FOOD AND ANIMAL FEEDS CONTAMINATION BY PCDDs-PCDFs IN ITALY IN YEARS 1999 AND 2000: INCIDENCE AND TEQ CONGENERS PATTERNS

Giampiero Scortichini, Gianfranco Diletti, Luigi Torreti, Anna Francesca Forti, Rossana Scarpone and Giacomo Migliorati

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" Via Campo Boario 64100 Teramo, Italy

Introduction

As a consequence of the Belgian crisis, the European Community has planned a monitoring scheme to verify the contamination levels from PCDDs/PCDFs in foods and animal feeds. Following these dispositions, the Italian Ministry of Health has introduced the PCDDs/PCDFs monitoring in the National Residues Surveillance Plan (1), according to the Directive 96/23/EC (2). These tests were performed at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy (UNI CEI EN 45001 accredited), as authorised laboratory for the dioxins and furans analyses. There are various methods to carry out PCDDs/PCDFs tests using the isotopic dilution technique and the HRMS detection; it has been decided to adopt EPA Method 1613 rev. B (3), integrated by a results evaluation method basing on the criteria established by the Italian Institute of Health (National Reference Laboratory) (4).

Methods and Materials

Wet samples have been previously freeze-dried to make them suitable for the extraction with Dionex Accelerated Solvent Extractor (ASE) 200. For this purpose, previously homogenised freeze-dried aliquots were mixed with diatomaceous earth and spiked with EPA Method 1613 PCDDs/PCDFs internal standard solution (containing fifteen $^{13}C_{12}$-labeled 2,3,7,8-PCDD/PCDF) prior to extraction. Then samples were extracted in ASE

Obtained fat was thus purified according to EPA Method 1613; clean up consisted of an acid/base liquid/liquid partitioning, then samples were purified by means of the automatic Power Prep system (Fluid Management System, Inc.), flowing through three different cleanup columns (acid/base silica, alumina and carbon).

PCDDs/PCDFs eluate (40 mL in toluene) was evaporated to dryness, then the remainder was dissolved in nonane, fortified with 1,2,3,4-TCDD-$^{13}C_{12}$/1,2,3,7,8,9-HxCDD-$^{13}C_{12}$ recovery standard and analysed by High Resolution Gas Chromatography (HRGC)/ High Resolution Mass Spectrometry (HRMS), in the selected ion monitoring mode at a resolution of 10,000. The HRGC/HRMS system consisted of a MAT 95XL Finnigan coupled to a Trace Series 2000 capillary GC with AS 200 autosampler; analysis of the seventeen 2,3,7,8-substituted PCDDs/PCDFs was performed on a DB-5 MS (60 m × 0.25 mm, 0.1 μm) capillary column. The GC conditions were optimised to completely separate the various 2,3,7,8-Cl-substituted dioxins/furans: initial oven temperature, 140°C; injector temperature, 240°C; interface temperature, 290°C. Injection volumes of 1-2 μL were used for sample and calibration analyses; masses monitored for the determination of PCDDs/PCDFs were the same as those listed in EPA Method 1613 Table 3.

Results and Discussion

248 samples collected from 14 different Italian regions in 1999 and 2000 were analysed. With the aim of demonstrating the level of contamination of samples, the following criteria of distinction were applied:

1. negative samples: all those samples in which none congener showed concentration values exceeding the limits of detection (LOD);
2. positive samples: those samples in which at least one congener was found to exceed LOD;
3. LOD: it was systematically determined for each sample and for each specific congener, with the purpose of estimating the quantitative meaning of not detectable samples. In this regard, the resulting toxic equivalents were expressed as I-TEQ (NATO/CCMS); if specific congeners could not be detected their contribution to the total toxicity was calculated as half the value of the respective limit of detection (1/2 LOD).

Following these evaluation criteria, 99 samples out of 248 have not surpassed the LOD. Obtained data have been divided into groups of homogeneous matrices, in order to determine:

1. the average levels of contamination expressed as I-TEQ (5) and as analytical concentrations (pg/g fat);
2. the specific congeners patterns.

