A NOVEL SOURCE FOR DIOXINS PRESENT IN WASTE FAT FROM GELATIN PRODUCTION

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Introduction

During the last decade a number of dioxin related incidents have occurred in the feed and food chain. This started with the Brazilian citrus pulp, followed by the Belgian incident with PCBs and within that same year the discovery of elevated levels of dioxins in kaolinic clay used for animal feed. As a result monitoring programs in the Netherlands and other European countries were intensified. Subsequently, a number of other incidents occurred like those with cholin chloride, dried bakery waste and kaolinic clay used for selection of potatoes. These smaller incidents, although not resulting in a direct risk for the consumer, show that there are still a number of different sources for dioxins, which may prevent a further reduction of human exposure. Early identification will help to eliminate these sources. The present paper describes the most recent incident in the Netherlands, dealing with a very novel source that is still not fully disclosed. The incident with waste fat used for animal feed was first detected with the DR CALUX® assay and subsequently confirmed by the HRGC/HRMS reference method. The feed was primarily given to pigs and resulted in slightly elevated levels in these animals.

Materials and methods

Sampling

Sampling of feed, fat and animal products was performed at feed production plants, farms and slaughterhouses, and was performed by the Dutch Food and Consumer Product Safety Authority (VWA).

DR CALUX®

The DR CALUX® bioassay was applied on feed and animal fat as described previously[1]. Samples were tested in single and the response compared to a set of reference samples (screening approach). Samples were suspected if higher that 0.5 pg TEQ/g (pig fat), 1.5 pg TEQ/g (feed fat), 0.5 ng TEQ/kg (feed) or 2 pg TEQ/g (chicken).

HRGC/HRMS

The HRGC/HRMS method as described previously[2] but using a clean-up with the FMS system.

Results and Discussion

In the Netherlands, regular monitoring programs are carried out on feed and feed ingredients, sometimes extended by surveys, as was the case with the current incident. The survey dealt with fats and other ingredients reused for animal feed. Samples were screened with the DR CALUX® bioassay and if suspected analyzed with the HRGC/HRMS reference method. This resulted in the discovery of the incident, dealing with waste fat used by a feed company for the production of animal feed. Table 1 shows the results of the initial screening with the bioassay, demonstrating that an incident like this is easily detected. Subsequent analysis by HRGC/HRMS revealed a level of 50 pg TEQ/g fat with a very peculiar congener pattern, as shown in Figure 1. Additional sampling at the feed company eventually resulted in two fat samples with even higher levels, being 220 and 440 pg TEQ/g fat. The fats were reported to have been used at 1.5 to 5% in the feed, potentially resulting in feed levels up to 22 ng TEQ/kg. The fatty acid pattern of the fat confirmed that the fat was derived from pigs.

Analysis of feed samples resulted in a highest level of 8.4 ng TEQ/kg feed. Initially it could not be excluded that the fat was derived from pigs that had been exposed to dioxins at very high levels. This was supported by the fact that further HRGC/HRMS studies showed that the non-2,3,7,8 substituted dioxin congeners were hardly present in the fat (<5% of the 2,3,7,8-substituted congeners), with the exception of a compound with identical mass as the PCDFs.

Table 1. Result of the screening of the first fat sample (number 200163193; 50 ng TEQ/kg) with the DR CALUX® bioassay, showing a clearly elevated response. At RIKILT the response of the sample extract, tested in triplicate, is compared with that of a set of reference samples.

RIKILTnumber	Product	Signal (RLUs)						Decision
		1	2	3	mean	SD	Difference	
Blank fat		52	74	75	67	13	**0**	
Reference 1	0.38 pg TEQ/g	74	73	78	75	2	8	
Reference 2	0.75 pg TEQ/g	107	104	95	102	6	35	
Reference 3	1.5 pg TEQ/g	182	161	160	168	13	101	
Reference 4	3.0 pg TEQ/g	214	286	237	246	37	179	
200163193	Animal fat	888	1030	905	941	77	874	Suspected

