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Evaluation of PCDD/Fs characterization in animal feed and feed additives

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Abstract

Safety control of feed and feed additives is necessary to have safe food of animal origin. Based on media reports, nine incidents regarding dioxins and/or PCBs contaminations occurred worldwide during the last decade. Korea is a country which imports feed and feed additives. In this study, various kinds of feed and feed additives were analyzed to monitor the contamination level of dioxins. The level of PCDD/Fs in fish oil was the highest with a concentration of 23.33 ng kg^{-1}, which is equivalent to a toxicological concentration of 4.68 ng WHO-TEQ/kg. Feed from animals origin such as chicken meal, animal fat, fish meal, fish oil, and shell powder showed relatively higher concentrations of PCDD/Fs. Feed from plants origin, minerals, and additives ranged from non-detects for bit pulp and ethoxyquin to 8.28 ng kg^{-1} for DL-methionine. From a toxicological point of view, the highest concentration in vitamins was 0.08 ng WHO-TEQ/kg among the feed additives. 2,3,4,7,8-PeCDF was the dominant congener in samples of fish oil, fish meal, and shell powder. Animal fat showed that the pattern of PCDD/Fs depends on the sources of contamination. A sample of animal fat showed 1,2,3,4,7,8-HxCDF and the other sample showed 1,2,3,4,7,8-HxCDD as a primary congener. Generally, low levels of PCDDs were detected in feed additives. Patterns of PCDD/Fs in choline chloride were different with that in choline chloride from an incident in Europe in 2000.

Keywords: PCDD/Fs; Feed; Feed additives; Fish oil; Fish meal; Animal fat

1. Introduction

Food safety is a high priority in almost all countries. Food from animal origin has been contaminated with dioxins, polychlorinated dibenzo-*p*-dioxins, and polychlorinated dibenzofurans (PCDD/Fs), through contaminated animal feed in the USA and in Europe during the last decade. Intensive studies showed that animal feed/feed additives are one of the major sources of contamination. In 1996, contaminated ball clay from a mine in the southern USA was used as an anti-caking agent for chicken feed (Hayward et al., 1999; Ferrario et al., 2004). In 1997, contaminated citrus pulp from Brazil was used as feed material for ruminants in Germany. This citrus pulp, containing about 5–10 ng I-TEQ/kg of PCDD/Fs, was distributed in the global market. The main source of PCDD/Fs was a highly contaminated lime (with 2.5 million pg I-TEQ/kg) which was added to the wet peel to neutralize the citrus pulp (Malisch, 2000). During the last decade, the biggest incident was contamination of Belgian food in 1999. Polychlorinated biphenyls (PCBs) in animal fat were involved in this incident as well as PCDD/Fs, which were produced by age or heat degraded PCBs (Bernard et al., 2002; Covaci et al., 2002). In 2000, German authorities found PCDD/Fs in a choline chloride premix (manufactured in Belgium) which was processed into final products and distributed in Spain. The source of dioxin contamination was PCP-contaminated sawdust which carried choline chloride (Llerena et al., 2003). Two different incidents involved mineral supplements in the USA from 2002 to 2003. Carbosan

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Table 1
History of dioxin contaminations in animal feed and feed additives

Year	Source of contamination	Country	References
1996	Ball clay as anti-caking agent	USA	Hayward et al. (1999) Ferrario et al. (2004)
1998	Lime as neutralization for citrus pulp	Germany, Brazil	Malisch (2000)
1999	Waste oil with PCBs	Belgium	Bernard et al. (2002) Covaci et al. (2002)
2000	Choline chloride	Germany, Belgium, Spain	Llerena et al. (2003)
2002	Carbosan copper	UK, USA	FSA (2002)
2003	Zinc oxide & copper oxide	USA, Canada	FDA (2003)
2003	Dried bread waste	Germany, the Netherlands	Hoogenboom et al. (2004)
2004	Clay for potato sorting	the Netherlands, Belgium, Germany	MANF (2004)
2005	Gelatine, Hydrochloric acid	Belgium, the Netherlands	AFSCA (2006) MANF (2006)

