Decrease of PCDD/F and PCB concentrations in eggs after a natural contamination

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Introduction

According to the EU regulation, each member state is mandated to organize control plans or survey for dioxins and PCBs in various food and feed. During one of this national survey dedicated to free-range chicken eggs in France, it was observed that some samples contained quite abnormally elevated concentrations of dioxins. The origin of the samples was the North of France, an area that is well known for its industrial past. Few years ago, some dioxin contamination problems already occurred in this region [1]. Many decontamination studies have already been conducted but often after a controlled intake with contaminated feeding stuffs [2,3]. In this context, the purpose of this study was first to characterize the decrease of PCDD/F concentration in eggs after a natural contamination, and secondly to determine the delay necessary to reach back the maximum tolerable value of 3 pg WHO-TEQ/g fat for the PCDD/Fs. For this purpose, 30 laying hens were gathered from this farm after preliminary investigations to determine the origin of the contamination (soil and feeding stuff).

Materials and Methods

Study design and sampling

Thirty laying hens were reared in controlled poultry facility without any soil contact. They had unlimited access to low contaminated feed. The environmental conditions (light period, diet) were exactly comparable to industrial ones. The study was held during 22 weeks, considering the first day of the experiment in this new area as the initial day 0.

Eggs were collected every day and pooled for analysis approximately twice a week, in order to get a representative sample. The average eggs production was about 3.5 eggs/chicken/week.

The soil of the farm where the chickens came from was collected in different places and analyzed. The worms found in this soil were also analyzed since they are part of the chicken's intake. The feed given to the hens at the farm and the one given at the lab were also analyzed.

Extraction and clean-up

The extraction and purification methodology has already been described elsewhere[4]. Before extraction, egg samples were freeze-dried and 17 ^{13}C-labelled PCDDs/PCDFs and 18 ^{13}C-labelled PCBs were added. Samples were then extracted using an Accelerated Solvent Extraction System (ASE). The extraction solvent was a mixture of toluene/acetone 70:30 (v/v). Resulting extracts were evaporated to dryness, permitting the gravimetric determination of the fat content. Finally, a three-step purification was performed, using successively a multi-layer silica gel column, Florisil, and Celite/carbon columns. The external standards were added for the recovery calculation ($^{13}C_{12}$-1,2,3,4-TCDD for the PCDD/F, $^{13}C_{12}$-PCB #111 for the PCBs).

GC/HRMS analysis

The GC/HRMS detection was performed on a Hewlett-Packard 6890 gas chromatograph, equipped with a DB-5MS column (30 m x 0.25 mm, 0.25 mm film thickness), coupled to a Jeol JMS-700D high-resolution mass spectrometer. The mass analyzer operated at a resolution of 10,000 in the selected ion monitoring (SIM) in the Electron Ionization (EI) mode. This procedure integrated all the necessary quality assurance parameters to fulfill the requirements of the Commission Directive 2002/69/EC of July 2002 laying down the sampling methods and the methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs. Moreover, this research project

was conducted under a certified system (ISO 9001 v. 2000 standard) and analyses were performed upon an accredited system (ISO 17025).

Results and Discussion

Soils and feeds

As recommended in the case of non-compliant samples, investigations were done to determine the origin of the contamination observed in the first analyzed egg sample. The feeding stuff given during the growth period and the soil of the farm were collected and analyzed. The results reported in Table 1 clearly demonstrated a significant contamination of soil and worm samples. Even if the concentrations of PCDD/Fs and PCBs found in this area were higher than those usually found in a typical rural area, they were lower comparing to some industrial contaminated soils[5]. However, these values appeared sufficient to lead to the contamination of eggs from hens raised on this soil, as previously observed[5].

The PCDD/F and PCB concentrations in the feed given to the hens during the decontamination step are reported in Table 2. These feeds and vitamins were specifically prepared for hens and homogenized. All samples were found to be below the maximum tolerable level for dioxins (0.75 pg WHO-TEQ/g matrix with 12 % of moisture) and the PCB concentrations were very low. As hens had only access to these feeding stuffs during all the experiment, the possibility of additional contamination through the diet was excluded.

Table 1

Measured concentrations in soil and worms collected in the farm (pg WHO-TEQ/g dried matrix for dioxins and DL-PCBs and ng/g dried matrix for Marker PCBs)

	PCDD/Fs	Dioxin-like PCBs	Marker PCBs
Soil (upper)	11.42	2.26	14.99
Soil (deeper)	6.69	1.46	10.41
Worms	4.13	5.27	58.49
Feeding stuff	0.04 (12% moisture)	0.01 (12% moisture)	0.12 (12% moisture)

Table 2

Observed concentrations in the feeding stuffs given to the laying hens during the experiment (pg WHO-TEQ/g dried matrix for dioxins and DL-PCBs and ng/g dried matrix for Marker PCBs)