Collected data have highlighted that the frequency of positive samples results higher in fish and fish feed samples (100% of examined cases for both matrices; see Fig. 1), with an average detected level of 6.60 I-TEQ for fish feed and of 5.28 I-TEQ for fish. These values could be due to the use, in the farming fish species, of feeds with an elevated content in flours and fats of animal origin, that could cause an accumulation effect owing to the feed-back mechanism in the food chain (see Fig. 2).

80% of the positive animal feeds, apart from those for fish, present an average level of 0.63 I-TEQ. This value does not produce a significant accumulation effect on the matrices of the same food chain (milk, meat, fat, eggs). In animal species other than fishes, it could be attributed to the use of foodstuffs with a lower content of fatty substances and to the ban of animal flours in the preparation of foods. In fact milk, meat, fat and eggs showed I-TEQ values of 0.81, 0.73, 0.59 and 0.25, respectively.

From the study of the distribution patterns for the various matrices, it should be noticed that the presence of 1,2,3,4,6,7,8-HpCDD and OCDD is probably due to the introduction in the national territory of choline chloride, proportionally contaminated by the same congeners; in fact choline chloride is usually employed in the industrial preparation of animal rations. Moreover, these isomers are also present in the same ratios in the typical contamination patterns for products (milk, eggs, fat, meat) deriving from animals fed with those kinds of aliments (see Fig. 3). In this contest, it is useful to highlight that profiles comparable to those reported in the present study have been published (6,7,8,).

Figure 1: calculated I-TEQ values for examined samples

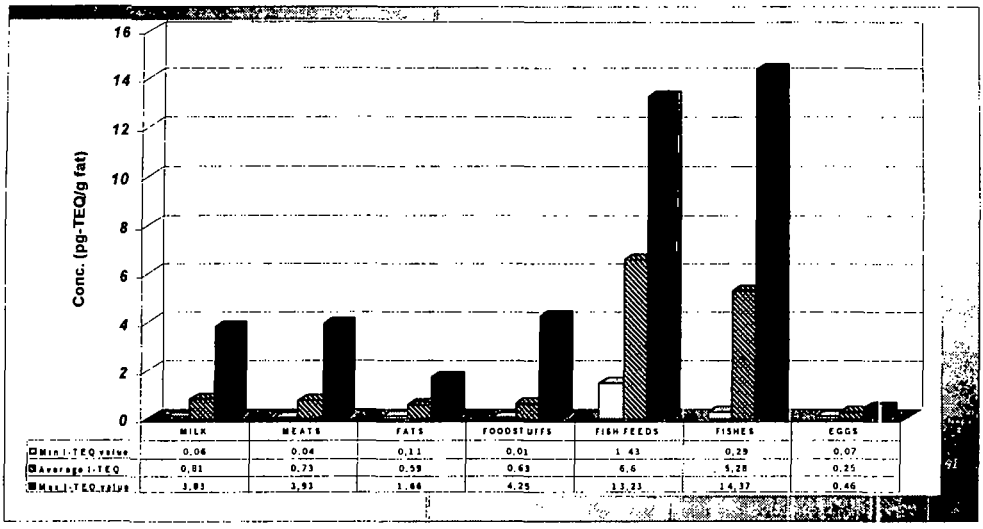


Figure 2: correlation between the PCDD/F homologue profiles for fish and fish feed