copper from a company in the USA was contaminated with dioxin that was used in the UK for cattle feed, principally for dairy animals (FSA, 2002). Contaminated copper oxide and zinc oxide were also used in livestock, aquaculture, and poultry feed. The FDA of the USA suggested that the sources of dioxin were by-products or co-products of industrial metal production (FDA, 2003). In 2003, bakery waste used as animal feed was contaminated by dioxin from waste wood that was used to dry bakery waste in Germany. A Dutch company used the bakery waste to produce feed for pigs, cattle, poultry, and duck husbandry (Hoogenboom et al., 2004). In the Netherlands in 2004, clay used in potato sorting caused dioxin contamination in cattle, pig, sheep, and goat feed (MANF, 2004). The contaminated potato feed was exported to Belgium and Germany. The clay originated from Germany. The latest incident was found by regular tests in a feed company in the Netherlands in 2005. Contaminated feed originated from Belgium. A technical error occurred during gelatin production. Gelatin was made with dioxin contaminated by spirit of salt, hydrochloric acid (AFSCA, 2006; MANF, 2006). The nine reported incidents are organized in Table 1.

Currently, Korea imports 75% of its animal feed and feed additives. A survey is needed for systematic monitoring in the future. This study presents the survey results of PCDD/Fs in animal feed and feed additives used in Korea. Environmental contaminants such as PCDD/Fs, PCBs, toxic metals, and toxins are a global problem and must be monitored in each country. Hazard Analysis Critical Control Point (HACCP) should be used in the production of animal feed (den Hartog, 2003). The levels of PCDD/Fs in feed and feed additives must be thoroughly assessed in order to find strategic ways to ensure animal feed safety. Many surveys or monitoring would aid assessment of feed safety.

2. Materials and methods

Samples were provided from producers or distributors of animal feed and feed additives in Korea. There were 32 samples, including seven different samples of fish meal, two samples of animal fats, shell powder, calcite, choline chloride, sodium bicarbonate, calcium phosphate, and others. These were collected between February and August 2004. An automatic shaker extracted the oily samples for a two-hour period using hexane and acidic silica. The other samples were extracted using a Soxhlet extractor for 18 h with toluene/acetone (9:1, v/v) (Abad et al., 2002). Before extraction, ^{13}C-PCDD/Fs were spiked into the samples as internal standards. An isotope dilution method based on USA EPA method 1613B was used for analysis. Clean-up was performed by silica, alumina, and carbon columns using a Power-Prep™ (Fluid Management Systems, Waltham, MA, USA) automated column procedure. The sample was purified by elution from the silica and alumina columns with 90 ml of hexane and 60 ml of 2% methylene chloride/hexane followed by elution from the silica, alumina and carbon columns with 120 ml of 50% methylene chloride/hexane. Ethyl acetate/benzene (4 ml, 1:1, v/v) was then used to elute the sample from the carbon column followed by 10 ml of hexane. The final fraction containing PCDD/Fs was collected by reverse elution of the carbon column with 80 ml of toluene. The eluate was concentrated until almost dry and re-dissolved in 40 μl of nonane and 10 μl of ^{13}C-labeled 1,2,3,4-TCDD and ^{13}C-labeled 1,2,3,7,8,9-HxCDD (typically 20 ng ml^{-1}) were added as the internal standards. The extract was analyzed by a HR-GC/MS (Autospec Ultima, Micromass Co., UK) equipped with a DB5MS capillary column (50 m × 0.25 mm I.D., 025 μm film thickness, J&W Scientific, USA). The GC oven temperature was held for 1 min at 180 °C. Next, the temperature was increased to 240 °C at 30 °C/min and held for 18 min and followed by an increase to a final temperature of 290 °C at a rate of 5 °C/min. The final temperature was held for 14 min. Injector and transfer line temperatures were 260 °C and 280 °C, respectively. The results were calculated using 0 for non-detects. Recoveries of internal standards were 75–130%. Quality control was done by blanks, recovery standards, and certified reference material WMF-1 (Wellington lab. Inc., Ont., Canada). The limit of detection (LOD) was ranged from 0.006 to 0.098 ng kg^{-1} fat basis in samples of feedingstuffs.

