PROBLEMS WITH DIOXINS AND DIOXIN-LIKE PCBS IN FREE RANGE EGGS

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Introduction

Since 2001 RIKILT performs a monitoring program of animal derived food products like meat, milk and eggs. In the first year it was discovered that eggs from laying hens with access to soil may contain elevated levels of dioxins. This confirmed previous studies[1] and shows that in addition to feed also the environment of the hens is very important. A carry-over study with contaminated feed showed that even low intake of dioxins may already result in non-compliant levels in the eggs[2]. Follow-up studies by Kijlstra et al.[3] showed that a problem especially refers to the smaller farms and also led to a series of measures to prevent the contamination of soil and to reduce the uptake of soil. In particular for organic farming systems, the foraging outside is a prerequisite. A strict monitoring program was installed in Germany for eggs sold in this country, including those imported from other countries (so-called KAT-program). Eggs are regularly tested, the frequency depending on the observed levels. Until recently this program only focused on dioxins but now also dioxin-like PCBs are included. This may explain the frequent problems, both in Germany and the Netherlands, with eggs that are compliant for dioxins but not for the sum of dioxins and dl-PCBs. The question arises what the source for the PCBs is. Previous studies by Kijlstra et al.[3] showed a relationship between dioxins in soil and eggs but for dl-PCBs this was much less clear. Similar was observed in studies in Belgium[4].

In the Netherlands a non-compliant eggs sample was discovered in the monitoring program midst of 2011 and reported through the RASFF system since the eggs were exported to Belgium. A large number of egg and soil samples were analyzed to investigate the problems at this farm and the effects of the various measures taken to reduce the levels. Beginning of 2012 there was another farm with elevated PCB-levels, initially discovered by self-control. Also on this farm a large number of samples were analyzed to reveal the source. To further investigate the relationship between intake and excretion into the eggs, another carry-over study was performed with feeds containing 10% of soil from two contaminated locations.

Materials and methods

Samples of eggs, soil and other materials were obtained from two farms in the Netherlands. Levels of dioxins, dl-PCBs and the 6 ndl-PCBs (indicator-PCBs) were analyzed by GC/HRMS as described previously[5]. TEQ-based levels are calculated using TEFs from 1998. CALUX analysis was performed as described previously, level estimated by comparison with a set of reference samples of butter fat[6].

For the hen study, soil from two locations was obtained, one being a farm with free-ranging hens, one being a dairy farm North of a MWI, known for its past problems. Feeds were prepared by mixing the feed with 10% of this soil.

Results and discussion

Carry-over study

Three feeds were prepared, one blank (A) and two that were mixed with 10% of low (B) or high (C) contaminated soil. The dioxin and dl-PCB levels in the low contaminated soil were 1.83 and 0.26 ng TEQ/g dm, in the high contaminated soil 13.73 and 1.24 ng TEQ/kg dm. Levels of ndl-PCBs were respectively 5.81 and 1.16 µg/kg. Levels in the feeds were about 10-fold lower, showing upper/lowerbound dioxin levels of 0.17/0.00, 0.25/0.20, 1.32/1.32 ng TEQ/kg, dl-PCB levels of 0.02/0.00, 0.05/0.04, 0.14/0.13 ng TEQ/kg and ndl-PCB levels of 0.6/0.0, 0.6/0.0 and 0.8/0.6 µg/kg (all based on 88% dm).

Figure 1 shows the levels of dioxins, dl-PCBs and ndl-PCBs in eggs from the hens fed with the higher contaminated feed C. Levels increase very rapidly in the beginning and then start to level off. After 3 weeks the levels for dioxins, dl-PCBs and total TEQ were respectively 7.9, 0.9 and 8.8 pg TEQ/g, that for ndl-PCBs 5.0 ng/g fat. However, prolonged exposure will result in somewhat higher levels. The levels of dioxins and total

TEQ would exceed the old EU maximum levels of 3 and 6 pg TEQ/g fat, based on TEFs 1998. The highest observed level of ndl-PCBs is 1/8 of the recently introduced limit of 40 ng/g fat.
The upperbound levels observed on day 21 in eggs of hens fed with the blank feed were respectively 0.27, 0.10 and 0.37 for dioxins, dl-PCBs and total TEQ and 0.8 ng/g fat for the ndl-PCBs. In eggs from hens fed the lower contaminated feed B, levels on day 21 were 1.55, 0.30 and 1.85 for dioxins, dl-PCBs and total TEQ and 1.6 ng/g fat for the ndl-PCBs. These levels do not exceed the limits, indicating that consumption of about 10 gram of soil with dioxin levels around 1.8 ng TEQ/kg does not present a risk. Nevertheless, in practice farms with such soil levels do produce eggs that exceed the maximum limits[x]. The question is whether this is due to specific hot spots in the backyard or the consumption of much higher amounts of soil by the hens, especially in the summer time.

