# The Determination of Dioxins and PCBs in Animal Feedstuffs. Discussion of Requirements for Compliance with the New EU Regulations 2375/2001

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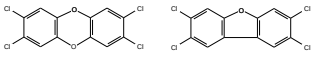
## Introduction

The EU recently promulgated regulation 2375/2001 detailing the maximum allowable levels of Dioxins in various foodstuffs from July 2002. There are also maximum levels for these compounds noted in Council Decision 2001/102/EC relating to the components of animal feeds. These have been set at levels close to the best achievable limits of detection of the current analytical methods. This paper describes the compounds that are regulated and the methods for their determination.

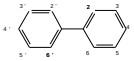
## What are Dioxins and PCBs?

'Dioxins' is a generic name given to a series of seventeen specific chlorinated dibenzo-p-dioxins and dibenzofurans in which chlorines are present in the 2,3,7,8 positions of the molecules. The most toxic of these is the tetrachlorinated dioxin, 2,3,7,8-TCDD. The other sixteen have been given toxic equivalent factors[1] (TEF) relative to the most toxic, allowing the total effect of exposure to be determined from a single numerical value. Dioxins and Furans are the by-product of incineration processes and some chemical reactions. They have no useful purpose.



**2,3,7,8-TCDD** **2,3,7,8-TCDF**

Polychlorinated biphenyls (PCBs) are a series of compounds that were manufactured by chlorinating the biphenyl molecule. Millions of tonnes of these compounds were manufactured from the early part of the last century until the mid-1980s when their production was banned. Of the 209 possible PCB congeners twelve have been assessed[1] to have toxic effects similar to Dioxins and have been given TEFs in the WHO assessment.



**Polychlorinated BiPhenyl**

Both Dioxins and Furans are essentially insoluble in water, and bio-accumulate in fatty tissue as a consequence. They are extremely resilient and do not bio-degrade easily.

The main exposure pathway for humans is through food ingestion (circa 90%). This is particularly the case for fatty foods. The WHO[1] have set a tolerable daily intake for Dioxins of 1 ng/kg body weight per day.

The measurement of these compounds involves a highly specific analysis. The method must differentiate the compounds of interest from others present at much higher concentrations in the matrix and other 'non toxic' Dioxin and PCB congeners.

## What are the regulatory limits?

Examples of the regulatory limits taken from the recent regulation for control of animal feedstuffs are given in the table below.

| Type of Feed | Regulatory Limit ng/kg PCDD/F WHO TEQ |
|---|---|
| All feeds of plant origin including vegetable oils and by-products | 0.75 |
| Minerals | 1.0 |
| Animal fat, including milk fat and egg fat | 2.0 |

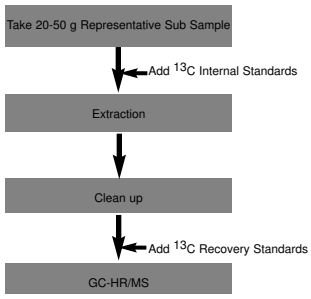
These limits are therefore in the region of 1 ng/kg, or one part per trillion. To put this measurement in context, it is equivalent to finding one small grain of sand in 1000 tonnes of feed!

These limits are 'upper bound' concentrations. Even with the best available technology there are circumstances in which the methods do not detect certain congeners, particularly in vegetables. In these cases, the conservative view is adopted and the congeners are reported as if they were present at the limit of detection (LOD). Hence, it is essential that the method employed achieves a limit of detection which is significantly below the regulatory limits.

## Detection Methods

Currently, the recognised most sensitive and reliable method is that of isotope dilution high-resolution gas chromatography - high resolution mass spectrometry (HRGC-HRMS). A synopsis of the test method is given below.

**Dioxin Analysis Scheme**




A High Resolution Mass Spectrometer
Micromass Ultima

## Quality Control

The QC measures ensure that:

- The chromatographic stage successfully isolates the compounds of interest from other non-toxic Dioxin and PCB congeners.
- The mass spectrometer obtains sufficient sensitivity for the analysis. (LOD < 1/5th of the regulatory limit).
- The recovery of the toxic compounds is within acceptable limits.
- Confidence in any positive results reported is assured by the routine analysis of method blanks. These must subsequently be determined to be very much less than the regulatory limits.

## Performance Criteria Specified in Directives 2002/69/EC and 2002/70/EC

- Coefficient of variation for replicate analysis <15%.
- Accuracy of results must be demonstrated to be within 20%.
- Specialist equipment capable of differentiating between species differing by less than 50 ppm in mass (A High Resolution Mass Spectrometer) is required to ensure the reported results are reliable and not subject to false positives.

## Conclusions

Specialised laboratories can achieve routine determination of Dioxins and PCBs to the limits of detection required for comparison of results with the new regulatory limits. However it should be noted that the analysis is very demanding of the equipment and facilities used.

Care should also be taken in interpreting results close to the regulatory limits as inherent uncertainties in the measurements may not always be taken into account. For example, for a 'true' value of 1.0 ng/kg, a sample may be reported anywhere between 0.65 and 1.35 ng/kg, when taking the performance criteria of the method into account. Therefore, it is strongly recommended that at the very least, duplicate analysis be performed when samples are reported above, or indeed just below, the limits.

## References

1. VAN LEEUWEN, F.X.R., and YOUNES, M.M. Consultation on assessment of the health risk of dioxins: re-evaluation of the tolerable daily intake (TDI): Executive summary. Food Additives and Contaminants, Vol. 17, no. 4 (April 2000). pp. 223 - 240.