3. Results and discussion

The concentrations of PCDD/Fs by weight in animal feed and feed additives are presented in Table 2. The upper and lower bound levels were calculated with the value of non-detectable congeners taken as equal to the LOD and equal to zero, respectively. The numbers after the sample name are the different sample sources for the same type of sample. Seven different sources of fish meal were analyzed and the concentrations ranged from 12.10 ng kg^{-1} to 0.50 ng kg^{-1} in the lower bound. The average concentration in the lower bound was 4.38 ng kg^{-1}. Generally, one or two samples of the same type were analyzed and the concentrations were quite different. The highest concentration of PCDD/Fs (23.33 ng kg^{-1}) was found in fish oil. Animal fat, shell powder, sodium bicarbonate, and DL-methionine showed relatively high levels of PCDD/Fs concentrations by weight. A sample of DL-methionine had a concentration of only OCDD of 8.28 ng kg^{-1}. Feed additives including minerals and limestone showed low levels of only PCDDs. PCDD/Fs were not found in a sample of a preservative ethoxyquin in the lower bound calculation. Low levels of PCDD/Fs were found in a sample of cotton seed and PCDD/Fs were not found in bit pulp at

Table 2
Concentrations of PCDD/Fs in animal feed and feed additives (ng kg^{-1})

Ingredient	Concentration[a]		Ingredient	Concentration	
	Lower bound	Upper bound		Lower bound	Upper bound
Chicken meal	4.13	4.59	Shell powder-1	7.11	7.43
Meat meal	0.19	0.69	Shell powder-2	3.57	4.03
Dried blood	0.86	1.34	Minerals	0.43	0.93
Plasma protein	1.40	1.84	Limestone-1	0.88	1.38
Animal fat-1	16.89	17.29	Limestone-2	0.01	0.51
Animal fat-2	5.84	6.30	Limestone-3	0.37	0.87
Fish meal-1	12.10	12.47	Sodium bicarbonate-1	0.14	0.64
Fish meal-2	5.76	6.16	Sodium bicarbonate-2	6.06	6.56
Fish meal-3	3.83	4.29	Bicalcium phosphate-1	2.03	2.52
Fish meal-4	2.23	2.73	Bicalcuim phosphate-2	0.41	0.91
Fish meal-5	4.15	4.61	Salt	0.44	0.94
Fish meal-6	2.12	2.53	Vitamins	3.0	3.46
Fish meal-7	0.50	0.96	Choline chloride-1	3.03	3.49
Fish oil	23.33	23.76	Choline chloride-2	0.23	0.61
Cotton seed	1.16	1.62	Ethoxyquin	nd	0.54
Bit pulp	nd	0.54	DL-Methionine	8.28	8.78

[a] Mean concentrations were calculated at non-detects = 0 for lower bound and non-detects = LOD for upper bound values. nd: not detected.

Table 3
Mean concentrations of PCDD/Fs in types of matrices (ng TEQ/kg product)[a]

Congener	Animal origin ($n = 6$)		Fish origin ($n = 10$)		Plant origin ($n = 2$)		Minerals & additives ($n = 14$)	
	Lower bound	Upper bound	Lower bound	Upper bound	Lower bound	Upper bound	Lower bound	Upper bound
2,3,7,8-TCDD	0	0.007	0	0.007	0	0.007	0	0.007
1,2,3,7,8-PeCDD	0	0.035	0	0.034	0	0.035	0	0.035
1,2,3,4,7,8-HxCDD	0.115	0.115	0.077	0.077	0.026	0.027	0.023	0.024
1,2,3,6,7,8-HxCDD	0	0.005	0	0.005	0	0.005	0	0.005
1,2,3,7,8,9-HxCDD	0	0.004	0	0.004	0	0.004	0	0.004
1,2,3,4,6,7,8-HpCDD	0.008	0.008	0.008	0.008	0	0.0004	0	0.0004
OCDD	0	0.0001	0	0.0003	0	0.00004	0	0.0001
2,3,7,8-TCDF	0	0.001	0.032	0.032	0	0.001	0	0.001
1,2,3,7,8-PeCDF	0	0.002	0	0.002	0	0.002	0	0.002
2,3,4,7,8-PeCDF	0	0.029	0.744	0.761	0	0.028	0	0.029
1,2,3,4,7,8-HxCDF	0.133	0.137	0	0.005	0	0.005	0	0.005
1,2,3,6,7,8-HxCDF	0	0.004	0	0.004	0	0.004	0	0.004
2,3,4,6,7,8-HxCDF	0	0.003	0.003	0.006	0	0.003	0	0.003
1,2,3,7,8,9-HxCDF	0	0.004	0	0.004	0	0.004	0	0.004
1,2,3,4,6,7,8-HpCDF	0	0.003	0.002	0.013	0	0.0004	0.001	0.001
1,2,3,4,7,8,9-HpCDF	0	0.0003	0	0.0003	0	0.0003	0	0.0003
OCDF	0	0.00001	0	0.00001	0	0.00001	0	0.00001
Sum of PCDD/Fs	0.256	0.357	0.866	0.963	0.026	0.126	0.024	0.125

[a] Mean concentrations were calculated at non-detects = 0 for lower bound and non-detects = LOD for upper bound values.

the lower bound. Based on the results of this study, feed from animal origin must be given priority to maintain the safety of animal feed. The European Union (EU) Scientific Committee on Animal Nutrition also mentioned that fish meal and fish oil are heavily contaminated by PCDD/Fs and dioxin-like PCBs (Malisch, 2001).

