L.N. 241 of 2005

VETERINARY SERVICES ACT
(CAP. 437)

Sampling Methods and methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in Foodstuffs Rules, 2005

IN exercise of the powers conferred under article 25 of the Veterinary Services Act, the Minister for Rural Affairs and the Environment, in agreement with the Minister of Health, the Elderly and Community Care, has made the following rules:-

Title and scope.

1. (1) The title of these rules is the Sampling Methods and methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in Foodstuffs Rules, 2005.

(2) The scope of these rules is to implement the rules found under European Union Council Directive 2002/69/EC on the sampling methods and the methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs.

Definitions.

2. For the purposes of these rules, and unless context otherwise requires -

"aggregate sample" means the combined total of all the incremental samples taken from the lot or sublot;

"the commission" means the European Commission;

"the community" means the European Community as established under the Treaty establishing the European Community;

"the competent authority" means the Veterinary Services within the territory of Malta as provided under article 2 of the Veterinary Services Act;

"incremental sample" means a quantity of material taken from a single place in the lot or sublot;

"laboratory sample" means a representative partċquantity of the aggregate sample intended for the laboratory;

"lot" means 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.

"Member State" means a State which is a Member within the European Community;

"sublot" means the 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;

"third country" means a State which is not a Member within the European Community;

"Veterinary Services" means the competent authority within the territory of Malta as established under article 2 of the Veterinary Services Act;

Sampling for official control.

3. Malta 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 Schedule I hereto.

Sample preparation and methods of analyses.

4. Malta 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 Schedule II hereto.

SCHEDULE I

METHODS OF SAMPLING FOR OFFICIAL CONTROL OF THE LEVELS OF DIOXINS (PCDD/PCDF) AND THE DETERMINATION OF DIOXIN-LIKE PCBsIN 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.

Congerer	TEF value	Congerer	TEF value
Dibenzo-p-dioxins(PCDDs)		**'Dioxin-like' PCBsNon-ortho PCBs + Mono-ortho PCBs**	
2,3,7,8-TCDD	1	**Non-ortho PCBs**	
1,2,3,7,8-PeCDD	1	PCB 77	0,0001
		PCB 81	0,0001
1,2,3,4,7,8-HxCDD	0,1	PCB 126	0,1
1,2,3,6,7,8-HxCDD	0,1	PCB 169	0,01
1,2,3,7,8,9-HxCDD	0,1		
1,2,3,4,6,7,8-HpCDD	0,01		
OCDD	0,0001		
Dibenzofurans(PCDFs)		**Mono-ortho PCBs**	
2,3,7,8-TCDF	0,1	PCB 105 0,0001	
1,2,3,7,8-PeCDF	0,05	PCB 114 0,0005	
2,3,4,7,8-PeCDF	0,5	PCB 118 0,0001	

1,2,3,4,7,8-HxCDF 0,1	PCB 123 0,0001
1,2,3,6,7,8-HxCDF 0,1	PCB 156 0,0005
1,2,3,7,8,9-HxCDF 0,1	PCB 157 0,0005
2,3,4,6,7,8-HxCDF 0,1	PCB 167 0,00001
1,2,3,4,6,7,8-HpCDF 0,01	PCB 189 0,0001
1,2,3,4, 7,8,9-HpCDF 0,0001	
Abbreviations used: T = tetra; Pe = penta; Hx = hexa; Hp = hepta; O = octa; CDD = chlorodibenzodioxin; CDF = chlorodibenzofuran; CB = chlorobiphenyl.	

2. **General provisions**

2.1. *Personnel*

Sampling shall be performed by an authorised qualified person as specified by the Veterinary Services.

2.2. *Material to be sampled*

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

2.3. *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.

2.4. *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 2.8.

2.5. *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.

2.6. *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 homogenized 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.

2.7. *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.

2.8. *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.

3. **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 2.5). 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).

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.

SCHEDULE 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).

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. **Requirementsfor 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. **Requirementsto be met by analytical procedure for dioxinsand 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 pictogram 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:

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 13C-labelled 2,3,7,8-chlorine substituted internal PCDD/F standards (and of 13C-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 13C-labelled 2,3,7,8-substituted

internal PCDD/F standards and all 12 13C-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 13C-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 methodsof 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 R2 > 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

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