A PECULIAR CASE OF PCB CONTAMINATION IN YOUNG ORGANIC HENS

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Introduction

Dioxins and PCBs are an important threat to the health of consumers with food of animal origin being the most important source of these compounds for the average consumer. To further reduce the intake, the EU has set maximum levels for various food products and feed according to the principle strict but feasible. Official control programs and especially company self-control should allow for detection of samples with elevated levels and the identification and removal of novel sources. During the last decade a serious of larger or smaller incidents occurred that were somehow linked to the feed chain with sources like improper fuels for drying, the use of certain clays or contaminated chemicals. However, in addition to feed, also the housing and environment may be important sources of dioxins and PCBs. Animals foraging outside may be especially affected by such sources and laying hens appear to be extremely sensitive, concentrating the contaminants in the eggs. Soil levels that would not be considered different from the background could already lead to levels exceeding the limits. At Dioxin 2012, we presented a thus far unknown source, being the use of contaminated building debris for foundation and paths in the courtyard. This source caused problems with elevated PCBs at egg farms that previously were considered to be without problems. In the second part of 2012, RIKILT was contacted in relation to another case with elevated PCB-levels in eggs. The present paper describes this case in more detail.

Materials and methods

Samples were analysed by GC/HRMS for dioxins and PCBs by routine methods applied at RIKILT[1]. Egg samples were extracted using the ASE 350 (Dionex, USA) with hexane/acetone (1:1) as extraction solvent. The extraction program consists of 3 cycles, including heating at 100°C during 5 min at a pressure of 1500 PSI. Solvent residue was evaporated and the remaining lipid was kept in the refrigerator for further analysis. For soil the same procedure using the ASE 350 was applied, however with toluene/ethanol as the extraction solvent. Livers were extracted according to Smedes[2] by blending the sample with sodium chloride saturated water 2-propanol and cyclohexane. Fat was obtained by evaporating the cyclohexane phase. Soil and liver samples were spiked prior to extraction with ^{13}C labeled standards , while for the egg samples the obtained fat was spiked with the ^{13}C labeled standards. The animal fat samples were directly after spiking with the ^{13}C labeled standards diluted with hexane. All extracts were purified using the automated PowerPrep™ system (FMS) which was composed of disposable pre-packed columns (Jumbo silica, mixed bed silica, alumina and carbon) to aid in the isolation of the different fractions. The clean-up resulted in the collection of two purified fractions, one with mono-ortho dl-PCBs and non-dioxin-like PCBs and the other with dioxins and non-ortho dl-PCBs.
The fractions were concentrated using the Power-Vap (FMS) and analyzed by GC-HRMS using an Agilent (Wilmington, USA) gas-chromatograph 6890N (GC column DB5 MS 60m, 0.25mm i.d., 0.25µm; J&W, Folson, USA) and an AutoSpec Ultima high resolution mass spectrometer (Waters, Milford, USA) operated in electron impact ionization mode using selected-ion monitoring and controlled by Masslynx data system. GC-HRMS data were processed using DIOXNOP software to determine the concentrations and subsequently the TEQ levels, based on WHO-TEFs 2005. The performance of the methods is regularly checked by participation in PT-tests.

Results and discussion

Increased levels in eggs by an organic farm led to the discovery that the hens were already contaminated before delivery to the farm. As a precautionary measure several hens had been slaughtered at the age of 15 weeks and abdominal fat samples had been stored. A sample was reanalyzed and showed dioxin and dl-PCB levels of respectively 7 and 431 pg TEQ/g fat, and an ndl-PCB level of 652 ng/g fat (Table 1).

Subsequently the farm was visited and new samples were taken from the new flock of hens being raised at the farm, aged 17.5 weeks at that time. There were two stables , the previous contaminated hens were raised at stable 1. This stable was divided in two parts, one with brown and one with white hens. Pooled abdominal fat samples of 10 brown hens showed dioxin, dl-PCB and ndl-PCB levels of respectively 2 pg TEQ/g, 80 pg TEQ/g and 169 ng/g, that from 10 white hens of 4 pg TEQ/g, 232 pg TEQ/g and 469 ng/g (Table 1). Livers of these hens showed two-fold higher dioxin levels, slightly lower dl-PCB levels and much lower ndl-PCB levels. Overall, this confirmed the findings with the previous hens that there was obviously a PCB-contamination at the farm. Hens from stable 2 showed low levels. Samples of abdominal fat from individual hens were also tested with the DR CALUX assay and showed a large variation between the hens, confirming that the source is only accessible for part of the hens (Figure 1).

