RESIDUES OF DIOXINS AND COPLANAR PCBs IN EGGS OF FREE RANGE CHICKENS

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Introduction

Since the dioxin crisis in 1999 RIKILT has been involved in a number of monitoring programmes aiming at the early detection of contaminated feed and food ingredients and the elucidation of possible new sources of dioxins. In one of the programmes, at least 400 animal feeds and feed ingredients are screened by the CALUX-bioassay and samples showing a higher response than a citrus pulp sample with 400 pg TEQ/kg or a fat sample containing 5 pg TEQ/g dioxins and PCBs, are further investigated by GC/MS. In addition 5-10% of the negative samples are investigated by GC/MS. In general samples showing a positive response are fish oil and fish meal, and clay minerals.

In 2001 a new programme was started, aiming at investigating products of animal origin, like meat and eggs. Over 300 samples are screened in the CALUX-assay and suspected samples investigated by GC/MS. This part of the programme results in an estimation of the fraction of non-compliant samples. In addition samples collected in each quarter of the year are pooled per type of sample and analysed by GC/MS, in order to investigate the average background level of dioxins and co-planar PCBs in these products. Results are presented on the internet: www.rikilt.dlo.nl/dioxinen. In 2001 this resulted in the discovery that an egg obtained from a farm with free-range chickens contained dioxin levels above the current Dutch limit of 5 pg TEQ/g fat. The initial observation was followed by a focussed action on these type of eggs. The present paper will focus on the actual levels in these eggs and discuss possible sources.

Materials and methods

Samples

Egg samples, coming from a known source, were collected from packing stations throughout the Netherlands or directly from a farm. Egg fat was extracted with pentane from egg yolk, following mixing with anhydrous sodium carbonate.

CALUX analysis

An aliquot of 0.5 g fat was mixed with hexane and extracted on acid silica as described previously[1]. In each test series a blanc butter fat sample and butter fat samples containing 2, 5 and 7 pg TEQ/g fat were included. The response obtained with the butter fat sample of 5 pg TEQ/g, containing 2.7 pg TEQ/g dioxins and 2.3 pg TEQ/g non-ortho PCBs, was used as the reference signal. Samples showing a lower response were declared negative, samples showing a higher response suspected.

Dioxin analysis

Dioxins, non-ortho and mono-ortho PCBs were analysed following clean-up of the fat by GPC, aluminium oxide and carbon as described by Tuinstra *et al* 1994. The carbon-eluate containing the mono-ortho PCBs was analysed separately from the dioxins and non-ortho PCBs.

Results and Discussion

Dioxins in eggs

In 2001 a total of 57 egg samples was screened with the CALUX-assay. Two of these eggs (3.5 %) showed a positive response and were shown to contain dioxin levels of respectively 5.0 and 7.7 pg TEQ/g fat, and total TEQ levels of 5.5 and 11.3 pg TEQ/g. The latter sample was traced back to a small farm with free ranging chickens, which sold its eggs locally. Table 1 shows the levels of dioxins and dioxin-like PCBs in this sample (egg 1). For comparison, dioxin levels determined in 4 pool samples prepared from the 57 egg samples varied between 0.5 and 0.7 pg TEQ/g, and total TEQ levels between 0.8 and 1.5 pg TEQ/g.

Since this observation indicated that many more farms with free-range chickens might produce eggs with dioxin levels over the residue limit, 17 farms were selected and visited. Eight of these farms, termed free-range, allowed but didn't force their chickens to go outside. As a result, these chickens forage outside for only a limited part of the day. Nine other farms were so-called organic farms, forcing their chickens to forage outside the stable for at least eight hours per day. Screening of the 17 egg samples, prepared from 6 eggs/farm, revealed 6 positive samples. All these samples came from organic farms. In general, the samples from this type of farm showed the nine highest CALUX responses. The six positive samples were analyzed by GC/MS (Table 1, eggs 2-7) and showed that 1 sample had a dioxin level above the Dutch level of 5 pg TEQ/g, and 2 more samples a level at or above the EU limit of 3 pg TEQ/g. Total TEQ levels varied between 2.9 and 10.5, showing that dioxin-like PCBs may contribute significantly to the residue levels.

