分析時間が短く、分析費用がかからないこと。

・中立機関による実証試験の実施が可能であること。

（３）応募機関の要件

・生物検定法による測定技術の開発者又は当該開発者から依頼を受けた機関。
ただし、ダイオキシン類の検出・測定技術のみの開発者／機関については、前処理技術開発／実施機関と共同で応募すること。（なお、当該開発者／機関において、技術情報を提供／説明できる前処理を前処理実施機関に依頼して実施する場合に限り、当該開発者／機関だけで応募することができる。）

・１つの生物検定法による測定技術*つき、１機関又１グループの応募に限る。

*：細胞の株等が異なる場合は別の方法と見なす。

（４）提出資料

・応募様式（別紙参照）
（技術の名称、概要、特徴、公定法との比較データ、特許・ライセンス関係、応募機関の連絡先等）
・会社概要等応募機関に関する資料
・関連論文等技術資料
・その他（必要に応じて）

（５）募集期間

・第１回検討会終了後、４週間程度。
・審査等の結果は個別に連絡するものとする。

（６）その他

・応募機関における資料作成、分析に係る費用は、応募機関が負担するものとする。
・特許に関する調整事項がある場合は事前に調整を済ませておくこと。
・応募機関名は、有望な測定方法に係る場合のみ公表する場合がある。

３．分析試験方法

（１）第一次分析試験

環境省が依頼する機関から各応募機関に分析試験用試料を送付し、応募機関において分析したデータ等をもとに、各測定技術の基本的評価を行う。

＜標準試料分析＞

○目　的：測定技術の基本的評価
○分析者：応募機関において分析
○試　料：ダイオキシン類等の試薬を調製したもの
○試料数：２検体

＜実試料分析１＞

○目　的：前処理技術を含めた技術の基本的評価
○方　法：応募機関において分析
○試　料：一般的な廃棄物焼却施設から採取した排出ガス、ばいじん及び焼却灰について、粗抽出したもの
○試料数：排出ガス、ばいじん、焼却灰　各１検体

＊別途、環境省が依頼する２機関において、同一試料を公定法により分析する

（２）第二次分析試験

環境省が依頼する機関から第二次分析試験対象応募機関に分析試験用試料を送付し、応募機関及び中立機関において分析したデータ等をもとに、各測定技術の様々な廃棄物焼却炉への技術的適用性を評価する。

＜実試料分析２＞

○目　的：様々な廃棄物焼却炉への技術的適用性評価
○方　法：応募機関において分析
○試　料：複数の種類の廃棄物焼却炉から採取した排出ガス、ばいじん及び焼却灰について、粗抽出したもの
○試料数：排出ガス、ばいじん、焼却灰　各４検体

＊別途、環境省が依頼する２機関において、同一試料を公定法により分析する

＜中立機関による検証＞

○目　的：応募機関による分析の妥当性の検証
○方　法：環境省が依頼する中立的な分析機関における各応募機関の方法による分析
○試　料：実試料分析２における試料と同一の試料
○試料数：排出ガス、ばいじん、焼却灰　各４検体

４．ヒアリング

・第二次分析試験対象応募機関に対して実施する。

５．評価項目

・測定原理としての妥当性
・公定法との相関性（乖離の程度とその要因）
・再現性、ばらつきの程度
・感度（検出限界、定量限界）
・簡易性（コスト、時間、取扱い）　等

（別　紙）

生物検定法によるダイオキシン類簡易測定技術　応募様式

１．応募機関

応募機関	名　称	
	住　所	
	担当者	所属：　　氏名：
	連絡先	TEL：　（　　）　　FAX：　（　　）　　E-mail：
共同応募機関	名　称	
	住　所	
協力体制（分担）		

＊応募機関の会社概要等を添付して下さい。

２．応募技術

技術の名称	
技術の概要 ・内容・原理 ・技術フロー ・測定可能範囲 ・主な用途と利用実績 ・必要な設備等	
技術の特徴 ・選択性 ・迅速性 ・経済性 ・簡易性 ・その他	 （交差反応性等） （所要時間、同時分析可能検体数等） （市販価格、コスト等）
公定法との比較データ ・標準試料 ・排出ガス ・ばいじん ・燃え殻	
実用化状況	市販・近く市販予定（　年　月頃）・開発完了・その他（　　）
技術の特許・ライセンス関係	
現段階で判明している課題等	
中立機関による実証試験の可否	可（貴機関内／貴機関外）　・　否
備考・特記事項	

