SURVEY OF DIOXINS AND DIOXIN-LIKE COMPOUNDS IN ANIMAL FEED IN POLAND

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Introduction

The primary route of exposure to dioxin-like compounds for the general population is through the consumption of food of animal origin comprising above 90% of the total dioxin exposure in Europe. Because the feed contamination is a major route through which livestock is exposed to dioxins, to ensure consumer protection the EU established maximum dioxin levels in animal feed and different raw components. Besides setting the limits on the levels of dioxins in feedstuffs, EU has implemented monitoring systems. The EU directives[1] prescribing maximum permitted levels for dioxins in feed were implemented into Polish law[2].

National Veterinary Research Institute in Pulawy, Poland (NVRI) has completed a survey of dioxin-like compounds (including 17 dioxin and furans PCDD/PCDF and 12 dioxin-like PCB (dl-PCB) congeners) in mixed feeds and feed components from feed facilities around the Poland, sampling the overall mixture and the major and minor feed components. The survey was carried out to gain a representative overview of the actual dioxin levels in feedingstuffs and to describe the present situation.
The presented study were conducted in the years 2004-2007 in the frame of national monitoring program.

Materials and Methods

Since 2004, new Polish dioxin laboratory in National Veterinary Research Institute has surveyed the PCDD and PCDF levels in feed and from 2007 dl-PCB content was also analyzed. In this period over 600 samples were analyzed in the framework of the official dioxin surveillance. The samples were taken in a representative way by veterinary inspectors. The collected samples were grinded and the moisture content was determined.
Two accredited methods of dioxin analysis were used. Bioanalysis (screening method) was carried out with the XDS CALUX bioassay and HRGC/HRMS was used for confirmation of suspected samples. Both methods were validated and the requirements of dioxin and dl-PCB analysis laid dawn in the Commission Directive 2002/70[3] and Commission Regulation 1883/2006[4] were fulfilled.

Screening method. The in vitro assay is based on the ability of dioxin and related chemicals to activate the Ah receptor (AhR), a chemical–responsive DNA binding protein that is responsible for producing the toxic effects of these chemicals. Measurement of the activation level of AhR-depended gene expression by a chemical extract with resulting values expressed as TEQs. Fat extraction was done by toluene/methanol and toluene. Clean up and fractionation was performed using silica/sulfuric acid column and XCARB column. Samples extracts, standards and quality control samples were applied to genetically engineered cell on micro plates and induction of luciferase activity was quantified. Quality assurance was done by double sample determination, using reagent blank, spiked samples, certified and by natural contaminated control samples and control charts. Method was validated and uncertainty was estimated. Number of he false positive results was about 8% and no false negatives were found. The precision of the method was acceptable with CV < 30%, suggested for used cell based bioassays in compliance with the Commission Regulation 1883/2006.

HRGC/HRMS analysis. Analytical methodology has been previously described[5]. Feedstuffs were analyzed according to method using gas chromatography coupled to high resolution mass spectrometry. In brief, analytes were extracted with pressurized liquid extraction, and cleaned by multi-step procedure on acidic silica, Florisil and Carbopack C columns. PCDD/PCDF, non-orto PCBs and mono-orto PCBs fractions were analyzed on MAT 95XP (Thermo Scientific) with DB-5MS column. For quantification of analytes isotope dilution mass spectrometry was used. Method was comprehensively validated and expanded uncertainty was estimated at the level of interest.

Result and discussion
The results of the national monitoring have shown that feedingstuffs in Poland have dioxin contents and dioxin-like PCBs far below the maximum levels permitted in the European Union, with exception of fish meal samples. Overview of obtained results is presented on consecutive table. All values were recalculated to a feed moisture content of 12%. In general level of PCDD/PCDF in feed materials of plant and mineral origin, vegetable oils and milk powder was much below permitted levels (Table 1).

Out of 616 tested samples, to which a statutory maximum level applies, 21 fish meal samples (3% of all tested samples) gave cause for noncompliant (Table 2). PCDD/PCDF level was above permitted concentration in 21 samples and it was confirmed by HRGC/HRMS. PCDD/PCDF and dl-PCB level in contaminated fish meal samples is presented on table 3.

Application of screening method, as the first step of analysis, allowed us to find 87% of compliant samples and only 78 different feed were analyzed by HRGC/HRMS. Twenty one of them (fish meal) were confirmed by HRGC/HRMS as noncompliant (as mentioned above) (Table 3). The profile of toxic dioxin and furan congeners was similar to dioxin profile in Baltic herrings described in our previous report[5] (Figure 1).

Application screening method, specifically designed to separate compliant and non-compliant samples and avoid false compliant results, allowed the pre-selection of samples suspected of being contaminated above limit values and led to reduced total costs of the monitoring program.
In general, obtained results showed no serious risk from dioxins presence in examined feed, but concluded that high concentrations dioxins in fish meal seems to be reasonably important dioxins source. In the future, national monitoring programs should take into consideration extending official control for fish meals as a potent dioxin source for farmed animals. Because the human exposure for dioxins comes mainly from contaminated food of animal origin it is necessary to continue the feedstuff control for the presence of this chemicals.

Acknowledgements
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References:
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Table 1: PCDD/PCDF levels in feed and feed components (ng WHO-TEQ/kg of feed with moisture content 12%)

Product	n	Range	Maximum content[1]
Compound feedingstuffs	386	0.02-0.37	0.75
Fish meal	128	0.27-0.75*	1.25
Feed materials of plant origin	46	0.02-0.12	0.75
Vegetable oils	15	0.04-0.23	0.75
Feed for fish, pet food	15	0.08-0.20	2.25
Milk powder	80	0.03-0.30	0.75
Fish oil	15	0.05-2.80	6.0
Feed materials of mineral origin	38	0.03-0.53	1.0
* except the samples exceeding the maximal allowed content			

Table 2: Number of non-compliant feed samples

Year	Number of samples analyzed (in %)				
	tested	CALUX positive	HRGC/HRMS confirmed	false positive	false negative
2004	204 (100)	25 (12)	14 (7)	11 (26)	0
2005	174 (100)	19 (11)	2 (1)	17 (10)	
2006	150 (100)	31 (21)	3 (2)	28 (19)	
2007	88 (100)	3 (3)	2 (2)	1 (1)	
Total	616 (100)	78 (13)	21 (3)	47 (8)	

Table 3: Fish meal. Number of samples and dioxin level above the maximum content (1.25 ng WHO-PCDD/F-TEQ/kg and 4.5 ng WHO-PCDD/F-PCB-TEQ/kg)

Year	n	PCDD/ PCDF		∑ PCDD/PCDF/dl-PCB	
		HRGC/HRMS confirmed (%)	range ng WHO-TEQ/kg	HRGC/HRMS confirmed	range ng WHO-TEQ/kg
2004	38	13 (34%)	1.25-3.53	nt*	-
2005	37	2 (5%)	1.41-1.56	nt*	-
2006	35	3 (9%)	2.99-3.65	nt*	-
2007	18	2 (2%)	2.77-3.24	2 (2%)	5.33-5.95
* not tested					

Figure 1: Fish meal. PCDD/PCDF profile