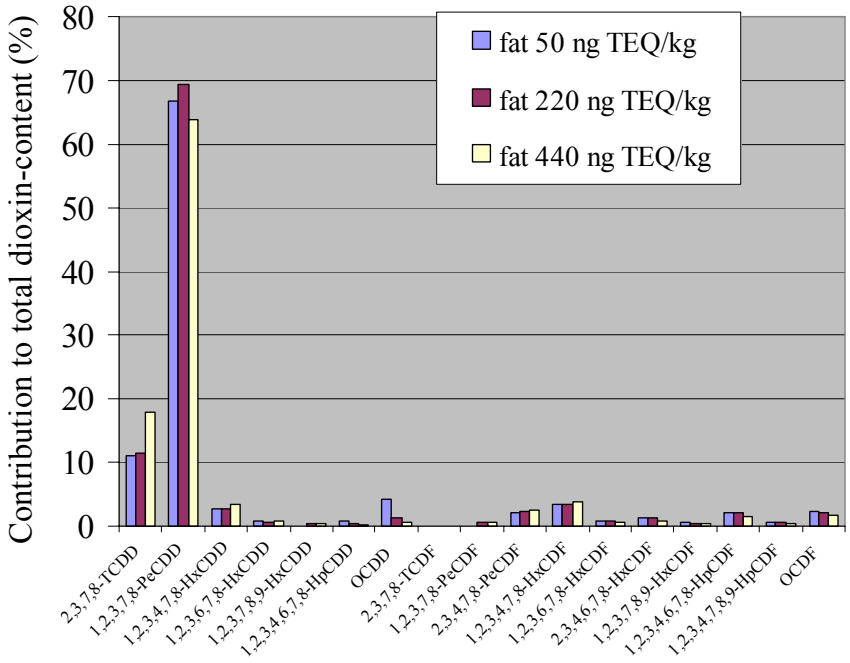

Figure 1. Congener patterns of the 3 most contaminated fat samples, expressed in absolute amounts.

It is well-known that dioxin sources, like e.g. contaminated clay, do contain such non-2,3,7,8 substituted congeners. However these congeners are readily metabolized by animals and humans, unlike the 2,3,7,8-substituted ones, thus resulting in a kind of biological filtering and selection of the 2,3,7,8-substituted congeners. The pattern in the fat, dominated by TCDD and PeCDD in a relative contribution of 1 to 6, was not recognized from the literature or by any other expert in the field. Additional studies of the fat with GC/TOF-MS did not provide a clear source of the dioxins, although some chlorinated phenols and several PAHs were detected.

Tracking and tracing showed that the fat was delivered to the Dutch feed company by a Belgium fat recycler. Follow-up studies by the Belgian FAVV revealed that the fat was a waste product derived from the production of gelatin. The source of the dioxins was subsequently disclosed as being the hydrochloric acid (HCl) used for dissolving the pig bones used for the gelatin production. The HCl was derived from the production of chemicals but was routinely filtered. A malfunction of the filters had caused the contamination. At present it is still unknown which chemical production process results in the very specific synthesis of 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD.

Impact of the incident

The contaminated fat was primarily used for the production of pig feed. Based on worst-case assumptions, being the mixing of the highest contaminated fat of 440 ng TEQ/kg at 5% into pig feed and feeding this material for 8 weeks to the animals just before slaughter, it was estimated that the levels in the pig fat could be as high as 40 pg TEQ/g fat. The 8 weeks was based on the time length between the use of contaminated fat for the production of feed and the confirmation of the analysis result. A pharmacokinetic model for pigs, developed at RIVM, was used for these calculations. In practice the highest level detected in feed was 8.4 ng TEQ/kg. This can be explained by the fact that according to the feed company, fat was also mixed at less than 5% into feed. Highest levels detected in pig fat were around 3 pg TEQ/g fat and in general were just above the limit of 1 pg TEQ/g fat. Most samples, were however clearly above the normal background levels of 0.1 pg TEQ/g and showed the typical congener pattern observed in the fat and feed. The difference between predicted worst-case levels and observed levels can easily be explained by a more realistic scenario, being the feeding of the highest contaminated feed of 8.4 ng TEQ/kg for a period of 2 weeks followed by a period on clean feed of e.g. 6 weeks prior to the sampling at slaughter (Figure 2, Table 2).