＊前処理技術を含めてご記入下さい。
＊欄内に書ききれない場合は、別紙に記入の上、添付して下さい。
＊関連論文等技術資料を添付して下さい。

資料４

今後のスケジュールについて（案）

平成 15 年５月 28 日　第１回検討会（検討会の進め方等）

平成 15 年５～６月　生物検定法の公募

書類審査

平成 15 年７～９月　第一次分析試験

平成 15 年 10 月　第２回検討会（第一次分析試験結果の評価）

平成 15 年 11 月～平成 16 年１月　第二次分析試験

平成 16 年１月　第３回検討会（ヒアリング）

平成 16 年３月　第４回検討会（第二次分析試験結果の評価、取りまとめ）

＊これまで環境省において検討してきた低分解能ＧＣ／ＭＳ法などのその他の簡易測定法については、第２回及び第４回検討会において併せて検討するものとする。

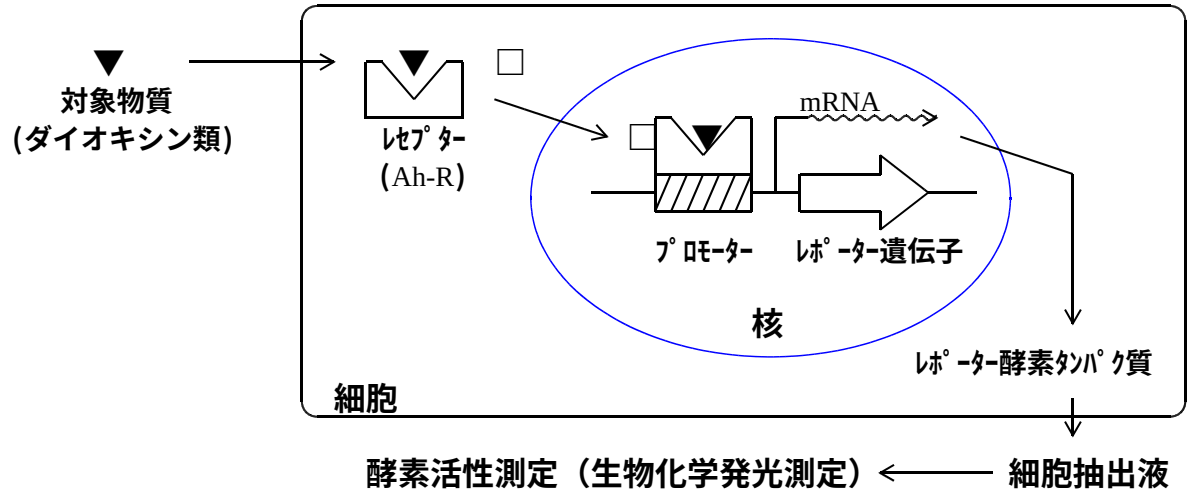
参考資料１

主な生物検定法について

１．レポーター遺伝子アッセイ

遺伝子発現を調節する転写プロモーターの特性や活性、又はそのプロモーターに結合する転写因子の活性を生物学的に測定する手法。目的遺伝子の転写プロモーターをβ－ガラクトシダーゼやルシフェラーゼ等のレポーター遺伝子上流に挿入した人工遺伝子を作成し、細胞内に導入して、レポーター遺伝子の発現を酵素の活性や生物化学発光を測定することによって定量化する。

ダイオキシン類の生理活性を検出する系としては、プロモーターにダイオキシン受容体結合配列、レポーター遺伝子として発光生物に由来するルシフェラーゼ遺伝子や緑色蛍光タンパク質（ＧＦＰ）遺伝子が用いられることがある。