Source identification

The first farm was subsequently resampled and 20 different eggs were first screened with CALUX, showing that 75 % of the samples were suspected. The samples were randomly pooled per 5 eggs and analysed by GC/MS, revealing dioxin levels of 3.0 (4 CALUX-negative eggs), 3.6 (1 CALUX-negative egg), 5.8 and 6.7 pg TEQ/g and total TEQ levels of 5.0, 5.9, 8.9 and 9.2 pg TEQ/g. At the same time 5 chickens were slaughtered and abdominal fat collected. One of the samples showed a slight positive response in the CALUX assay. A pool sample of the 5 chicken fat samples was analysed by GC/MS and shown to contain 0.9 pg TEQ dioxins/g and 3.1 pg total TEQ/g, i.e. much lower than the egg samples.

Samples of soil and straw collected outside and inside the stable contained dioxin levels of 1.1 and 1.6 ng TEQ/kg and total TEQ levels of 1.3 and 3.0 ng TEQ/kg. Water samples did not contain significant levels. Feed samples were negative in the CALUX-assay, i.e. lower than 0.4 ng TEQ/kg. Furthermore, similar feed is used in farms without free-ranging chickens and as such seems unlikely to contribute more than 1 pg TEQ/g fat. Therefore, it seems likely that the source of the contamination is outside the stable and possibly related to the ingestion of contaminated soil. However, assuming a complete transfer of dioxins to the egg and a fat content of 6 gram per egg, a total of at least 60 pg total TEQ should be ingested per day, or at least 46 gram of the soil contaminated at 1.3 ng TEQ/kg. Therefore, it cannot be excluded that worms or insects, accumulating dioxins, may play a role in the contamination. Similar has been suggested by other

Investigators, like Shuler *et al.* (1997), who performed a field survey on chickens from five farms[2]. Soil samples, taken from a depth of 0-10 cm were analysed and shown to contain 11 (A), 13 (B), 1.8 (C), 1.3 (D) and 1.4 (E) ng I-TEQ/kg dry weight. Corresponding levels in single eggs were 3.1 and 6.1 (A), 19 and 12 (B), 4.6 and 2.3 (C), 6.1 (D) and 3.5 (E) pg I-TEQ/g fat. The relatively low levels in eggs from farm A were hypothesised to be due to the high density of chickens on this particular farm as compared to the other farms, resulting in the disappearance of soil organisms. Harnly *et al.* (2000)

Table 1. Dioxin levels in a number of egg samples collected from farms with free-range chickens