The TEQ levels in congener-specific mean concentrations are shown in Table 3. Twenty-nine samples from a total of 32 samples showed less than 1.0 ng WHO-TEQ/kg from a toxicological point of view. In the mean values of group of matrices, many of the congeners were shown zero in the lower bound. Low levels of PCDDs were only shown in feed of plant origin and feed additives except bicalcium phosphate-1 and choline chloride. A low level of 1,2,3,4,6,7,8-HpCDF was found in a sample of bicalcium phosphate. Two samples of choline chloride showed different congener profiles. Choline chloride-1 contained 1,2,3,4,7,8-HxCDD and OCDD. Choline chloride-2 contained 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 2,3,4,6,7, 8-HxCDF, 1,2,3,4,6,7,8-HpCDF, and OCDF. The different formulations and carriers of choline chloride resulted in different profiles of PCDD/Fs contamination. PCP-contaminated pine sawdust, a carrier for choline chloride, was the source of PCDD/Fs contamination in Germany, Belgium, and Spain in 2000 (Llerena et al., 2003). Limestone showed 0.04 ng WHO-TEQ/kg of only 1,2,3,4,7,8-HxCDD. The typical congener, 1,2,3,7,8,9-HxCDD in ball clay and kaolin samples was not found in any samples in this study (Rappe et al., 1998; Rappe and Anderson, 2000; Abad et al., 2002). Eljarrat et al. (2002) reported that levels of PCDD/Fs in samples of animal origin were higher than the levels in samples of mineral origin with the exception of kaolin. The total contributions of PCDDs were greatest in the kaolin samples (Abad et al., 2002). Kaolin was not included in this study. The TEQ levels found in all samples in this study were below the maximum levels that the EU has set for animal feed (Commission directive 2006/13/EC, 2006). The EU limits for dioxin are 0.75, 2, 6, and 1.25 ng WHO-TEQ/kg in feedingstuffs of animal origin, animal fat, fish oil, and fish and their products and by-products, respectively.

The congener profiles of the samples showing high concentrations of PCDFs in the lower bound are presented in Fig. 1. PCDD/Fs were found with 4.68, 1.59, 1.40, 0.85 and 0.33 ng WHO-TEQ/kg in fish oil, shell powder-1, fish meal-1, animal fat-1 and fish meal-1, respectively. PCDFs were the dominant congeners for samples with relatively high concentrations in feed of animal origin. 2,3,4,7,8-PeCDF in fish meals, fish oil, and shell powder and 1,2,3,4,7,8-HxCDF in animal fat showed the highest levels of dioxins. Only 1,2,3,4,7,8-HxCDD with 0.5 ng WHO-TEQ/kg was found in a sample of animal fat-2 which is not shown in Fig. 1. It indicates that the contamination source is different for feed from fish and other animals. The amount (4.42 ng WHO-TEQ/kg) of 2,3,4,7,8-PeCDF in a fish oil was 94% of the total TEQ concentration found in fish oil. However, the detected concentration was 38% of the total amount found in fish oil. 1,2,3,4,6,7,8-HpCDD, OCDD, and 2,3,7,8-TCDF were found in fish meal-1 and fish oil. 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD were often found to have low levels in the feed from animal origin. 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD were not found in any samples. Seven different fish meals were analyzed and their congener profile is shown in Fig. 2. 1,2,3,4,7,8-HxCDD was found in all samples. 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, and 1,2,3,