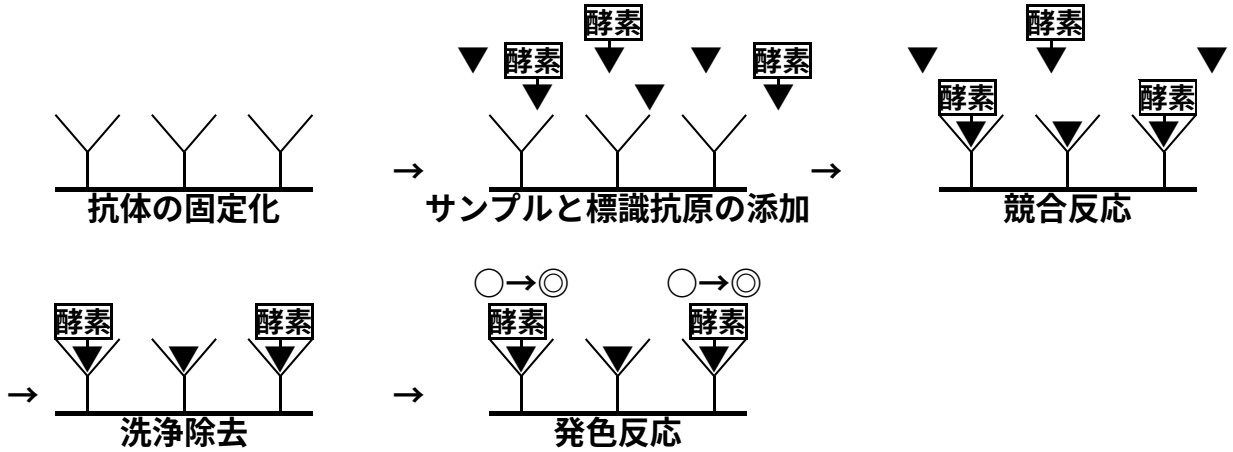


２．酵素免疫測定法（ELISA:Enzyme-Linked Immuno Sorbent Assay）

酵素を標識として用い、抗原抗体反応を利用した測定法。

（例）直接競合 ELISA

抗体をマイクロプレートや試験管にコーティング（固定化）したものに、対象物質（サンプルや標準物質）および標識抗原を添加して競合反応させ、抗体に結合しなかった対象物質や標識抗原を洗浄除去後、酵素基質を添加して酵素による発色反応をさせ、ついで発色の程度を比色計等で測定し、対象物質の標準品の発色度と比較することにより対象物質の濃度を測定する方法。



参考資料２

諸外国における生物検定法の利用状況について

１．ＥＵにおける食品・飼料規制への利用

2002 年 7 月 1 日に施行された「食品・飼料のダイオキシン類基準」を担保する公定測定法が、2002 年 7 月のＥＣ指令で規定。この中で、スクリーニング手法として生物検定法を採用。

＜ＥＣ指令の概要＞

- 本格測定法とスクリーニング測定法を定め、スクリーニング法での測定で、基準値の６～７割以上の場合は、本格測定法で再度測定し、判定する。これ以下の値であれば基準を満たしているとする。
- 最終確認のための本格測定法は、高分解能ＧＣ／ＭＳ法。
- スクリーニング測定法として、
  ①細胞を用いる生物検定法（cell-based bioassay）
  ②キットを用いる生物検定法（kit-based bioassay）
  を指定。

２．米国におけるスクリーニング法としての利用

ヒトの細胞を用いたスクリーニング測定用生物検定法として、米国ＥＰＡは、2000 年 1 月に Method4425 を制定。

COMMISSION DIRECTIVE 2002/69/EC

of 26 July 2002

laying down the sampling methods and the methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs

(Text with EEA relevance)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 85/591/EEC of 20 December 1985 concerning the introduction of Community methods of sampling and analysis for the monitoring of foodstuffs intended for human consumption [1], and in particular Article 1 thereof,

Whereas:

(1) Commission Regulation (EC) No 466/2001 [2], as last amended by Regulation (EC) No 563/2002 [3], and amended by Council Regulation (EC) No 2375/2001 [4] establishes maximum limits for dioxins and furans in certain foodstuffs.

(2) Council Directive 89/397/EEC of 14 June 1989 on the official control of foodstuffs [5] lays down the general principles for the performance of control of foodstuffs. Council Directive 93/99/EEC of 29 October 1993 on the subject of additional measures concerning the official control of foodstuffs [6] introduces a system of quality standards for laboratories entrusted by the Member States with the official control of foodstuffs.

(3) Directive 85/591/EEC has fixed general criteria for methods of sampling and analysis. However, in certain cases it is necessary to lay down more specific criteria and or requirements with which the method of analysis should comply in order to ensure that laboratories use methods of analysis with comparable levels of performance.

(4) The provisions for sampling and methods of analysis have been drawn up on the basis of present knowledge and they may be adapted to take account of advances in scientific and technological knowledge.

(5) The provisions laid down in this Directive relate only to the sampling and analysis of dioxins and dioxin-like PCBs for the implementation of Regulation (EC) No 466/2001 and do not affect the sampling strategy, sampling levels and frequency as specified in Annexes III and IV to Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC [7]. They do not affect the targeting criteria for sampling as laid down in Commission Decision 98/179/EC of 23 February 1998 laying down detailed rules on official sampling for the monitoring of certain substances and residues thereof in live animals and animal products [8].

(6) An active approach should be pursued in order to obtain comprehensive reliable data on the presence of dioxin-like PCBs in foodstuffs. Requirements should therefore be laid down as regards the methods of analysis to be used for the determination of dioxin-like PCBs in foodstuffs.

(7) A screening method of analysis with proven, widely acceptable validation and high throughput could be used to select the samples with significant levels of dioxins. The levels of dioxins in these samples need to be determined by a confirmatory method of analysis. It is therefore appropriate to establish strict requirements for the confirmatory methods of analysis and minimum requirements for the screening method.