Table 1. Dioxin, dl-PCB and ndl-PCB levels in eggs from the ovarian, abdominal fat and livers of 5 brown hens

	Dioxins (pg TEQ/g vet)			dl-PCBs (pg TEQ/g fat)			ndl-PCBs (ng/g fat)		
	eggs	fat	liver	eggs	fat	liver	eggs	fat	liver
Previous									
pool		7.2			431			652	
1/10									
Br pool		1.9	3.3		80	52		169	58
Wh pool		4.2	11.0		234	215		469	181
22/10									
Hen 1	1.9	1.6	1.4	76.5	78.9	48.3	161	170	68
Hen 2	0.3	0.2	0.4	1.4	1.4	1.1	4	4	4
Hen 3	1.5	1.3	1.6	78.8	74.2	50.7	121	115	64
Hen 4	1.3	1.0	1.5	51.5	43.4	34.9	84	80	49
Hen 5	0.5	0.3	0.8	14.4	11.5	12.3	23	19	13

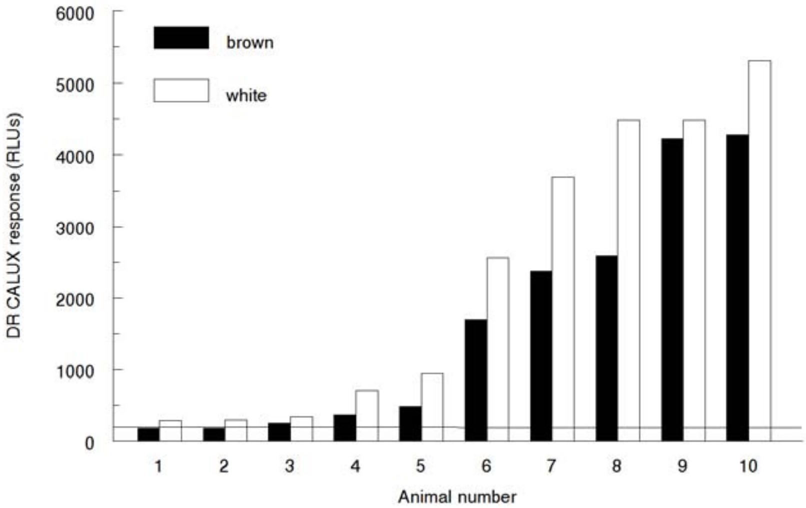

Figure 1. Results obtained with fat from individual hens, 10 of each kind, and analysed by DR CALUX-assay. Results are expressed in relative light units but not converted to BEQs. The line indicates the response corresponding to the ML of 3 pg TEQ/g fat.

Various soil samples were also taken from the courtyard used by the brown hens and from the inside floor from both parts. Levels are shown in Figure 2. A highly suspected path with small stones showed no real contamination, similar to soil samples taken at longer distance from the stable. However, soil samples taken close to the wall showed very high levels. Recently new soil had been transferred to this part but this soil itself was actually clean. Considering the samples taken inside, a mixture of manure, litter and soil, the higher level was observed at the side of the white hens.

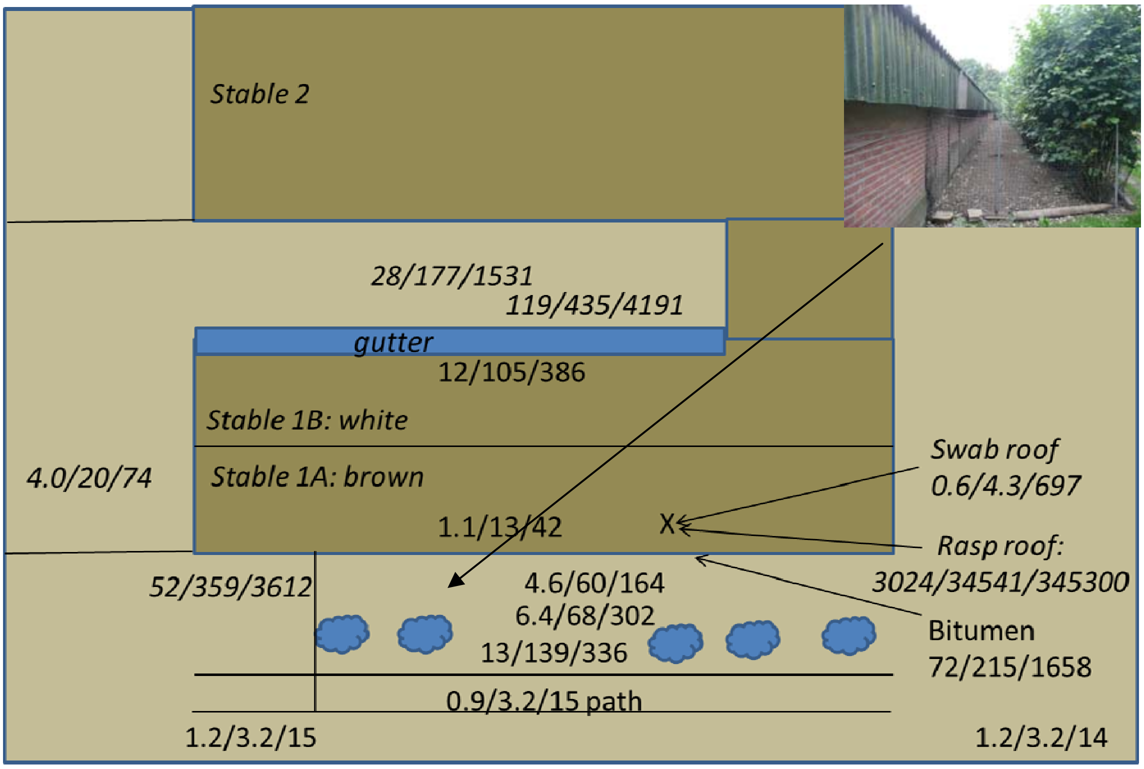

Figure 2. Dioxin, dl-PCB and ndl-PCB levels in soil and roof samples around stable 1. Dioxin and dl-PCB levels in soil expressed in pg TEQ/g dm and absolute pg TEQ levels in swabs and rasp. The ndl-PCB levels are in ng/g dm or ng absolute.

