# ANIMAL FEED CONTAMINATION BY PCDDs-PCDFs IN ITALY IN YEARS 2002-2003

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**Introduction**

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), are ubiquitous toxic contaminants mainly originating from thermal and incineration processes and representing a potential risk for human health. Various studies show that environmental levels have decreased during the last 20 years[1]. In contrast to this trend several cases of specific contamination have caused high PCDD and PCDF levels in feedstuffs[2,3,4,5]. It is important to monitor the dioxin contamination of feed to avoid large scale feed contamination and to decrease human exposure to dioxins. In Italy PCDDs and PCDFs monitoring has been introduced in the National Residues Surveillance Plan (NRSP) since 1999 and all relevant laboratory tests have been carried out at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (ISO/IEC 17025 accredited), following designation by the Ministry of Health.

The aim of this study was to evaluate PCDD/Fs levels and congener distribution patterns in different animal feed in Italy, collected in the period 2002-2003.

## *Materials and methods*

According to NRSP sampling plan, a total of 252 animal feed samples (see Table 1 for details) were collected by the regional veterinary services, covering the national territory, during the years 2002 and 2003.

Feeding-stuffs samples containing a high water amount were left to dry at room temperature before the analysis. Moisture content was determined by drying samples in oven at 103°C, thus allowing the calculation of PCDD/Fs concentration considering a 12% moisture content, as requested by the EU legislation[6]. After grinding, feed-stuffs were homogenized and a representative aliquot of sample was mixed with diatomaceous earth and spiked with $^{13}$C-labelled 2,3,7,8-congeners (Wellington Laboratories, Ontario, Canada). Sample extraction was performed by accelerated solvent extraction (ASE) using an ASE 200 (Dionex, Sunnyvale, CA, USA) extractor, with a mixture of n-hexane/acetone 80:20 (v/v). Collected extract was then evaporated to dryness in a Zymark Turbovap. After the acid/base partitioning a further purification step (multilayer silica, alumina and carbon) was then applied according to EPA Method 1613 Rev. B[7] by means of the automatic Power-Prep system (Fluid Management System, Watertown, MA, USA). The eluate

containing PCDD/PCDF congeners was dried under nitrogen stream and the residue was taken up in nonane and added of the recovery standards.
Purified extracts were then analyzed by high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) operated with electron impact (EI) ionization, by selected ion monitoring (SIM) mode at a static resolution of 10000. The HRGC/HRMS system was a MAT 95XL spectrometer (ThermoFinnigan, Bremen, Germany) coupled to a Trace Series 2000 gas chromatograph, (ThermoQuest, Milan, Italy).
GC separation of the seventeen PCDD/PCDF congeners was carried out on a DB-5 MS capillary column (60 m, 0.25 mm i.d., 0.1 μm film thickness, J&W Scientific, Folsom, USA). Quantification of the seventeen 2,3,7,8 chlorine-substituted dioxins/furans was made by isotope dilution method. TEQ values were calculated using WHO-TEFs[8]. According to the European legislation, WHO-TEQs were calculated as upper bound concentrations, i.e. assuming that all values for the specific congeners below the limit of determination (LOD) are considered equal to their specific LOD.

## *Results and discussion*

In Table 1, the TEQ values, the number and kind of tested samples are presented.
The WHO-TEQ values were below the mean values reported in other EU Countries[1]. Among all examined samples, one rabbit feed (1.53 ng WHO-TEQ/kg) did not comply with regulation limits[6]. As reported in other papers, the highest concentration was found in fish feeds (0.44 ng WHO-TEQ/kg) and in fats used as raw materials for animal feedingstuffs (0.41 ng WHO-TEQ/kg), while feed of vegetable origin were the least contaminated among all others (0.037 ng WHO-TEQ/kg) [2,9,10,11].
Comparing the congeners profiles (Figure 1), two kinds of pattern distribution can be pointed out. The first distribution pattern shows an higher overall concentration of PCDDs with respect to PCDFs, with a ratio PCDDs/PCDFs ($R_{PCDDs/PCDFs}$) = 23 for animal fat and $R_{PCDDs/PCDFs}$ = 4.5 for vegetable feeds. In such feedingstuffs OCDD and 1,2,3,4,6,7,8-HpCDD congeners are predominant, representing 95% and 80% respectively.
In the second pattern, referred to fish feeds and mineral feeds, a more equilibrated PCDDs/PCDFs congeners distribution was found, resulting in $R_{PCDDs/PCDFs}$ = 0.82 and 1.2 respectively. Furthermore, the analytical contribution of the single congeners was different taking into account the considered matrices. In fact, the most abundant congeners in fish feed are represented by OCDD (31%), 2,3,7,8-TCDF (28%) and 2,3,4,7,8-PeCDF (12%). For mineral feed, a significant presence of the congeners characterised by a high degree of chlorination such as OCDD (42%), 2,3,4,6,7,8-HpCDF (15%) and OCDF (13%) was found, even though this data should be considered purely indicative due to the small number of samples analysed.