(8) The measures provided for in this Directive are in accordance with the opinion of the Standing Committee on the Food chain and Animal Health,

HAS ADOPTED THIS DIRECTIVE:

Article 1

The Member States shall ensure that the sampling for the official control of the levels of dioxins and furans and the determination of the levels of dioxin-like PCBs in foodstuffs is carried out in accordance with the methods described in Annex I.

[1] OJ L 372, 31.12.1985, p. 50.
[2] OJ L 77, 16.3.2001, p. 1.
[3] OJ L 86, 3.4.2002, p. 5.
[4] OJ L 321, 6.12.2001, p. 1.
[5] OJ L 186, 30.6.1989, p. 23.
[6] OJ L 290, 24.11.1993, p. 14.
[7] OJ L 125, 23.5.1996, p. 10.
[8] OJ L 65, 5.3.1998, p. 31.

Article 2

The Member States shall ensure that sample preparation and methods of analyses used for the official control of the levels of dioxins and furans and the determination of the levels of dioxin-like PCBs in foodstuffs comply with the criteria described in Annex II.

Article 3

Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive by 28 February 2003 at the latest. They shall forthwith inform the Commission thereof.

When Member States adopt those provisions, they shall contain a reference to this Directive or be accompanied by such a reference on the occasion of their official publication. Member States shall determine how such reference is to be made.

Article 4

This Directive shall enter into force on the 20th day following that of its publication in the *Official Journal of the European Communities*.

Article 5

This Directive is addressed to the Member States.

Done at Brussels, 26 July 2002.

For the Commission

David BYRNE

Member of the Commission

ANNEX I

METHODS OF SAMPLING FOR OFFICIAL CONTROL OF THE LEVELS OF DIOXINS (PCDD/PCDF) AND THE DETERMINATION OF DIOXIN-LIKE PCBs IN CERTAIN FOODSTUFFS

1. Purpose and scope

Samples intended for the official control of the levels of dioxins (PCDD/PCDF) content, as well for the determination of the content of dioxin-like PCBs [1] in foodstuffs shall be taken according to the methods described below. Aggregate samples thus obtained shall be considered as representative of the lots or sublots from which they are taken. Compliance with maximum levels laid down in Commission Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs shall be established on the basis of the levels determined in the laboratory samples.

2. Definitions

Lot: an identifiable quantity of food delivered at one time and determined by the official to have common characteristics, such as origin, variety, type of packing, packer, consignor or markings. In the case of fish and fishery products, also the size of fish shall be comparable.

Sublot: designated part of a large lot in order to apply the sampling method on that designated part. Each sublot must be physically separated and identifiable.

Incremental sample: a quantity of material taken from a single place in the lot or sublot.

Aggregate sample: the combined total of all the incremental samples taken from the lot or sublot.

Laboratory sample: a representative part/quantity of the aggregate sample intended for the laboratory

[1] Table WHO TEFs for human risk assessment based on the conclusions of the World Health Organisation meeting in Stockholm, Sweden, 15-18 June 1997 (Van den Berg *et al.*, (1998) Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for Humans and for Wildlife. Environmental Health Perspectives, 106(12), 775).

Congener	TEF value
Dibenzo-p-dioxins (PCDDs)	
2,3,7,8-TCDD	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	0,0001
Dibenzofurans (PCDFs)	
2,3,7,8-TCDF	0,1
1,2,3,7,8-PeCDF	0,05
2,3,4,7,8-PeCDF	0,5
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	0,0001

Congener	TEF value
'Dioxin-like' PCBs Non-ortho PCBs + Mono-ortho PCBs	
Non-ortho PCBs	
PCB 77	0,0001
PCB 81	0,0001
PCB 126	0,1
PCB 169	0,01
Mono-ortho PCBs	
PCB 105	0,0001
PCB 114	0,0005
PCB 118	0,0001
PCB 123	0,0001
PCB 156	0,0005
PCB 157	0,0005
PCB 167	0,00001
PCB 189	0,0001

Abbreviations used: T = tetra; Pe = penta; Hx = hexa; Hp = hepta; O = octa; CDD = chlorodibenzodioxin; CDF = chlorodibenzofuran; CB = chlorobiphenyl.

3. **General provisions**

3.1. *Personnel*

Sampling shall be performed by an authorised qualified person as specified by the Member States.

3.2. *Material to be sampled*

Each lot, which is to be examined, must be sampled separately.

3.3. *Precautions to be taken*

In the course of sampling and preparation of laboratory samples precautions must be taken to avoid any changes, which would affect the content of dioxins and dioxin-like PCBs, adversely affect the analytical determination or make the aggregate samples unrepresentative.

