A case of eggs contamination by PCDD/Fs in Italy: analytical levels and contamination source identification

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Introduction

In the framework of the 2004 National Residues Surveillance Plan (NRSP), PCDD/Fs levels exceeding the European Union (EU) tolerance limit were detected in eggs from a farm located in the north of Italy.

In order to identify the source of contamination further samples were collected in the farm under investigation, including various food of animal origin (eggs, laying hens meat and cheese), animal feed for chicken, wood-shaving litter and wood-shavings. Cheese and feed samples presented dioxin contamination at background levels, while high PCDD/Fs concentrations were found in eggs, meat, litter and wood shavings.

As a consequence, the Italian Ministry of Health planned a specific dioxins survey in eggs from farms where hens were raised on wood-shaving litters.

Here we report preliminary data on PCDD/Fs contamination levels and profiles in the samples of interest.

Materials and methods

Samples were homogenized and tested by a validated method routinely used for dioxins monitoring in food and animal feed and successfully verified in a number of inter-laboratory studies. Samples were extracted by accelerated solvent extraction (ASE) using an ASE 300 Dionex instrument with a mixture of n-hexane and acetone 80:20 (v/v). The clean-up procedure was based on an acid/base partitioning and a further purification was then performed according to EPA Method 1613 Rev. B by means of an automated clean-up process with Power-Prep system (Fluid Management System) using disposable columns (multilayer silica, alumina and carbon). PCDD/Fs were separated by high resolution gas chromatography (HRGC) on a DB-5 MS capillary column (60 m x 0.25 mm, 0.1 µm) and determined by high resolution mass spectrometry (HRMS), at a resolution of 10000 in the selected ion monitoring (SIM) mode. The HRGC/HRMS system consisted of a GC Trace Series 2000 (ThermoQuest) coupled with a MAT 95 XL (ThermoFinnigan). The quantification of the seventeen 2,3,7,8 chlorine-substituted dioxins/furans was accomplished by the isotope dilution method. TEQ values were calculated using WHO-TEFs. According to the European legislation, WHO-TEQs were calculated as upper bound concentrations assuming that all values of specific dioxins congeners below the limit of determination (LOD) are equal to the respective LOD.

Results and discussion

The dioxin levels found in the first egg sample was equal to 88.11 pg WHO-TEQ/g fat, with a major abundance of PCDDs in respect to PCDFs. The further samples taken in the same farm gave the following results:

- eggs 33.00 pg WHO-TEQ/g fat, laying hens meat 45.16 pg WHO-TEQ/g fat, thus confirming the contamination found in the previously analysed egg sample;
- cheese (from free grazing cattle) 0.54 pg WHO-TEQ/g fat, excluding pollution of environmental origin;
- chicken ration 0.050 pg WHO-TEQ/g, excluding contamination due to feed products;
- wood-shaving litter 50.82 pg WHO-TEQ/g, wood shavings 40.10 pg WHO-TEQ/g, with similar congener profiles, thus obtaining a clear identification of the contaminated material from which dioxins were transferred to poultry.

The PCDD/Fs profiles found in eggs, meat, litter and wood shavings are showed in Figure 1.

All samples were characterized by a predominance of PCDDs over PCDFs. In eggs, wood shavings and litter the PCDFs levels amounted to 8% of PCDDs concentration. In meat PCDFs contribution raised to 16%.

The congeners profile was the same in all contaminated matrices. OCDD was the most abundant congener among dioxins (range 54% - 82%), followed by 1,2,3,4,6,7,8-HpCDD (range 11% – 24%). OCDF was predominant among furans (range 4% - 7%), followed by 1,2,3,4,6,7,8-HpCDF (range 1% - 5%). In eggs and meat, 1,2,3,6,7,8-HxCDD (2% and 5%, respectively) and 1,2,3,4,7,8-HxCDF (1% and 2%, respectively) were also present in significant relative concentrations.

From a toxicological point of view, the congener which most contributed to TEQ values in eggs, litter and wood shavings was 1,2,3,4,6,7,8-HpCDD (range 28% - 61%), followed by 1,2,3,6,7,8-HxCDD (range 14% - 24%). On the contrary, 1,2,3,6,7,8-HxCDD was prevalent (33%) in meat, followed by 1,2,3,4,6,7,8-HpCDD (17%).

The HpCDD/F and OCDD/F congeners abundance induced to hypothesize that the primary contamination source was pentachlorophenol (PCP), a biocide highly persistent in the environment used in paintings for wood preservation, in pulp, paper, textile and leathers industries[1, 2]. PCP and its derivatives may contain high levels of

PCDD/Fs as impurities, with a predominance of HpCDD/F e OCDD/F[3].
In June 2000 a similar problem was found in Germany where high levels of dioxin were detected in a choline choride premix used as animal food component[4]. The conclusion was that PCP-contaminated sawdust used as carrier for choline choride was the source of dioxin contamination in feedingstuff.
In the framework of the dioxins survey in eggs coordinated by the Italian Ministry of Health, 90 samples have been analysed until now; two samples were not compliant with the EU tolerance limit[5] and they were characterized by a congener pattern similar to those encountered in the previous contaminated samples. The present results highlight the problem of contaminated wood shavings used by unaware breeders in chicken litters: even very low amounts of litter particles ingested by chicken could cause serious contamination of food for human consumption, such as eggs and meat.

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References

1. Alcock R. E., Jones K. C. 1997. Pentachlorophenol (PCP) and Chloranil as PCDD/F sources to sewage and sludge amended soils in the UK. Chemosphere 35:2317-2330.
2. Wild S. R., Harrad S. J., Jones K. C. 1992. Pentachlorophenol in the UK environment: a budget and source inventory. Chemosphere 24:833-845.
3. Masunaga S., Takasuga T., Nakanishi J. 2001. Dioxin and dioxin-like PCB impurities in some Japanese agrochemical formulations. Chemosphere 44:873-885.
4. Llerena J. J., Abad E., Caixach J., Rivera J. 2003. An episode of dioxin contamination in feedingstuff: the choline chloride case. Chemosphere 53:679-683.
5. Council Regulation (EC) No 2375/2001 of 29 November 2001 amending Commission Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs. Official Journal L 321, 06/12/2001, 1

Figure 1: PCDD/Fs congener profiles in different samples