	PCDD/Fs	Dioxin-like PCBs	Marker PCBs
Cereals	0.053	0.003	0.050
Feedstuffs	0.058	0.004	0.041
Vitamins	0.031	0.011	0.162

Eggs

Eggs were collected every day. Whole eggs from each collection time were mixed together (leading to pools of 12 eggs in average) in order to minimize inter-individual variations. During the first week, the hens did not lay normally due to the stress of the travel from farm to lab. Considering the time needed for adaptation to their new environment, the eggs collected during this first week have not been analyzed. The first sample included in the study showed a high concentration in dioxins, dioxin-like and marker PCBs, in the same range than those obtained for the egg sample analyzed during the official survey. During the following weeks the contamination level observed in the analyzed samples decreased exponentially. The measured concentrations for dioxins and PCBs are presented on

Figures 1 and 2. For PCDD/Fs, these results demonstrated that a two-month period of clean feeding was necessary to reach a concentration below the maximum tolerated value of 3 pg WHO-TEQ/g of fat. Considering the total TEQ that includes the DL-PCBs TEQ, 2 months were needed to be under the 6 pg WHO-TEQ/g fat value (future limit for total TEQ, which will take effect in the EU in 2005). The observed decreasing curves for PCDD/Fs and PCBs were found to be very similar, indicating that the elimination of these two classes of contaminants via the eggs is comparable.

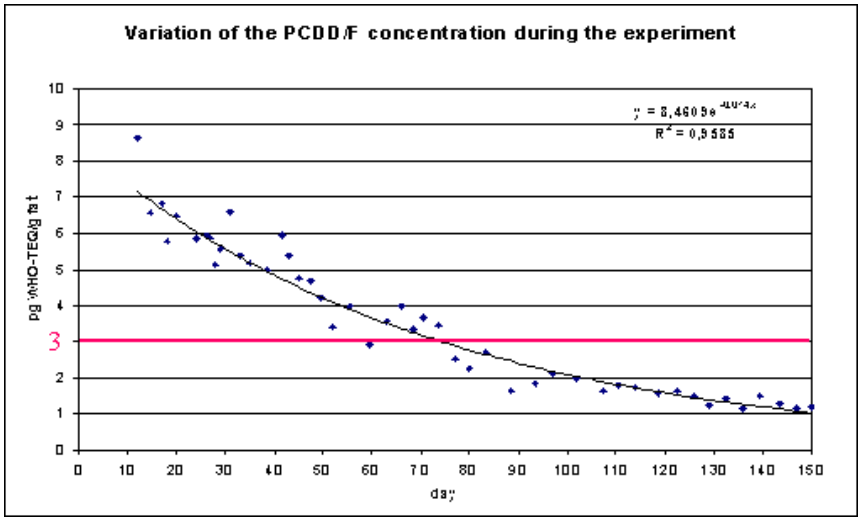

Fig.1. PCDD/Fs decrease curve in eggs (in pg WHO-TEQ/g fat)

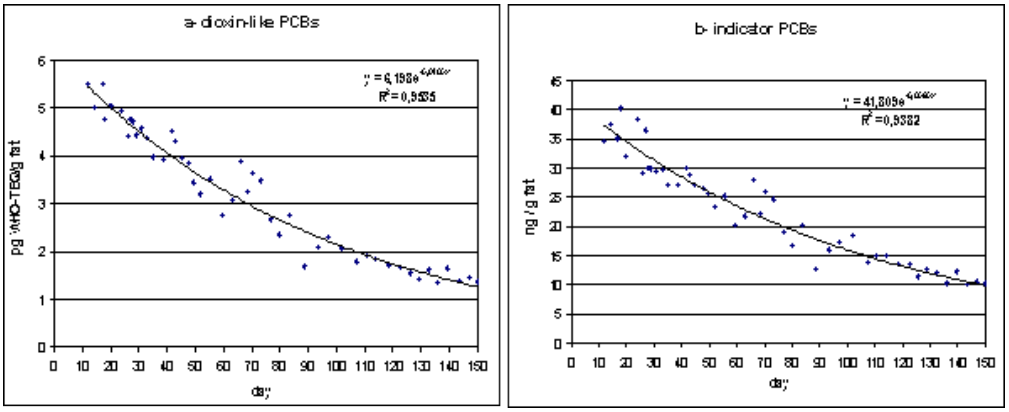

Fig.2. Decrease in eggs, a: dioxin-like PCBs (pg WHO-TEQ/g fat), b: marker PCBs (ng/g fat)

Conclusion

In this study, thirty laying hens naturally contaminated by PCDD/Fs and PCBs were fed for 22 weeks with low contaminated feeding stuff. Eggs were collected every day and analyzed to determine the presence of PCDD/Fs and PCBs. The depletion curves observed for PCDD/F and PCB concentrations were found to be exponential and very similar for the two groups of contaminants. In the present experimental conditions, two months were necessary to reach the maximal tolerated level fixed by the EU for PCDD/Fs in eggs.

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