3.4. *Incremental samples*

As far as practical incremental samples shall be taken at various places distributed throughout the lot or sublot. Departure from this procedure must be recorded in the record provided for under 3.8.

3.5. *Preparation of the aggregate sample*

The aggregate sample is made up by uniting all incremental samples. It shall be at least 1 kg unless not practical, e.g. when a single package has been sampled.

3.6. *Subdivision of aggregate sample in laboratory samples for enforcement, defence and referee purposes*

The laboratory samples for enforcement, trade (defence) and referee purposes shall be taken from the homogenised aggregate sample unless this conflicts with Member States' regulations on sampling. The size of the laboratory samples for enforcement shall be sufficient to allow at least for duplicate analyses.

3.7. *Packaging and transmission of aggregate and laboratory samples*

Each aggregate and laboratory sample shall be placed in a clean, inert container offering adequate protection from contamination, from loss of analytes by adsorption to the internal wall of the container and against damage in transit. All necessary precautions shall be taken to avoid change of composition of the aggregate and laboratory samples, which might arise during transportation or storage.

3.8. *Sealing and labelling of aggregate and laboratory samples*

Each sample taken for official use shall be sealed at the place of sampling and identified following the Member States' regulations. A record must be kept of each sampling, permitting each lot to be identified unambiguously and giving the date and place of sampling together with any additional information likely to be of assistance to the analyst.

4. **Sampling plans**

The sampling method applied shall ensure that the aggregate sample is representative for the lot that is to be controlled.

Number of incremental samples

In the case of milk and oils, for which a homogeneous distribution of the contaminants in question can be assumed within a given lot, it is sufficient to take three incremental samples per lot which forms the aggregate sample. Reference to the lot number shall be given. For other products, the minimum number of incremental samples to be taken from the lot shall be as given in Table 1.

The aggregate sample uniting all incremental samples shall be at least 1 kg (see point 3.5). The incremental samples shall be of similar weight. The weight of an incremental sample should be at least 100 grams. The weight of the incremental sample is dependent on the size of the particles in the lot. Departure from this procedure must be recorded in the record provided for under 3.8. In accordance with the provisions of Commission Decision 97/747/EC of 27 October 1997 fixing the levels and frequencies of sampling provided for by Council Directive 96/23/EC for the monitoring of certain substances and residues thereof in certain animal products [1], the sample size for hen eggs is at least 12 eggs (for bulk lots as well for lots consisting of individual packages, Tables 1 and 2).

[1] OJ L 303, 6.11.1997, p. 12.

TABLE 1

Minimum number of incremental samples to be taken from the lot

Weight of lot (in kg)	Minimum number of incremental samples to be taken
< 50	3
50 to 500	5
> 500	10

If the lot consists of individual packages, then the number of packages, which shall be taken to form the aggregate sample, is given in Table 2.

TABLE 2

Number of packages (incremental samples) which shall be taken to form the aggregate sample if the lot consists of individual packages

Number of packages or units in the lot	Number of packages or units to be taken
1 to 25	1 package or unit
26 to 100	about 5 %, at least 2 packages or units
> 100	about 5 %, at maximum 10 packages or units

5. **Compliance of the lot or sublot with the specification**

The control laboratory shall analyse the laboratory sample for enforcement in duplicate analysis in case the obtained result of the first analysis is less than 20 % below or above the maximum level, and calculate the mean of the results. The lot is accepted if the result of the first analysis is more than 20 % below the maximum level or, where duplicate analysis is necessary, if the mean conforms to the respective maximum level as laid down in Regulation (EC) No 466/2001.

ANNEX II

SAMPLE PREPARATION AND REQUIREMENTS FOR METHODS OF ANALYSIS USED IN OFFICIAL CONTROL OF THE LEVELS OF DIOXINS (PCDD/PCDF) AND THE DETERMINATION OF DIOXIN-LIKE PCBs IN CERTAIN FOODSTUFFS

1. **Objective and field of application**

These requirements should be applied where foodstuffs are analysed for the official control of the levels of dioxins (polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF)) and the determination of dioxin-like PCBs.

Monitoring for the presence of dioxins in foodstuffs can be performed by a strategy involving a screening method in order to select those samples with levels of dioxins and dioxin-like PCBs that are less than 30-40 % below or exceed the level of interest. The concentration of dioxins in those samples with significant levels needs to be determined/confirmed by a confirmatory method.

Screening methods are methods that are used to detect the presence of dioxins and dioxin-like PCBs at the level of interest. These methods have a capacity for a high sample throughput and are used to sift large numbers of samples for potential positives. They are specifically designed to avoid false negatives.