During a second visit, more targeted samples were taken from the soil at the other side of the stable and from the roof. The soil at the "white" side was more contaminated than that at the "brown" side where the clean soil had been added, whereas a soil sample more to the left, that was not mixed showed comparable levels as those at the "white" side. The highest levels were detected in swab and especially rasp samples from the roof. Samples from the roof on stable 2 and replaced parts on stable 1 were negative. Therefore the cause of the PCB contamination appeared to be the coating of the roof plates of stable 1 which originated from the beginning of the seventies. The absence of gutters caused the run-off of the rain water onto the soil in front of the stable walls, since only a few years ago a gutter had been placed on the "white" side of the stable. The requirement to allow young laying hens to have access to an outdoor courtyard was relatively new, possibly being the reason that the high contamination had gone unnoticed. It is unclear to what extent similar contaminated roof plates had been applied to other farms.

Follow-up studies

In the past, studies were performed with hens already laying eggs, in order to study the carry-over of dioxins and PCBs to eggs. The question was however, if the results from these studies could be used for predicting the levels in the eggs of the hens and the time needed to obtain levels below the existing EU maximum levels. As a start, a number of brown hens were slaughtered at the start of the laying and liver, abdominal fat and fat from preliminary eggs in the ovarian were analysed (Table 1). Levels in egg and abdominal fat were rather comparable, with slightly lower levels in fat extracted from the liver. There was strong variation in the levels between the hens, probably reflecting the tendency to go outside.

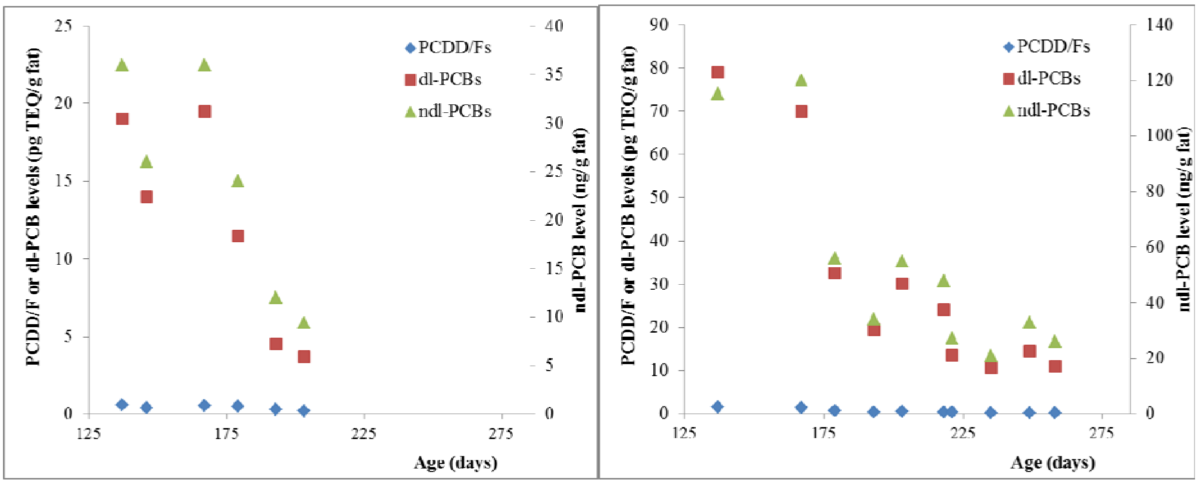

Figure 3. Levels of dioxins, dl-PCBs and ndl-PCBs in eggs from brown (left) and white (right) laying hens.

Once the hens started to produce eggs they could no longer be kept at the farm and were transferred to two different egg producing farms, one for the brown hens, one for the white hens. At regular time periods, eggs were sampled and analysed (Figure 3). In the case of the brown hens, total TEQ levels started around 20 pg TEQ/g fat, remained around that level for some time in the period when more and more hens started to lay eggs, and then decreased within a couple of weeks to levels below the ML. At that stage sampling of eggs from brown hens was ceased.

The levels in eggs from white hens were much higher, in agreement with the differences in abdominal fat and soil. Levels subsequently dropped rapidly but then entered a slow phase with a half-life around 50 days. This half-life was comparable to that observed previously in a study with laying hens[3]. Since it was estimated that levels would remain above MLs for several months, it was decided to euthanize the hens.

Conclusions

A thus far unknown source was discovered, being roof plates coated with a PCB-containing material. This is important for dealing with potential new incidents with a.o. eggs from free-ranging hens. Also the present study revealed valuable information on the behavior of dioxins and PCBs in hens that became contaminated before they started the production of eggs.

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