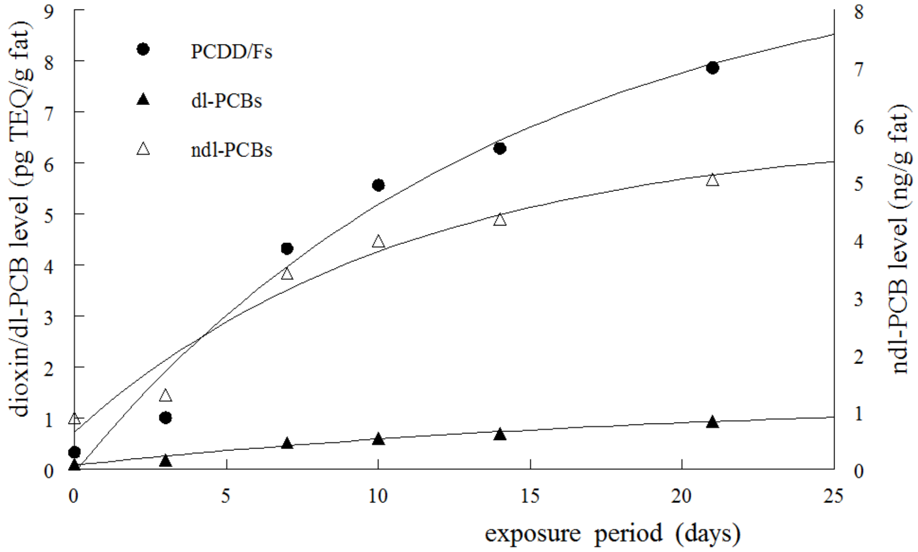

Figure 1. Levels of dioxins (PCDD/Fs, dl-PCBs and ndl-PCBs in eggs from hen fed with the feed containing the high contaminated soil.

Elevated levels in eggs from farm A
During the summer period of 2011, screening of samples with DR CALUX revealed a suspected sample. GC/HRMS analysis confirmed the finding and showed levels of 1.5, 7.4 and 8.9 pg TEQ/g fat for dioxins, dl-PCBs and total TEQ, i.e. a total TEQ level exceeding the ML due to the presence of dl-PCBs (Table 1). Also the level of ndl-PCBs was clearly elevated, being 96 ng/g fat. The eggs were derived from a small farm that also participated in the study of Kijlstra et al. and at that time showed no problems at all. Since that study, the farm was extended by building a second stable adjacent to the first one.
New samples were obtained from the farmer, this time two boxes of 10 eggs from each of the two stables. One box of each stable was analyzed with GC/HRMS showing much higher dl-PCB levels in stable 2, 2.1 versus 0.8 pg TEQ/g fat (see Table 1). The remaining eggs were tested individually with DR CALUX and showed a large variation (see Figure 2). Pooling of these individual samples and analysis by GC/HRMS showed a much higher level for stable 1, similar to that in stable 2 (second value shown in Table 1). It was decided to keep the hens inside, since also based on the large variation, soil seemed most likely to be the source.
Eggs collected 2 weeks later on 5th September showed a decline in the levels but still above the ML. Also based on analysis of litter samples inside of the stables, the interior was cleaned. Levels further declined but on 13nd September still exceeded the ML. New samples were collected on 5th October, this time also sampling the two sub-departments in each stable. This time, only one of the 4 samples was still non-compliant. Samples on 14nd October confirmed that the levels had finally declined below the existing limits. This slow decline is in agreement with results from carry-over studies with laying hens, showing initially a rapid decline but subsequently a very slow decline with a half-line around one month.

Table 1. Levels of dioxins, dl-PCBs and ndl-PCBs in egg samples from different dates after discovery of the non-compliant level. In most cases 20-30 were used to prepare the sample.

Date	Stable	PCDD/Fs (pg TEQ/g fat)	dl-PCBs (pg TEQ/g fat)	ndl-PCBs (ng/g fat)
28-jun	mix	1.5	7.4	96
12-aug	1	0.8/1.9*	3.9/10.0*	52/130*
	2	2.1/2.7*	10.2/12.0*	157/173*
24-aug	1	1.8	6.9	100
	2.1	1.5	8.6	126
	2.2	1.5	8.1	111
5-sep (hens inside)	1	1.5	6.3	83
	2	0.9	5.3	69
13-sep	1	1.6	6.7	92
	2	1.1	5.5	69
5-oct	1.1	1.1	4.8	62
	1.2	0.7	3.6	44
	2.1	0.7	3.9	48
	2.2	0.8	7.0	90
14-oct	1.1	1.0	4.6	55
	1.2	0.7	4.5	46
	2.1	0.7	4.5	50
	2.2	0.7	4.8	54

* second result based on a pooled sample of individual eggs first analyzed with CALUX (Figure 2).