Confirmatory methods are methods that provide full or complementary information enabling the dioxins and dioxin-like PCBs to be identified and quantified unequivocally at the level of interest.

2. **Background**

Because environmental and biological samples (including samples of foodstuffs) in general contain complex mixtures of different dioxin congeners, the concept of Toxic Equivalency Factors (TEFs) has been developed to facilitate risk assessment. These TEFs have been established to express concentrations of mixtures of 2,3,7,8-substituted PCDDs and PCDFs, and more recently, some non-ortho and mono-ortho chlorine substituted PCBs which possesses dioxin-like activity in toxic equivalents (TEQs) of 2,3,7,8-TCDD (see Annex I, footnote 1).

The concentrations of the individual substances in a given sample are multiplied by their respective TEF and subsequently summed to give the total concentration of dioxin-like compounds expressed as TEQs.

The concept of 'upperbound' requires using the limit of quantification for the contribution of each non-quantified congener to the TEQ.

The concept of 'lowerbound' requires using zero for the contribution of each non-quantified congener to the TEQ.

The concept of 'mediumbound' requires using half of the limit of quantification calculating the contribution of each non-quantified congener to the TEQ.

3. **Quality assurance requirements to be complied with for sample preparation**

— Measures must be taken to avoid cross-contamination at each stage of the sampling and analysis procedure.

— The samples must be stored and transported in glass, aluminium, polypropylene or polyethylene containers. Traces of paper dust must be removed from the sample container. Glassware should be rinsed with solvents previously controlled for the presence of dioxins.

— The sample storage and transportation has to be performed in a way that maintains the integrity of the foodstuff sample.

— Insofar as relevant, finely grind and mix thoroughly each laboratory sample using a process that has been demonstrated to achieve complete homogenisation (e.g. ground to pass a 1 mm sieve); samples have to be dried before grinding if moisture content is too high.

— Perform a blank analysis by carrying out the entire analytical procedure omitting only the sample.

— Sample weight used for the extraction must be sufficient to fulfil the requirements with respect to sensitivity.

— There are many satisfactory specific sample preparation procedures, which may be used for the products under consideration. The procedures have to be validated according to internationally accepted guidelines.

4. **Requirements for laboratories**

— Laboratories shall demonstrate the performance of a method in the range of the level of interest, e.g. 0,5 ×, 1 × and 2 × the level of interest with an acceptable coefficient of variation for repeated analysis. For details of acceptance criteria, see point 5.

— Limit of quantification for a confirmatory method should be in the range of about one fifth of the level of interest, to make sure that acceptable coefficients of variations are met in the range of the level of interest.

— Regular blank controls and spiking experiments or analysis of control samples (preferably, if available, certified reference material) should be performed as internal quality control measures.

— Successful participation in interlaboratory studies that assess laboratory proficiency is the best way to prove the competence in specific analyses. However successful participation in interlaboratory studies for, e.g. soil or sewage samples, does not necessarily prove the competence also in the field of food or feedingstuff samples, which present lower contamination levels. Therefore, the continuous participation in interlaboratory studies for the determination of dioxins and dioxin-like PCBs in the relevant feed/food matrices is mandatory.

— In accordance with the provisions of Directive 93/99/EEC, laboratories should be accredited by a recognised body operating in accordance with ISO Guide 58 to ensure that they are applying analytical quality assurance. Laboratories should be accredited following the ISO/IEC/17025:1999 standard.

5. **Requirements to be met by analytical procedure for dioxins and dioxin-like PCBs**

Basic requirements for acceptance of analytical procedures:

— *High sensitivity and low limits of detection*. For PCDDs and PCDFs, detectable quantities have to be in the picogram TEQ (10^{-12} g) range because of extreme toxicity of some of these compounds. PCBs are known to occur at higher levels than the PCDDs and PCDFs. For most PCB congeners sensitivity in the nanogram (10^{-9} g) range is already sufficient. However, for the measurement of the more toxic dioxin-like PCB congeners (in particular non-ortho substituted congeners), the same sensitivity must be reached as for the PCDDs and PCDFs.

— *High selectivity (specificity)*. A distinction is required for PCDDs, PCDFs and dioxin-like PCBs from a multitude of other, coextracted and possibly interfering compounds present at concentrations up to several orders of magnitude higher than those of the analytes of interest. For gas chromatography/mass spectrometry (GC/MS) methods a differentiation among various congeners is necessary, such as between toxic (e.g. the seventeen 2,3,7,8-substituted PCDDs and PCDFs and dioxin-like PCBs) and other congeners. Bioassays should be able to determine TEQ values selectively as the sum of PCDDs, PCDFs and dioxin-like PCBs.