For all the considered feedingstuffs, the highest contribution to the TEQ was due to the 2,3,4,7,8-PeCDF (range 24-40%), followed by 1,2,3,7,8-PeCDD (range 9-22%) and 2,3,7,8-TCDD (range 6-11%). It must be remarked the significant presence of 2,3,7,8-TCDF (19%) in fish feed and of 1,2,3,4,6,7,8-HpCDD (25%) in animal fat.

Table 2 shows the mean values of the congeners found in vegetable feed materials, grouped into five main categories (feed for poultry, cattle, pig, sheep and rabbit). The mean TEQ values and contamination profiles of feed for poultry, cattle, pig and sheep were similar (range 0.019–0.035 ng WHO-TEQ/kg). The TEQ level in rabbit feed was ten times higher (0.319 ng WHO-TEQ/kg), even

though the small number of samples and the strong influence of a violative specimen has to be taken into account.
The contribution of PCBs dioxin-like to the total TEQ was not determined. Since the limited data of the contamination of feedingstuffs by PCBs dioxin-like indicates their important contribution to the TEQ value, it is necessary to extend the monitoring to these substances in order to comply with the recommendations of the Scientific Committee on Animal Nutrition (SCAN)[1].

## *References*


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**Table 1: PCDD/Fs levels (ng WHO-TEQ/kg)* in feed samples**

| | N° of samples | Mean | Min | Max |
|---|---|---|---|---|
| Fish feed | 73 | 0.44 | 0.033 | 1.6 |
| Mineral feed | 6 | 0.073 | 0.0060 | 0.25 |
| Vegetable feed | 165 | 0.037 | 0.0020 | 1.5 |
| Animal fat | 8 | 0.41 | 0.069 | 1.3 |

* referred to 12% moisture content, upper bound concentrations

**Table 2: average PCDD/Fs concentrations in vegetable feed materials samples***

| Compounds | Poultry n = 16 | Cattle n = 88 | Pig n = 48 | Sheep n = 8 | Rabbit n = 5 |
|---|---|---|---|---|---|
| 2,3,7,8-T4CDD | 0.004 | 0.003 | 0.002 | 0.003 | 0.028 |
| 1,2,3,7,8-P5CDD | 0.004 | 0.007 | 0.003 | 0.003 | 0.051 |
| 1,2,3,4,7,8-H6CDD | 0.012 | 0.008 | 0.003 | 0.003 | 0.029 |
| 1,2,3,6,7,8-H6CDD | 0.012 | 0.013 | 0.015 | 0.008 | 0.039 |
| 1,2,3,7,8,9-H6CDD | 0.009 | 0.009 | 0.004 | 0.007 | 0.029 |
| 1,2,3,4,6,7,8-H7CDD | 0.128 | 0.183 | 0.073 | 0.079 | 0.152 |
| O8CDD | 0.718 | 1.365 | 0.515 | 0.549 | 0.506 |
| 2,3,7,8-T4CDF | 0.010 | 0.031 | 0.019 | 0.023 | 0.215 |
| 1,2,3,7,8-P5CDF | 0.006 | 0.013 | 0.004 | 0.007 | 0.186 |
| 2,3,4,7,8-P5CDF | 0.007 | 0.020 | 0.012 | 0.012 | 0.281 |
| 1,2,3,4,7,8-H6CDF | 0.015 | 0.015 | 0.009 | 0.010 | 0.161 |
| 1,2,3,6,7,8-H6CDF | 0.013 | 0.018 | 0.007 | 0.008 | 0.177 |
| 1,2,3,7,8,9-H6CDF | 0.003 | 0.005 | 0.003 | 0.004 | 0.022 |
| 2,3,4,6,7,8-H6CDF | 0.010 | 0.018 | 0.007 | 0.011 | 0.182 |
| 1,2,3,4,6,7,8-H7CDF | 0.031 | 0.058 | 0.026 | 0.034 | 0.270 |
| 1,2,3,4,7,8,9-H7CDF | 0.009 | 0.006 | 0.002 | 0.006 | 0.031 |
| O8CDF | 0.067 | 0.087 | 0.045 | 0.053 | 0.109 |
| WHO-TEQ | **0.022** | **0.035** | **0.019** | **0.021** | **0.319** |

* referred to a 12% moisture content, upper bound concentrations

Figure 1: PCDD/Fs congener profiles in different feeding stuffs