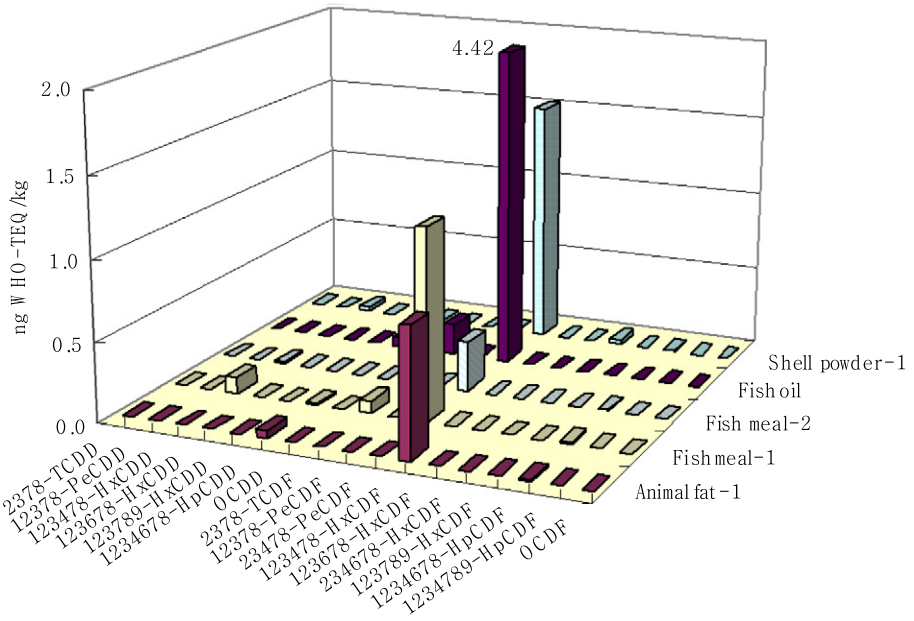

Fig. 1. Congener profiles in feed of animal origin showing intensive PCDFs. 2,3,4,7,8-PeCDF had the highest concentration in fish oil with 4.42 ng WHO-TEQ/kg.

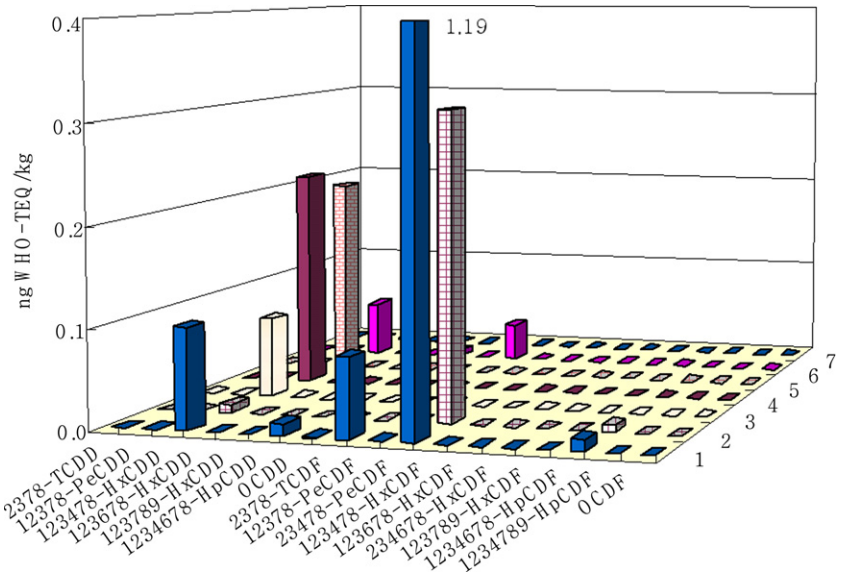

Fig. 2. Congener profiles in seven fish meal samples from different sources. 2,3,4,7,8-PeCDF had the highest concentration in fish meal sample 1 which had 1.19 ng WHO-TEQ/kg.

4,6,7,8-HpCDF were found in several samples. In fact, 2,3,4,7,8-PeCDF in fish meal-1 had a relatively high concentration compared to fish oil. However, there was no significance or different trend among samples. Samples 1 and 5 were from the same country. Samples 3 and 4 were from the same country. Samples 2, 6, and 7 were from different countries. The origin of the products did not show any trends although the source of fish was unknown.

In most cases, food contaminations of PCDD/Fs through feed additives are probably due to incident or anthropogenic sources. This means that the sources of contamination from feed additives are avoidable. However, feed of animal origin might be a common source of PCDD/Fs until almost all PCDD/Fs are removed from the environment. There are two different effects for final assorted feed. Contaminants are either diluted or concentrated from high or low levels of dioxins in additives, respectively or single ingredient to be an assorted. One major source of contamination for food of animal origin is feed or feed additives, and monitoring should find the general contribution of contaminants to food of animal origin. This survey showed that the primary source of PCDD/Fs in feed was animal materials used in the ordinary production process.

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