— *High accuracy (trueness and precision)*. The determination should provide a valid estimate of the true concentration in a sample. High accuracy (accuracy of the measurement: the closeness of the agreement between the result of a measurement with the true or assigned value of the measurement) is necessary to avoid the rejection of a sample analysis result on the basis of poor reliability of the estimate of TEQ. Accuracy is expressed as trueness (difference between the mean value measured for an analyte in a certified material and its certified value, expressed as percentage of this value) and precision (precision is usually calculated as a standard deviation including repeatability and reproducibility, and indicates the closeness of agreement between the results obtained by applying the experimental procedure several times under prescribed conditions).

Screening methods can comprise bioassays and GC/MS methods; confirmatory methods are high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) methods. Following criteria have to be complied with on total TEQ value:

	Screening methods	Confirmatory methods
False negative rate	< 1 %	
Trueness		– 20 % to + 20 %
CV	< 30 %	< 15 %

6. **Specific requirements for GC/MS methods to be complied with for screening or confirmatory purposes**

— Addition of ^{13}C-labelled 2,3,7,8-chlorine substituted internal PCDD/F standards (and of ^{13}C-labelled internal dioxin-like PCB standards, if dioxin-like PCBs have to be determined) must be carried out at the very beginning or start of the analytical method e.g. prior to extraction in order to validate the analytical procedure. At least one congener for each of the tetra to octa-chlorinated homologous groups for PCDD/F (and at least one congener for each of the homologous groups for dioxin-like PCBs, if dioxin-like PCBs have to be determined) must be added (alternatively, at least one congener for each mass spectrometric selected ion recording function used for monitoring PCDD/F and dioxin-like PCBs). There is a clear preference, certainly in case of confirmatory methods, of using all 17 ^{13}C-labelled 2,3,7,8-substituted internal PCDD/F standards and all 12 ^{13}C-labelled internal dioxin-like PCB standard (if dioxin-like PCBs have to be determined).

  Relative response factors should also be determined for those congeners for which no ^{13}C-labelled analogue is added by using appropriate calibration solutions.

— For foodstuffs of plant origin and foodstuffs of animal origin containing less than 10 % fat, the addition of the internal standards is mandatory prior to extraction. For foodstuffs of animal origin containing more than 10 % fat, the internal standards can be added either before extraction or after fat extraction. An appropriate validation of the extraction efficiency should be carried out, depending on the stage at which internal standards are introduced and on whether results are reported on product or fat basis.

— Prior to GC/MS analysis, 1 or 2 recovery (surrogate) standard(s) must be added.

— Control of recovery is necessary. For confirmatory methods, the recoveries of the individual internal standards should be in the range of 60 % to 120 %. Lower or higher recoveries for individual congeners, in particular for some hepta- and octa- chlorinated dibenzodioxins and dibenzofurans, are acceptable on the condition that their contribution to the TEQ value does not exceed 10 % of the total TEQ value (based on PCDD/F only). For screening methods, the recoveries should be in the range of 30 % to 140 %.

— Separation of dioxins from interfering chlorinated compounds such as PCBs and chlorinated diphenyl ethers should be carried out by suitable chromatographic techniques (preferably with a florisil, alumina and/or carbon column).

— Gaschromatographic separation of isomers should be sufficient (< 25 % peak to peak between 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF).

— Determination should be performed according to EPA Method 1613 revision B: Tetra- through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS or another with equivalent performance criteria.

— The difference between upperbound level and lower bound level should not exceed 20 % for foodstuffs with a dioxin contamination of about 1 pg WHO-TEQ/g fat (based on PCDD/PCDF only). For foodstuffs with a low fat content, the same requirements for contamination levels of about 1 pg WHO-TEQ/g product have to be applied. For lower contamination levels, for example 0,50 pg WHO-TEQ/g product, the difference between upperbound and lowerbound level may be in the range of 25 to 40 %.

7. **Screening methods of analysis**

7.1. *Introduction*

Different analytical approaches can be performed using a screening method: a pure screening approach and a quantitative approach.

Screening approach

The response of samples is compared to that of a reference sample at the level of interest. Samples with a response less than the reference are declared negative, those with a higher response are suspected positives. Requirements:

— A blank and a reference sample(s) have to be included in each test series, which is extracted and tested at the same time under identical conditions. The reference sample must show a clearly elevated response in comparison to a blank.

— Extra reference samples 0,5 × and 2 × the level of interest should be included to demonstrate the proper performance of the test in the range of interest for the control of the level of interest.