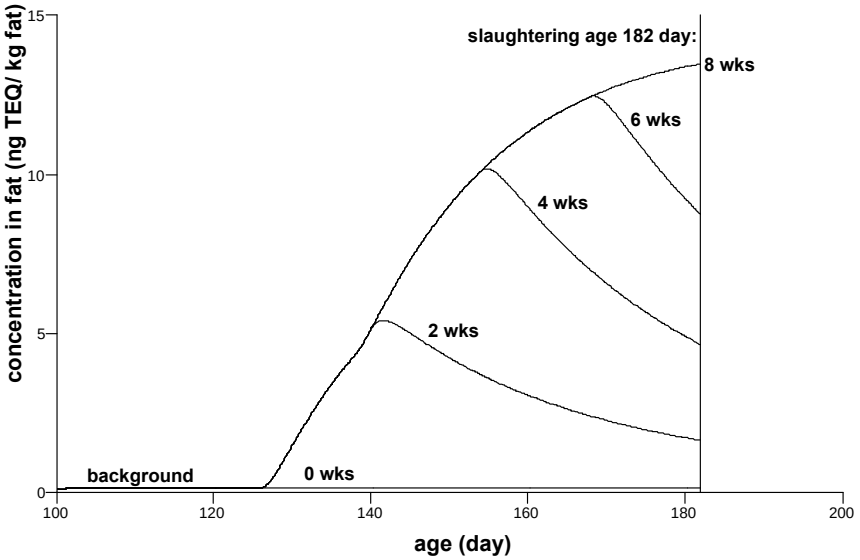

Figure 2. Concentration in fat of pigs exposed from age 126 days to feed with 8.4 ng TEQ/ kg for 0 (background), 2, 4, 6 and 8 weeks, followed by feed at background levels (0.1 ng TEQ/kg feed) for respectively 8, 6, 4, 2 and 0 weeks before being slaughtered at the age of 182 days. Calculated concentration time course (2, 4, 6, 8 weeks) is bent at day 138 because of the higher intake (2.8 kg feed/day) of the finisher diet as compared to the grower diet (2.1 kg feed/ day).

Tabel 2. Dioxin levels at the day of slaughter for the 5 different exposure scenarios in Figure 2.

	#weeks from age 126 day				
	0	2	4	6	8
Level (ng TEQ/kg fat)	0.2	1.7	4.7	8.7	13.5

Screening and confirmation of samples

Initially the analysis was focussed on a number of different fat and feed samples, in order to trace down the spreading of the contamination. Based on these data many samples of pig fat were examined by DR CALUX® and HRGC/HRMS. In order to save time, the latter analysis was not limited to suspected samples. Overall 218 samples of feed (42), animal fat for feed (12), pig fat (161) and chicken (3) were tested with the DR CALUX®

assay. Of these samples, 78 tested suspected and 140 negative. All suspected samples were analyzed by HRGC/HRMS, and 80 of the negative samples. Table 3 compares the DR CALUX® and HRGC/HRMS data based on the conclusion from the reference method. In the latter case, samples were declared positive if the levels were above the tolerance limit extended with the measurement uncertainty of the method. For feed e.g., the tolerance limit is 0.75 ng TEQ/kg but with measurement uncertainty this amounted to 1 ng TEQ/kg. Table 3 also includes the results without measurement uncertainty (between brackets). Our test strategy is to identify with DR CALUX® all samples that may exceed the action limit. This eventually results in two types of false negatives and true positives. Type 2 positive samples refer to samples tested as suspected and declared positive based on the GC/MS analysis. Type 1 positives are samples tested suspected and exceeding the action limit but not the tolerance limit. In the case of negative samples, type 2 samples are those declared negative with DR CALUX® but above the tolerance limit with GC/MS. According to the guidelines this figure should be below 1%. Type 2 results are samples declared negative but exceeding the action limit.

Table 3. Comparison of DR CALUX® and GC/MS based on the interpretation of the result. Decision based on limits including or without (between brackets) the measurement uncertainty for GC/MS.

DR CALUX®	Negative samples (n=80)			Suspected samples (n=78)		
GC/MS	True negative	False negative type 1*	False negative type 2*	False positive	True positive type 1*	True positive type 2*
With measurement uncertainty	75 (68)	5 (11)	0 (1)	16 (9)	26 (23)	36 (46)

* type 1: GC/MS result between action limit and tolerance limit; type 2 GC/MS result above tolerance limit

From Table 3 it is evident that 16 real false positive results were obtained but no false negatives. A relatively large number of samples (26) turned out to exceed the action limit but not the tolerance limit (type 1). Another 5 samples, screened as negative, exceeded the action limit but not the tolerance limit. Overall 75 of the 80 samples were tested correctly negative and 5 of the negative samples exceeded the action limit but not the tolerance limit. Similarly 62 samples were declared suspected and did exceed the action limit, whereas 21% (16) of the 78 suspected samples or 7% of the total number of samples were false positive. This seems to be a rather acceptable result for a screening assay. Overall this implies that the use of the assay was very suitable for selecting the suspected samples and filtering out negative samples that did not require GC/MS analysis.

Conclusion

The present incident shows once again that novel sources and pathways for contamination of the food chain with dioxins may occur. This requires proper evaluation of production processes in the food and feed chain, starting with the control of all products used for feed and food production. The reuse of chemicals and waste materials remains a high risk factor, as shown in most of the incidents during the last 8 years.

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