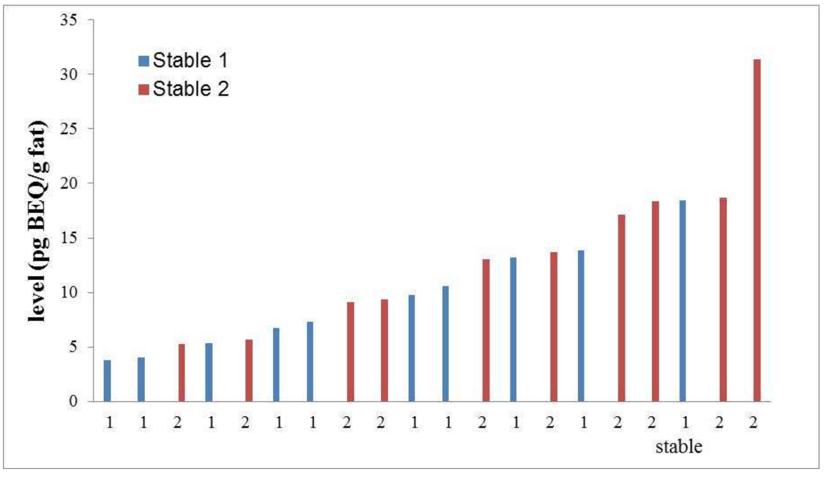

Figure 2. Levels in individual eggs from the tow stables, as estimated from the DR CALUX analysis.

Of course the major issue is the source of the contamination which seemed to be introduced after the studies of Kijlstra et al. around 2004/2005. The farm was visited several times and soil samples were collected from the barn yard and inside. Figure 3 shows all the levels measured at the various locations. Initial samples were collected at about 5-10 meters from the stables and showed in some cases some elevated levels of dl-PCBs and ndl-PCBs (parts 1.2, 2.1 and inside). At a later occasion the farmer collected samples of the far end of the barnyard (100 meters from the stables) and these samples showed much lower levels of PCBs. After the eggs declined below the limits and were sold again for consumption, another visit was paid and new samples were taken, this time closer to the stables. Especially at this part, there was a lot of building debris which was primarily used under the new stable 2 but the remaining material was spread out close to both stables to fortify the soil. These samples showed quite high levels of PCBs, the highest sample being a sample of stones collected from underneath stable 2 (level of ndl-PCBs 450 ng/g dm). Although the real source is still not entirely clear, the

question is whether the use of such old building debris, produced when demolishing old buildings, may still contain remains of e.g. old paints or sealants containing PCBs. In January 2012 another farm in the east of the Netherlands also showed increased levels of PCBs. This farm also participated in the Kijlstra study and at that time showed low levels. Quite recently, the soil on the barnyards was replaced with new soil but also building debris to fortify the soil for allowing the trucks to transport the soil.
Another investigation on farm A involves material used for building the roof. Analysis of some spare material kept at some distance from the stables and contaminated soil showed elevated dioxin and PCB-levels. The coating of the material is made from a kind of acrylate but thus far there were no indications that this material contains PCBs. Also the levels appeared too low to explain the observed soil levels. Remaining question is whether dioxins and PCBs may actually be formed from this material under certain weather conditions.

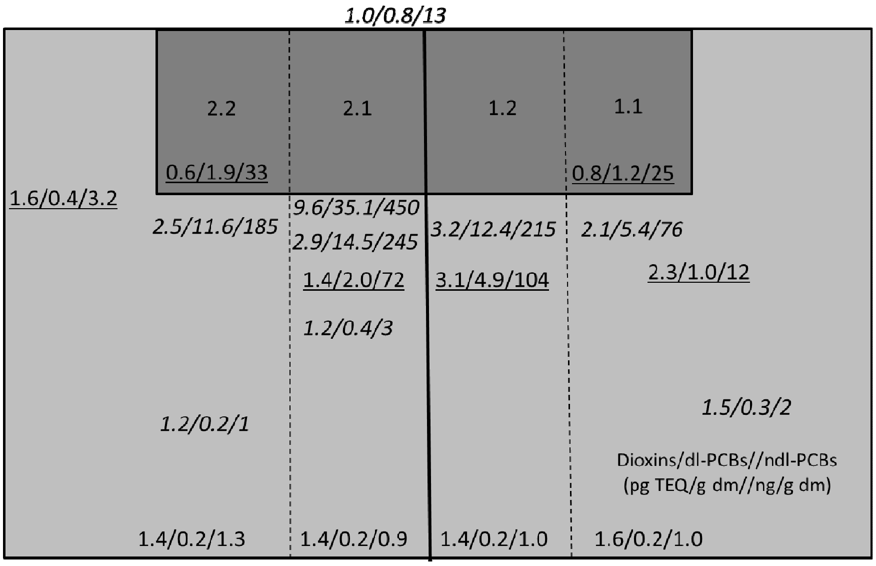

Figure 3. Levels of dioxins, dl-PCBs and ndl-PCBs in samples of soil at different positions of the barnyard and stables. Samples underlined were collected by RIKILT on 5th September, samples in italics on 10th November. The others were collected by the farmer.

Conclusions

The present paper shows that more attention should be paid to potential contamination of eggs with PCBs including dl-PCBs. It appears that these may not in all cases have a historic cause but that the problem may actually be introduced due to the use of old building materials in the barnyard. This deserves further attention in order to prevent further contamination of agricultural areas with these persistent contaminants. Another important conclusion is that in some cases, the new EU ML for ndl-PCBs (40 ng/g fat) may be more restrictive than that for the sum of dioxins and dl-PCBs, as shown in the present case.

Acknowledgements

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