— When testing other matrices, the suitability of the reference sample(s) has to be demonstrated, preferentially by including samples shown by HRGC/HRMS to contain a TEQ level around that of the reference sample or else a blank spiked at this level.

— Since no internal standards can be used in bioassays, tests on repeatability are very important to obtain information on the standard deviation within one test series. The coefficient of variation should be below 30 %.

— For bioassays, the target compounds, possible interferences and maximum tolerable blank levels should be defined.

Quantitative approach

The quantitative approach requires standard dilution series, duplicate or triplicate clean up and measuring as well as blank and recovery controls. The result may be expressed as TEQ, thereby assuming that the compounds responsible for the signal correspond to the TEQ principle. This can be performed by using TCDD (or a dioxin/furan standard mixture) to produce a calibration curve to calculate the TEQ level in the extract and thus in the sample. This is subsequently corrected for the TEQ level calculated for a blank sample (to account for impurities from solvents and chemicals used), and a recovery (calculated from the TEQ level in a quality control sample around the level of interest). It is essential to note that part of the apparent recovery loss may be due to matrix effects and/or differences between the TEF values in the bioassays and the official TEF values set by WHO.

7.2. *Requirements for methods of analysis used for screening*

— GC/MS methods of analysis and bioassays may be used for screening. For GC/MS methods the requirements as laid down in point 6 are to be used. For cell based bioassays specific requirements are laid down in point 7.3 and for kit-based bioassays in point 7.4.

— Information on the number of false-positive and false-negative results of a large set of samples below and above the maximum level or action level is necessary, in comparison to the TEQ content as determined by a confirmatory method of analysis. Actual false negative rates should be under 1 %. The rate of false positive samples should be low enough to make the use of a screening tool advantageous.

— Positive results have always to be confirmed by a confirmatory method of analysis (HRGC/HRMS). In addition, samples from a wide TEQ-range should be confirmed by HRGC/HRMS (approximately 2 % to 10 % of the negative samples). Information on correspondence between bioassay and HRGC/HRMS results should be made available.

7.3. *Specific requirements for cell-based bioassays*

— When performing a bioassay, every test run requires a series of reference concentrations of TCDD or a dioxin/furan mixture (full dose-response curve with a $R^2 > 0{,}95$). However, for screening purposes an expanded low level curve for analysing low level samples could be used.

— A TCDD reference concentration (about 3 × limit of quantification) on a quality control sheet should be used for the outcome of the bioassay over a constant time period. An alternative could be the relative response of a reference sample in comparison to the TCDD calibration line since the response of the cells may depend on many factors.

— Quality control (QC) charts for each type of reference material should be recorded and checked to make sure the outcome is in accordance with the stated guidelines.

— In particular for quantitative calculations, the induction of the sample dilution used must be within the linear portion of the response curve. Samples above the linear portion of the response curve must be diluted and re-tested. Therefore, at least three or more dilutions at one time are recommended to be tested.

— The percent standard deviation should not be above 15 % in a triplicate determination for each sample dilution and not above 30 % between three independent experiments.

— The limit of detection may be set as 3 × the standard deviation of the solvent blank or of the background response. Another approach is to apply a response that is above the background (induction factor 5 × the solvent blank) calculated from the calibration curve of the day. The limit of quantification may be set as 5 × to 6 × the standard deviation of the solvent blank or of the background response or to apply a response that is above the background (induction factor 10 × the solvent blank) calculated from the calibration curve of the day.

7.4. *Specific requirements for kit-based bioassays* ([1])

— Manufacturer's instructions for sample preparation and analyses have to be followed.

— Test kits should not be used after the expiration date.

— Materials or components designed for use with other kits should not be used.

— Test kits should be kept within the specified range of storage temperature and used at the specified operating temperature.

— The limit of detection for immunoassays is determined as 3 × the standard deviation, based on 10 replicate analysis of the blank, to be divided by the slope value of the linear regression equation.

— Reference standards should be used for tests at the laboratory to make sure that the responsiveness to the standard is within an acceptable range.

8. **Reporting of the result**

Insofar as the used analytical procedure makes it possible, the analytical results should contain the levels of the individual PCDD/F and PCB congeners and be reported as lowerbound, upperbound and mediumbound in order to include a maximum of information in the reporting of the results and thereby enabling the interpretation of the results according to specific requirements.

The report should also include the lipid content of the sample as well the method used for lipid extraction.

The recoveries of the individual internal standards must be made available in case the recoveries are outside the range mentioned in point 6, in case the maximum level is exceeded and in other cases upon request.

([1]) No evidence have yet been submitted of commercially available kit-based bioassays having sufficient sensitivity and reliability to be used for screening for the presence of dioxins at the required levels in samples of foodstuffs and feedingstuffs.