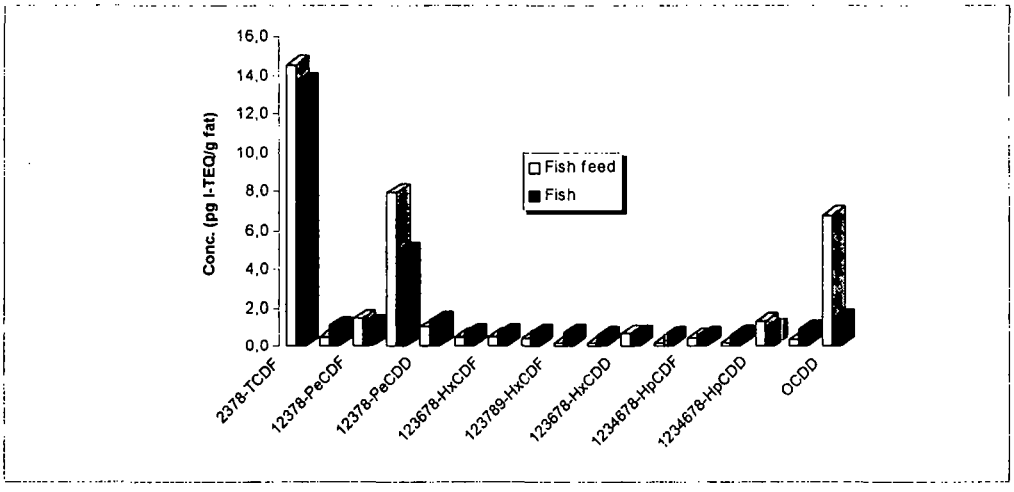
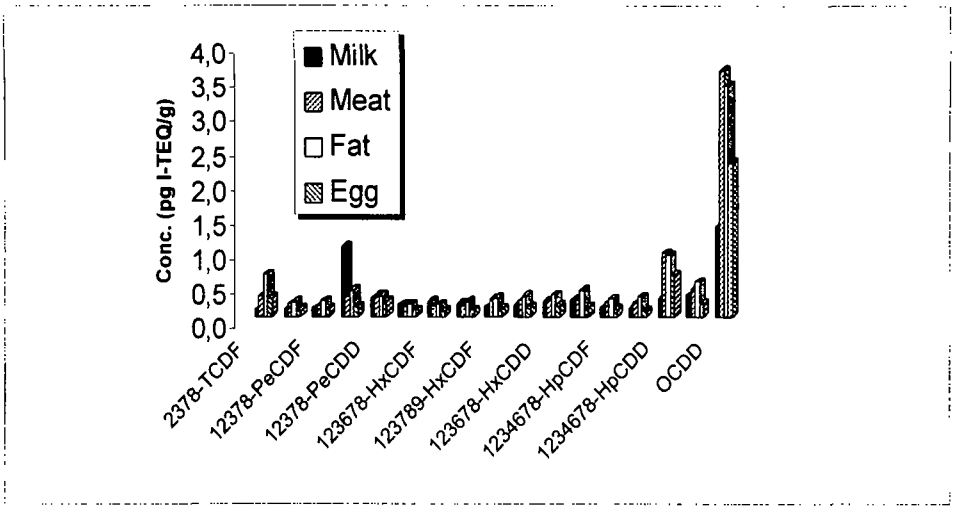


Figure 3: PCDD/F data patterns for different analysed matrices



References

1. Decreto Legislativo n. 336 del 4 agosto 1999. Attuazione delle direttive 96/22/CE e 96/23/CE concernenti il divieto di utilizzazione di talune sostanze ad azione ormonica, tireostatica e delle sostanze β-agoniste nelle produzioni di animali e le misure di controllo su talune sostanze e sui loro residui negli animali vivi e nei loro prodotti. G.U. n. 230 del 30 settembre 1999;
2. 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 *Official Journal L 125 , 23/05/1996 p. 0010 – 0032;*
3. U.S. Environmental Protection Agency, Office of Water Engineering and Analysis Division (4303) - Washington, D.C. 204600, Method 1613 Rev. B "Tetra through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGG/HRMS";
4. Istituto Superiore di Sanità. 1999. Linee-guida per interventi analitici mirati al rilevamento di PCB, PCDD, PCDF in prodotti alimentari (ISS- XEN-99-4; Version 01/07/99). [http://www.iss.it/diossina/diossina.htm];
5. NATO/CCMS (North Atlantic Treaty Organization, Committee on the Challenges of Modern Society); (1988a) International toxicity equivalency factor (I-TEF) method of risk assessment for complex mixtures of dioxins and related compounds. Report No. 176;
6. Mallisch, R.; Gleadle, A; Wright, C; (1999) Organohalogen compounds, 43, 265-269;
7. Bayarri, S.; Turrio Baldassarri, L.; Iacovella, N.; Rodriguez, F.; Di Domenico, A.; (1999) Organohalogen compounds, 43, 289-294;
8. Concejero, M.A.; Jimenez, B.; Eljarrat, E.; Rivera, J.; Gonzalez, M.J.; (1999) Organohalogen compounds, 43, 271-274;