congener	egg 1	egg 2	egg 3	egg 4	egg 5	egg 6	egg 7
2,3,7,8-TCDF	1.12	1.68	2.71	0.63	2.84	1.03	1.26
1,2,3,7,8-PeCDF	0.48	1.17	2.65	0.28	2.46	**	1.13
2,3,4,7,8-PeCDF	0.77	1.51	2.99	0.44	3.56	1.11	1.33
1,2,3,4,7,8-HxCDF	1.02	1.72	2.54	0.35	.78	1.06	1.12
1,2,3,6,7,8-HxCDF0.54	1.24	1.93	0.26	2.88	0.77	0.81	
2,3,4,6,7,8-HxCDF	0.80	1.71	2.11	0.36	3.98	0.95	0.86
1,2,3,7,8,9-HxCDF	<0.10	<0.10	0.71	<0.10	0.15	<0.10	0.16
1,2,3,4,6,7,8-HpCDF	9.46	14.80	6.63	1.58	27.70	3.66	1.39
1,2,3,4,7,8,9-HpCDF	<0.25	0.29	0.38	<0.25	0.62	0.17	<0.25
OCDF	3.07	.55	4.40	0.68	6.62	5.15	0.65
2,3,7,8-TCDD	3.86	0.24	0.40	0.07	0.55	0.34	0.15
1,2,3,7,8-PeCDD	2.29	0.65	1.00	0.12	2.00	0.40	0.59
1,2,3,4,7,8-HxCDD	2.93	0.82	1.21	0.16	2.64	0.34	0.52
1,2,3,6,7,8-HxCDD	2.03	2.50	2.84	0.39	11.50	1.04	1.50
1,2,3,7,8,9-HxCDD	0.63	0.74	1.20	0.20	3.15	0.40	0.53
1,2,3,4,6,7,8-HpCDD	9.76	10.50	11.20	2.19	40.90	4.26	3.48
OCDD	39.40	20.50	35.00	8.51	74.50	33.70	5.58
Total (pg TEQ/g)	**7.66**	**3.01**	**4.74**	**0.70**	**8.25**	**1.95**	**2.18**
PCB 77	146.8	2.7	2.7	1.2	1.73	3.3	1.1
PCB 81	1988.3	36.9	47.5	16.1	21.7	23.3	12.8
PCB 126	22.7	9.7	50.0	44.6	15.9	15.5	5.9
PCB 169	1.0	1.4	8.6	6.2	2.82	1.2	0.9
Total (pg TEQ/g)	**2.49**	**0.98**	**5.09**	**4.53**	**1.62**	**1.57**	**0.60**
PCB 105	120	160	20	<10	20	10	<10
PCB 114	5090	1580	1770	850	1090	1110	430
PCB 118	170	20	20	<10	10	20	<10
PCB 123	2370	440	410	160	310	350	130
PCB 156	190	260	420	280	240	90	40
PCB 157	470	540	740	430	440	180	80
PCB 167	100	70	120	70	70	40	20
PCB 189	30	80	140	70	70	20	<10
Total (pg TEQ/g)	**1.13**	**0.54**	**0.67**	**0.36**	**0.41**	**0.26**	**0.10**
Total (pg TEQ/g)	**11.29**	**4.53**	**10.50**	**5.59**	**10.28**	**3.78**	**2.88**

described a more controlled study on a contaminated area close to a PCP wood treatment facility in Oroville[3]. Soil concentrations of around 6 ng I-TEQ/kg (range 1.5-46) resulted in egg levels of 20-50 pg I-TEQ/g fat (range 0.8-140). The fraction of eggs exceeding 10 pg I-TEQ/g was around 70-90 %. Modelling of the data indicated that for confined chickens, a soil level of 2.7 ng TEQ/kg was required to result in an egg level of 10 pg I-TEQ/g fat. However, a soil level of only 0.4 ng TEQ/kg was required for chickens that had a larger area to forage. Again, this suggests a role for worms and insects, since their presence is more likely at lower densities. In general these studies confirm that very low soil levels already result in high levels in eggs. At the same time these data suggest that the new EU feed

limit of 0.75 ng TEQ/kg may be too high to ensure that levels in eggs are lower than the limit of 3 pg TEQ/g.

Regulatory measures

An exposure assessment on eggs containing 10 pg TEQ/g fat showed that a daily consumption of one egg would result in an additional intake of 0.92 pg TEQ/kg bw. Since the intake of dioxins by the average consumer in The Netherlands from other sources is 1.14 pg TEQ/kg bw, the total exposure would be around the pTWI of 14 pg TEQ/kg bw/week. In this regard, it seems unlikely that these kind of eggs would be mixed with lower contaminated "battery" eggs. Therefore the first farm was visited by the Food Inspection and prohibited to sell its eggs. Similar was true for the other farm showing an elevated level. Subsequently, a large number of other "organic" farms were visited and sampled, revealing that the problem was of a more general nature. However, based on the new European legislation, making an exception for eggs from free-range chickens until 1st of January 2004, it was decided to lift the ban unless levels would result in an unacceptable high exposure of the consumer. At the same time it was decided to start additional research.

Conclusion and follow-up studies

The present study indicates that it may be very difficult to obtain eggs from free-ranging chickens with dioxin levels below the new European limit of 3 pg TEQ/g fat. This is based on the fact that the source of the contamination appears to be the soil, and the fact that the contamination of the soil on the first farm was relatively low when compared to the average contamination in the Netherlands. Future studies will be directed towards a better understanding of the relationship between intake of dioxins through feed or soil and dioxin levels in eggs. Furthermore, the possible role of intermediates like earthworms will be studied. Last but not least possible ways to interfere with either soil contamination or the absorption of dioxins from soil will be studied.

Reference

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